# SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in the Sails Pavilion of the San Diego Convention Center, on Tuesday, August 1 and Wednesday, August 2. All posters will be posted from 9:30am until 5:00pm. Presenting authors will be in attendance from 12:30pm until 1:30pm. Please refer to the onsite Abstracts Title Guide for a complete schedule of posters.

Below are the topics and their scheduled times.

---

## TUESDAY, AUGUST 1, POSTER SESSIONS

**9:30am – 5:00pm**



## WEDNESDAY, AUGUST 2, POSTER SESSIONS

**9:30am – 5:00pm**



Ed. Note: These abstracts have been reproduced without editorial alteration from the materials supplied by the authors. Infelicities of preparation, grammar, spelling, style, syntax and usage are the authors'.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Cancer/Tumor Markers

## A-001

### The expression of DAMP proteins HSP70 and cancer testis antigen SPAG9 in peripheral blood of patients with HCC and lung cancer

B. Ren[1], S. Luo[2], F. Xu[1], G. Zou[1], G. Xu[1], Y. Huang[3]. *[1]Hunan Provencial Second People's Hospital, Changsha, China, [2]Hunan University of Chinese Traditionary Medicine, Changsha, China, [3]Hunan University of Chinese Trandiational Medicine, Changsha, China*

**Background:** There are different views of how the immune system participates in the reaction to cancer. Here, we evaluated expression of DAMP proteins heat shock proteins70 (HSP70) and cancer-testis antigen SPAG9 in patients with hepatocellular carcinoma (HCC) and lung cancer to explore tumor immunity. According to the "danger" theory of immunology, damage-associated molecular patterns (DAMPs) are released by the body's cells as a signal that there is endogenous danger. DAMPs, such as HSPs, are released from damaged or necrotic tissue and by some activated immune cells. DAMPs and pathogen-associated molecular patterns (PAMPs) signal through toll-like receptors (TLRs) to activate antigen presenting cells (APCs) and initiate the adaptive immune response. This theory is a challenge to the traditional self/non-self discrimination (SNSD) theory, which holds that activation of the immune system occurs strictly based on whether or not an entity is foreign. The danger theory, in contrast, posits that the immune system is only triggered by "dangerous" entities. Extracellular HSP70 and SPAG9 are proteins released by tumor cells, but their characteristics and the roles played in the development of cancer are different. Like SPAG9, HSP70 levels are upregulated in certain cancer patients. In this study, levels of HSP70, HSP70 antibodies, and SPAG9 antibodies were compared in peripheral blood of patients with lung cancer and hepatocellular carcinoma (HCC) and healthy subjects. We compared the similarities and differences between expression profiles of antigens and auto-antibodies and evaluate the value of these markers in the diagnosis of lung cancer and HCC. We also speculate on how immune responses to these two proteins support the SNSD and danger theories of the immune response to cancer.**Methods:** ELISA was be used to analyze HSP70, HSP70 antibody and SPAG9 antibody levels in serum of 45 patients untreatd and 52 treated lung cancer patients, 31 patients with HCC,41 patients with hepatitis/cirrhosis and 54 healthy subjects. **Results:** levels of HSP70 and SPAG9 antibody were significantly higher in serum of lung cancer and HCC patients than in serum from healthy subjects ($P < 0.001$), but there were no differences in levels of HSP70 antibody in patients and controls. Levels of serum SPAG9 antibody in newly diagnosed lung cancer patients were significantly higher than in treated lung cancer patients ($P < 0.05$), but there were no differences in levels of HSP70 or HSP70 antibody. Levels of serum HSP70 and SPAG9 antibody, but not HSP70 antibody, were also higher in hepatitis/cirrhosis patients than in healthy subjects ($P = 0.005$, $P < 0.001$). Levels of serum SPAG9 antibody were significantly higher in HCC patients than in hepatitis/cirrhosis patients, but there were no differences in HSP70 or HSP70 antibody levels. Finally, levels of serum HSP70 and SPAG9 antibody were significantly higher in HCC patients than in lung cancer patients ($P < 0.05$, $P < 0.001$).**Conclusion:** Cancer-testis antigen SPAG9 induces a strong humoral immune response in cancer patients but HSP70 does not. SPAG9 has potential as a tumor-specific biomarker but HSP70 has not.

## A-002

### *Diagnostic significance of plasma and ascitic fluid cholesterol, albumin, protein, and serum ascites ascites gradient(SAAG) in differentiating malignant from cirrhotic ascites*

L. EKPE[1], E. Ekanem[2], K. INAKU[1]. *[1]University of Calabar Teaching Hospital, Calabar, Nigeria, [2]University of Lagos, Lagos, Nigeria*

**Background:**

Over the years, differential diagnosis of ascitic fluid has been a dilemma for practicing physicians. Many analytes have been assessed to enhance diagnosis. In developing countries, emphasis is laid on using less expensive biochemical parameters and methods to differentiate ascitic fluid. The aim of the present study was to assess the value of ascitic total cholesterol, albumin, protein and their gradients (SAAG ) in differentiating malignant and cirrhotic ascites.

**Materials/Methods:**

This cross sectional prospective study was carried over a 2 year period at the medical, surgical, emergency and gyneacology units of the Lagos University Teaching Hospital (LUTH). A total of 61 adult patient with ascites were recruited for the study. Serum and ascitic fluid were assessed for levels of cholesterol and albumin and their gradients.

**RESULTS:**

Of the total 61 adult patients recruited (35males, 26Females), the mean age of the study population was 46.84 ± 12.10, mean body mass index (BMI) was 24.43 ± 3.18, serum and ascitic value of cholesterol, protein and albumin mg/dL were 125.1 ± 79.30, 72.29 ± 6.65, 39.51 ± 7.98 and 36.34 ± 12.39…… with corresponding *p* value of 0.475, 0, and 0.072 respectively). The accuracy of serum cholesterol, protein and albumin was 81.99%, 37%, 60.6% when compared to ascitic cholesterol, protein and albumin (58.9%, 70.5%, 67.2%). Serum protein had a sensitivity of 51.4%. Mean SACG was 11.48 ± 8.7.

**CONCLUSION**

Differentiation of cirrhotic and malignancy- related ascites seem to have very little diagnostic values from analytes of cholesterol, albumin and protein.

**KEYWORDS:**

**Cholesterol, ascitic, cirrhosis, malignant, albumin, protein.**

## A-003

### The serum concentration of pro-GRP in diagnosis of lung cancer

N. Serdarevic[1], R. Serdarevic[2], J. Coric[1]. *[1]Institute for Clinical Chemistry and Biochemistry; Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]Department of Ophthalmology, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

Serum concentration of Pro-GRP in the Diagnosis of Lung Cancer

**Background:** Lung cancer is classified into two major entities depending on cell type: small cell lung cancer and non-small cell lung cancer (SCLC and NSCLC, respectively). SCLC accounts for up to 15% of all new lung cancer cases and differs biologically from NSCLC by the presence of neuroendocrine differentiation and a higher rate of tumor growth. Clinically, early metastatic spread is very common. SCLC is highly sensitive to initial chemotherapy and radiotherapy. In recent years, a precursor of the neuropeptide gastrin releasing peptide, progastrin-releasing peptide (ProGRP), has been reported as the most promising marker for SCLC. The aim of study was to investigate the diagnostic and prognostic significance of pro-gastrin-releasing peptide (ProGRP) in small cell lungcancer (SCLC) and compare this marker with lung healthy control group.

**Methods:** The concentrations of Pro-GRP in 100 serum samples were determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 Abbott diagnostic. All of 50 patients were hospitalized at Oncology department in Intensive Care Unit at the University Clinics Center of Sarajevo. Pro-GRP was determined in lung cancer detection with SCLC pathologically confirmed lung cancer serum. We considered Pro-GRP 50 pg/ml as the upper limit of normality. Other group of 50 patients was a lung healthy control group. Collected data were statistically analyzed using programs SPSS version 16.0 and MedCalc.

**Results:** Serum of patients with lung cancer group, Pro-GRP content (1550.01 ± 287.6 pg/ml) and positive (63% ) were higher than benign lesions of the lung healthy control group (33.18 ± 3.92 pg/ml). High concentration of Pro-GRP content have sensitivity of 82.85 % , a specificity of 76.67 % , the largest area under the ROC curve was 0.793. It was found significant difference between lung cancer group and healthy individuals using Mann-Whitney test p= 0.0037.

**Conclusion:** Our study supports ProGRP as an important marker of SCLC and points the way for further clinical studies to explore its value in monitoring response to therapy and patients' follow-up.

## A-004

### Comparison of Conventional Chemical Method to Immunochemical Method for Faecal Occult Blood Testing

B. K. T. P. Dayanath[1], L. W. B. Samarawickrama[2], D. I. Siriwardhana[2]. *[1]North Colombo Teaching Hospital, Ragama, Sri Lanka, [2]Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka*

**Introduction:**

Faecal occult blood testing (FOBT) is one effective method to screen for colorectal cancer and to assess for gastrointestinal bleeding in suspected patients. Differences in the sensitivity and the specificity among methods have result in significant effects on further investigations.

**Objective:**

To check whether there is a significant difference of the results of traditional chemical method with immunochemical method for faecal occult blood testing.

**Methods:**

We evaluated the analytical performance of two different methods (traditional chemical based method and immunochemical method) using 25 patient samples (3 consecutive samples). Colonoscopy finding of each of the patient was taken as the **"gold standard"**.

**Results**:

| | | **Patient with positive colonoscopy finding** | | **Patient with positive colonoscopy finding** | | | |
|---|---|---|---|---|---|---|---|
| | | Colonoscopy positive | Colonoscopy negative | Colonoscopy positive | Colonoscopy negative | | |
| **FOB screen test outcome (traditional Chemical method)** | Test outcome positive | True positive (7) | False positive (5) | True positive (12) | False positive (1) | Test outcome positive | **FOB screen test outcome (Immunochemical method)** |
| | Test outcome negative | False negative (7) | True negative (6) | False negative (2) | True negative (10) | Test outcome negative | |
| | | **Sensitivity 58.3%** | **Specificity 54.5%** | **Sensitivity 92.3%** | **Specificity 90.9%** | | |

The analytical sensitivity and specificity were measured and Chemical based FOBT gave 58.3% of sensitivity and 54.5% specificity. Immunochemical FOBT produced 92.3% of sensitivity and 90.9% of specificity.

**Conclusions:**

These results demonstrate significant differences in the analytical performance among two FOBT methods. The immunochemical FOBT which showed superior sensitivity and specificity is more suitable than the chemical based FOBT when selecting an FOBT method for screening for colorectal cancer or for the assessment of gastrointestinal bleeding in suspected patients.

## A-005

### Methylation of KLF2 Associates With Its Expression and Non-small Cell Lung Cancer Progression

W. Jiang. *Department of Clinical Laboratory, Daping Hospital, Third Military Medical University, Chongqing, China*

Background: Kruppel-like factor 2 (KLF2) is a putative tumor suppressor gene. Our study investigated its role and epigenetic mechanisms in human non-small cell lung cancer (NSCLC) in ex-vivo and in vitro.

Methods: A total of 47 paired NSCLC and normal tissues and six cell lines were analyzed using qRT-PCR for KLF2 expression. KLF2 methylation was assessed using the methylation specific PCR (MSP) or bisulfite sequencing PCR (BSP). Functional KLF2 region 4 (+567 to +906) was confirmed using the dual-luciferase reporter assay, while CCK-8 cell viability and flow cytometric assays were used to assess changes in cell viability, cell cycle distribution, and apoptosis after knockdown or re-expression of KLF2. Western blot was performed to analyze KLF2 expression and p15 and p21 expression in cells.

Results: KLF2 expression was significantly reduced in NSCLC cells and tissues via KLF2 methylation. Reduction of KLF2 expression was associated with KLF2 region 4 hypermethylation in 27 of 47 (57.45%) NSCLC tissues. Furthermore, methylation at KLF2 region 4 was significantly associated with lymph node metastasis and advanced TNM stage. Re-expression of KLF2 suppressed NSCLC cell viability, arrested cells at G0/G1 cell cycle by induction of p15 and p21 expression, and promoted apoptosis, whereas knockdown of KLF2 expression had the opposite effects on cells.

Conclusion: KLF2 possesses tumor suppressor functions in NSCLC and detection of KLF2 methylation should be further evaluated as a tumor or prognostic biomarker for NSCLC.

## A-006

### A comparative study of serum pseudocholinesterase activities in relation to transaminases, gamma glutamyl transaminase and alkaline phosphatase in liver disorders in hospital based study

A. S. A. J. Dhungana. *Kathmandu Medical College, Kathmandu, Nepal*

**Abstract**

**Background:** Liver is the main source of pseudocholinesterase. It is a non-specific cholinesterase found in the blood plasma and may be reduced in patients with advanced liver diseases. Measurement of serum pseudocholinesterase activity serves as a sensitive indicator of synthetic capacity of the liver and can be used as a prognostic marker for carcinoma.

**Objective:** To compare pseudocholinesterase activities in relation to transaminases, Gamma Glutamyl Transaminase (GGT) and Alkaline phosphatase (ALP) in liver diseases.

**Method:** A prospective study was conducted at College of Medical Sciences – Teaching Hospital (CMS-TH), from May 2011 to May 2013 with 25 healthy (controls) and 25 diagnosed cases each of carcinoma of liver, cirrhosis of liver, infective hepatitis and obstructive jaundice among both sexes of same age group attending medical outpatient and inpatient departments. Serum pseudocholinesterase and other liver function test parameters were performed with semi - autoanalyzer and Roche Hitachi 902 respectively.

**Result:** Pseudocholinesterase level was significantly decrease in the order of control (mean ± SD = 6865.12 ± 928.41) > obstructive jaundice (mean ± SD = 5539 ± 791.05) > infective hepatitis (mean ± SD = 3800.69 ± 764.17) > cirrhosis of liver (mean ± SD = 1735.16 ± 433.82) > carcinoma of liver (mean ± SD = 1369.48 ± 276.64). The difference in the means was statistically significant ($p<0.001$). Similarly aspartate transaminase (AST) (Mean ± SD= 311.48 ± 177.15) and alanine transaminase (ALT) (Mean ± SD = 491.36 ± 308.92) were higher in hepatitis but in carcinoma of liver the AST (Mean ± SD = 292.55 ± 92.85) level was higher than ALT (Mean ± SD = 179.24 ± 70.25). The difference in the means was statistically significant ($p<0.001$). AST level was 1.6 time higher than ALT. Alkaline phosphatase (ALP) (Mean ± SD = 419.39 ± 90.49) and gamma glutamyl transaminase (GGT) (Mean ± SD = 257.81 ± 68.29) level were higher in obstructive jaundice and GGT (Mean ± SD = 195.54 ± 107.75) level was found to be higher in carcinoma of liver. The difference in the means was statistically significant ($p<0.001$)

**Conclusion:** There was significant decrease in pseudocholinesterase level in carcinoma of liver followed by cirrhosis of liver and less so in infective hepatitis. AST and ALT level were higher in hepatitis cases. The level of AST was found higher in carcinoma of liver than ALT. In obstructive jaundice ALP and GGT was raised and statistically significant. GGT level was raised in carcinoma of liver. Pseudocholinesterase level was found to be normal in obstructive jaundice. The study results indicated that with more severe liver cell destruction or cell degeneration, there was corresponding significant decrease in the level of pseudocholinesterase, which could be used as diagnostic marker of liver disease.

## A-007

### Demethylation of the *MIR145* Promoter Suppresses Migration and Invasion in Breast Cancer

Z. Lu, S. Liu, X. Li, W. Chen. *Central Hospital of Wuhan, Wuhan, China*

**Background:** Breast cancer is the most common and lethal malignancy in women, the metastasis to distant sites that is the major cause of mortality in breast cancer patients. Thus, searching for molecules that suppress the metastasis of breast cancer cells and that may provide novel targets for clinical therapies is a worthwhile undertaking. miR-145 has been implicated in the progression of breast cancer. However, the underlying mechanism for the miR-145 decrease in breast cancer has not been fully elucidated.

**Methods & Results:** Here, we report that miR-145 expression is decreased in breast cancer specimens and cell lines and that this low level of expression is associated with DNA methylation of its gene, *MIR145*. Methylation of *MIR145* has previously been correlated with cell migration and invasion, both in vivo and in vitro. We found that demethylation of *MIR145* to reactivate miR-145 contributes to the anti-cancer properties of 5-aza-2′-deoxyazacytidine (5-AzaC) and therefore is a potentially valuable biomarker for breast cancer.

**Conclusion:** In conclusion, the present study shows that the reduced expression of miR-145 in breast cancer is due to DNA methylation, and miR-145 promotes cell migration and invasion by targeting ANGPT2 in vivo and in vitro. Promoter methylation state and expression level of miR-145 are valuable biomarkers in breast cancer, and further investigation is underway to develop these biomarkers for clinical use.

## A-008

### Expression and Clinical significance of plasma MALAT1 in patients with breast cancer

F. Yan, J. Wu, L. Yu, X. Wang, M. Zhu. *Clinical Laboratory ,Nanjing Medical University Affiliated Cancer Hospital & Jiangsu Cancer Hospital, Nanjing, China*

**Objective:** To investigate the level of plasma MALAT1 in breast cancer patients and its clinical significance.

**Methods:** Plasma samples were collected from 102 breast cancer patients, 47 breast benign tumor and 50 healthy controls. The expressions of GAPDH and MALAT1 were determined by RT-qPCR. The potential association between plasma GAPDH levels and cases' clinicopathologic features were analyzed to evaluate the stability of GAPDH. A receiver operating characteristic (ROC) curve was constructed for differentiating breast cancer patients from healthy controls.Meanwhile,the association between MALAT1 and the clinicopathologic features were analyzed.*T*-test and one-factor ANOVA test were used for normal distribution of quantitative data. The rank sum test was used for non-normal distribution of data**.**

**Results:** GAPDH level was stable in female plasma and was not affected by age and pathology. GAPDH can be used as a reference for the detection of plasma lncRNAs. Levels of MALAT1 were significantly higher in BC patients[5.58(2.17~12.34)] compared with breast benign tumor patients and healthy controls [1.08(0.611~2.58) (Z=6.209,*P* <0.001), 1.63(0.98~3.51)(Z=4.871,*P* <0.001)].However,there was no significantly difference between breast benign tumor patients and healthy controls (Z=-1.675,*P* =0.094).Area under the ROC curve of MALAT1 was 0.744.The sensitivity and specificity were 54.1%,86.3%.Levels of MALAT1 were up-regulated in poor differentiation BC patients[8.55(3.14~22.40)],When they were compared with those in moderate or well differentiation BC patients[3.25(1.85~11.16) (Z=2.435,*P* =0.015)].

**Conclusion:** The expressions of MALAT1 were highly elevated in plasmas of BC patients and were associated with tumor differentiation.The level of plasma MALAT1 may be an important basis for the diagnosis of breast cancer.

**Key words:** Breast neoplasms;Long non-coding RNA;Plasma

## A-009

### Biclonal gammopathy detected not in immunohistochemical stain but in biochemical laboratory test in patient with multiple myeloma accompanying intravertebral plasmacytoma

Y. Han, S. Cho, J. Han, W. Lee. *Kyung Hee University Medical Center, Seoul, Korea, Republic of*

Background; Multiple myeloma is a plasma cell neoplasm which is characterized by clonal proliferation of plasma cells. Plasma cells secrete monoclonal immunoglobulins and/or free light chains, and this is referred to as monoclonal gammopathy. Protein electrophoresis is used for confirming monoclonal gammopathy and diagnosing multiple myeloma. While, biclonal gammopathy is characterized by simultaneous production of two different kinds of monoclonal components. It occurs in 3-6% of all monoclonal gammopathy. In biclonal gammopathy, the most common combination is known to be IgG and IgA (33%), followed by IgG and IgM combination (24%). We experienced a very rare case of biclonal gammopathy with IgA and IgM lambda type confirmed only by biochemical laboratory tests with quantitative difference of secreted monoclonal intact immunoglobulins in patient with multiple myeloma accompanying intravertebral plasmacytoma.

Methods; Bone marrow aspiration and biopsy examinations were done for diagnosing multiple myeloma. Monoclonal components in serum and urine were detected in capillary electrophoresis via capillary 2 (Sebia, France) and they were reconfirmed by a high-resolution gel electrophoresis in a Hydrasys analyzer (Sebia, France) using Hydragel 15 HR gels (Sebia, France). Also, immunohistochemical stains were performed on both bone marrow and intravertebral plasmacytoma specimens.

Results; An 81-year-old man who suffered from lower back pain and both leg weakness for 3 months was admitted to the department of neurosurgery. The patient was transferred to the department of hemato-oncology because plasmacytoma was confirmed by biopsy from vertebral lesion. Immunohistochemical stains in vertebral lesion revealed positive results for IgA and lambda, negative result for IgM. In bone marrow examination, plasma cells are counted upto 66.6%. Samely with vertebral lesion, IgA was positive and IgM was negative, respectively in immunohistochemical stain of bone marrow biopsy. However, serum protein and immunofixation electrophoreses confirmed the presence of two distinctly separated bands in IgA and IgM lambda resulting in biclonal gammopathy. This case showed the difference of the quantity between IgA and IgM serologically, which is that IgM secretion was considerably less than IgA. In urine, abnormal zone of restriction in lambda component was confirmed, too.

Conclusion; IgA positivity was confirmed in immunohistochemical stain in vertebral lesion. Meanwhile, serum electrophoresis showed the positive results of IgA and IgM. According to these results, we considered the possibility of double primary biclonal tumor, that is, IgA type plasmacytoma and IgM type multiple myeloma. To verify this, immunohistochemical stains of both tumor and bone marrow were performed. Eventually, only IgA was positive (and IgM was negative) in both tumor and bone marrow specimens. This suggests that clones from vertebral lesion and bone marrow have the same origin. Because IgM was secreted very small amount, IgM-producing clone cannot be detected by immunohistochemical staining. Instead, the presence of IgM-producing clone can be confirmed only by biochemical laboratory test, capillary electrophoresis. This seems to have occurred because biochemical laboratory tests have better detection limits and higher sensitivity than immunohistochemical staining. After his diagnosis, the patient is now undergoing anticancer treatment.

## A-010

### A novel biomimetic reference material for ctDNA

R. Peng[1], R. Zhang[2], Z. Li[2], J. Li[2]. *[1]National Center for Clinical Laboratories, Beijing Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, [2]National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:**

In recent years, liquid biopsy based on circulating tumor DNA (ctDNA) analysis has shed a new light on the molecular diagnosis and monitoring. However, the absolute and relative scarcity of ctDNA in plasma is problematic and thus the lack of proper detection methods has limited clinical variation. Here, we developed a set of Synthetic ctDNA Reference Materials (SCRMs) to emulate paired tumor-normal samples in real clinical tests as the quality control for somatic variation testing.

**Methods:**

We developed a set of SCRMs comprise spike-in M-cfDNA (sM-cfDNA) as simulated ctDNA reference materials and matched genomic DNA as genetic background. sM-cfDNA with somatic mutations was prepared in two spike-in approaches. On one hand, sheared site-directed mutagenesis DNA fragments were spiked in with cfDNA based on MNase digestion (M-cfDNA) to emulate SNVs and Indels. On the other hand, CRISPR/Cas9 edited HEK293T cells were blended with HEK293T cells and then digested by MNase to represent the fusion genes. To prove their reliability, the characteristics of SCRMs were compared to that of patient-derived plasma samples by next-generation sequencing. A collaborative study encompassing 11 laboratories was also done to prove the applicability of SCRMs.

**Results:**

Ten SCRMs with *KRAS* G12D, *EGFR* T790M, L858R, exon 19 deletion, exon 20 insertion and *EML4-ALK* rearrangement variant 2 and 3 were prepared. The allele frequencies of SNV and Indels obtained by theoretical calculation linearly related to that detected by digital PCR (Fig.1 a). Compared with patient-derived plasma samples, SCRMs have similar characteristics, such as size distribution (Fig.1 b). Furthermore, the collaborative study by 11 laboratories using different methodologies and workflows showed that most of the laboratories can correctly detected SCRMs in qualitative and quantitative assay (Fig.1 c, d).

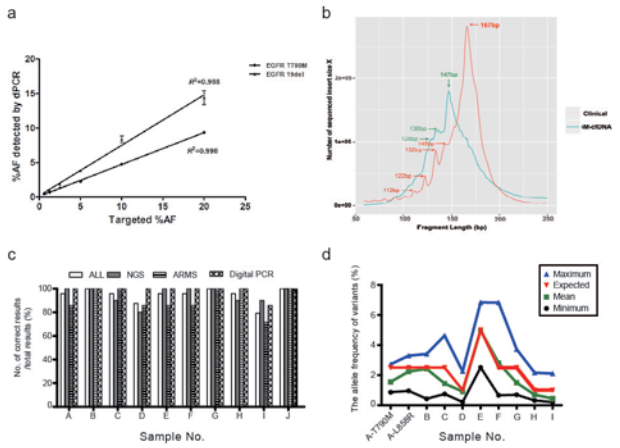



Fig 1: a) Allele frequency (AF) detection of sM-cfDNA was found to be linear with both SNVs and Indels. b) The fragment lengths derived from sM-cfDNA (blue) were similar to that of clinical controls (red). c) The correct rates of sample A-J reported by the laboratories using NGS, ARMS and digital PCR. d) The maximum, minimum and mean values of AFs of each variant reported by all the laboratories and the expected AFs of each variant.

**Conclusion:**

Owing to the high reliability and comparability, our SCRMs can be utilized as optimal quality controls in test performance assessments for ctDNA somatic mutation detection.

## A-011

### Validation of serum-based 4Kscore Test for detection of high grade prostate cancer

C. E. Higgins[1], P. Neybold[2], C. R. Barnes[3], V. Mathur[3], Y. Dong[1], M. Reeve[1], V. Linder[1], J. Weisberger[2]. *[1]OPKO Diagnostics, Woburn, MA, [2]Bioreference Laboratories, Elmwood Park, NJ, [3]OPKO Lab, Nashville, TN*

**Background**:The 4Kscore Test has been clinically demonstrated to improve shared decision making to proceed with a prostate biopsy, by providing a personalized, accurate risk of aggressive prostate cancer (Gleason score ≥7) on biopsy. A prospective study of 1,012 American patients scheduled for prostate biopsy (Parekh et al. 2015) showed excellent discrimination by the 4Kscore Test between men harboring aggressive prostate cancer and those with indolent tumors or no cancer (AUC 0.82). Since 2015, the 4Kscore Test has been recommended by the National Comprehensive Cancer Network Guidelines as a decision-making tool in the prostate cancer diagnosis process. The 4Kscore Test includes the assay of four biomarkers (total prostate specific antigen(tPSA), free PSA(fPSA), intact PSA(iPSA), and human kallikrein 2(hK2)) and was launched as a plasma-based blood test on the observation that some analytes are less stable in serum than in plasma (Woodrum et al. 1996, Christensson et al. 2011). Assay of the 4Kscore in serum allows for immediate reflex testing of a sample originally submitted for serum tPSA assay, with no additional blood draw. Here, we validate the use of 4Kscore in serum by: 1)demonstrating excellent analytical performance of the iPSA and hK2 assays in serum, 2)proving in a prospective clinical study equivalence between 4Kscore generated from serum and plasma samples, and 3)proving sufficient stability of serum for the 4Kscore when handled according to current serum tPSA protocols.

**Methods**:iPSA and hK2 custom assays were performed on the AutoDELFIA platform utilizing time-resolved fluorescence, and the assays' analytical performances were evaluated according to CLSI protocols at three independent laboratories (OPKO Diagnostics, OPKO Lab, and BioReference Laboratories). The tPSA and fPSA assays were performed with the Roche Elecsys assays on the Cobas instrument. Patient-matched plasma and serum samples from a cohort of 353 men scheduled to undergo prostate biopsy were collected in a prospective clinical study conducted at eight Veterans Administration Hospitals. Samples were measured in a CLIA-certified laboratory and were handled according to the procedures in place for accepting and processing samples submitted for tPSA assay. A subset of samples (N=57) were repeatedly measured over a three-day period.

**Results**:A complete characterization of analytical performance in three laboratories confirmed robust reproducibility, accuracy, and linearity at clinically relevant ranges of iPSA and hK2 in serum (iPSA LoQ:28.5pg/mL, hK2 LoQ:4.4pg/mL; repeatability and total precision for both assays ≤15%). Mean percent recovery over three days of repeated measurements in serum exhibited no significant change in a mixed model regression analysis (N=57,p>0.05); 4Kscore generated from serum three days after collection was equivalent to first day results (slope=1.014(CI:0.997-1.040,intercept=0.005(CI=.0005-0.012),Passing-Bablok). 4Kscore generated from serum were equivalent to those generated from the same patient's plasma (N=353,slope=1.011(CI:1.003-1.020),intercept=-0.001(CI:-.005-0.001),Passing-Bablok).

**Conclusion**:Analytical performance of the iPSA and hK2 custom assays in serum meets or exceeds plasma performance. The equivalence of 4Kscore whether generated from serum or plasma and the consistent performance of 4Kscore with routine serum sample handling in a CLIA environment allow the 4Kscore to be used as a reflex option on tPSA results.

## A-012

### Clinical implications and multiple antitumor effects of miR-651 and miR-708 in renal cell carcinoma

C. Zhang[1], Q. Yang[1], C. Wang[1], C. Wu[1], C. Zhang[2]. *[1]Jinling Hospital, Nanjing, China, [2]Nanjing University, Nanjing, China*

**Background:** The mechanisms involved in renal cell carcinoma (RCC) development and progression remain unclear, and new biomarkers are needed in routine practice to improve the diagnostic and/or prognostic accuracy. However, there is no standard serum biomarker to facilitate diagnosis or prognostic stratification in patients with RCC. There is increasing evidence that microRNAs (miRNAs) are involved in cancer development and progression and circulating miRNAs have great potential as biomarkers for diagnosis and prognosis in patients with several types of cancers. Our purpose was to search valuable biomarker for the diagnosis of RCC and to evaluate their functional significance and possible mechanism in RCC.

**Methods:** An initial microarray survey of 754 miRNAs was firstly performed using the TaqMan Low Density Array followed by a hydrolysis probe-based RT-qPCR validation from serum samples of 33 RCC cases and 33 normal controls to identify significantly dysregulated miRNAs in RCC. Subsequently, the diagnostic values of these miRNAs were evaluated by ROC analysis. The expression levels of miR-651 and miR-708 in tumor tissues (n=17 pairs) were also examined. Furthermore, *in vitro* experiments including CCK8 proliferation, transwell and wound healing assays were conducted to explore the potential functions of miR-651 and miR-708 in RCC. The databases including miRanda, TargetScan and PicTar were used to predict the common target genes of miR-651 and miR-708. Luciferase reporter assays combined with western blotting were employed to validate the target genes. Moreover, xenograft mouse models were used to demonstrate the role of miR-651 and miR-708 in RCC.

**Results:** The serum levels of four miRNAs were verified to be significantly increased, whereas the levels of four miRNAs were markedly decreased in RCC patients compared with the noncancer controls (at least $P < 0.05$). Of the eight dysregulated miRNAs, miR-651 and miR-708 exhibited the largest AUCs: 0.888 (95% CI 0.833-0.943) and 0.832 (95% CI 0.786-0.878), respectively. Luciferase reporter assays and western blotting showed that both miR-651 and miR-708 directly regulated RAP1B, a Ras-related small GTP-binding oncoprotein implicated in a variety of tumors. In RCC clinical specimens, the RAP1B protein level was inversely correlated with expressions. *In vitro* gain-of-function and loss-of-function studies in human renal carcinoma cell lines A498 and ACHN, demonstrated that miR-651 and miR-708 synergistically suppressed cell proliferation and migration by directly inhibiting RAP1B, and this effect was reversed by co-transfection with RAP1B. Furthermore, *in vivo* xenograft mouse model experiments demonstrated that the tumor weights in the miR-651 & miR-708 group were significantly lower than those in miR-651 or miR-708 group, and the miRNA transfected groups were all lower than the negative control.

**Conclusion:** miR-651 and miR-708 may potentially serve as novel biomarkers for RCC and may act as tumor suppressors in RCC progression by synergistically inhibiting the RCC cell proliferation and migration through targeting oncogene RAP1B. Our findings indicate that targeting miR-651 and miR-708 by a genetic approach may provide a novel strategy for the treatment of RCC.

## A-013

### FIT test prescription patterns and positive conversion rates of serial results of the target population (50-75 yo) in a prescription based colon cancer screening program in the province of Quebec, Canada.

J. Dube[1], F. M. Brouwers[2], F. Corbin[1], A. S. Çaku[1]. *[1]Université de Sherbrooke et CR-CHUS, Sherbrooke, QC, Canada, [2]Université de Sherbrooke, Sherbrooke, QC, Canada*

**Backgroud**

The fecal immunochemical test (FIT) uses antibodies to detect human hemoglobin protein in stool. This test has been offered in Quebec since 2013 as part of a Provincial Colon Cancer Screening Program and has implemented as a prescription-based colorectal screening strategy. The threshold for a positive test has been set at 175 ng/ml, a higher level as compared to other territories. This study aimed to investigate the prescription pattern and guideline adherence of physicians as well as the positive conversion rates in serial results of subjects with a negative first test.

**Methods**

In this retrospective study, we analyzed a database of anonymized data from 214,209 subjects with serial FIT testing. Our study population was selected based upon the following criteria: age ranging from 50-74 years and a minimal interval of 90 days between the two tests. To investigate the prescription pattern, intervals between serial tests were calculated for every subject. To evaluate the positive conversion rate in serial testing, subjects were categorized into 8 groups depending on the result of their initial test (A: 0-30, B: 31-49, C: 50-74, D: 75-99, E: 100-124, F: 125-149, G: 150-174, H: 175 ng/ml). The positive conversion rate was calculated for group A to G. Data were analyzed with the statistical package R.

**Results.**

Our study population included 174,362 subjects (90719 women, 83628 men) with a median age of 62 years. After a 3-year period of the implementation of FIT testing in Quebec, the prescription interval pattern had a bimodal distribution with peaks at 13 and 26 months following the initial test, with a median at 16 months. Furthermore, our data also showed that subjects with an initial FIT result of less than 30 ng/ml had a lower probability of conversion on repeat testing as compared to those of group G (2.6% vs 25.7%, $p<0.001$) The positive conversion rate increased significantly for each concentration category (A- 2.6%, B-9.1%, C-11.8%, D-15.3%, E-18.4%, F-21.2%, G-25.7)

**Conclusion.**

In Quebec, the FIT test is currently prescribed with a shorter median interval than that recommended by provincial guidelines. The bimodal distribution of prescription pattern suggests that there may be an ongoing change towards the recommended two-year interval. Furthermore, there is an increased trend in positive conversion rates for each subsequent concentration category. Our results suggest that a multiple threshold screening strategy based on initial results might be considered in FIT screening programs.

## A-014

### Serum thyroid hormone profile in Breast Cancer of local Libyan Patients.

J. R. Peela[1], A. Jarari[2], H. Y. Ali[2], N. M. Jarari[3], A. Rawal[1], J. Kooriyil[1], M. G. I. El Fituri[4], S. Awad[2], S. A. Abdulla[2], A. Zakokoo[2]. *[1]St Matthews University, Grand Cayman, Cayman Islands, [2]Department of Biochemistry,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [3]Department of Pharmacology,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [4]Department of Global Health,College of Global Public Health,New York University, New York, NY*

**Background**: Recent studies indicate a possible relationship between thyroid diseases and breast cancer in vivo. In addition, estrogen-like effects of thyroid hormones on breast cancer cell growth are seen in vitro. Therefore, this study evaluated thyroid function in breast cancer patients, women with fibrocystic disease and healthy controls. **Material and methods:** pretreatment group consisted of breast cancer patients who at the initial time of diagnosis (n=28), Post treatment group comprising of patients who had undergone either chemotherapy/ radiotherapy or hormone therapy for their disease (n=37) and fibrocystic group comprising of patients with benign breast tumor (n=21) and 51 healthy control group consisted of members of the public with no prior history of breast cancer or other cancer related disorders (n=51) were included in the study. Thyroid history was reported. Thyroid hormones (fT4, fT3, TSH, T3, T4) and thyroid antibodies (TPO and TG) were determined. Statistical analysis was performed by Kruskal-Wallis test and Mann-Whitney U ($p<0.005$ significant). **Results:** FT4 which were significantly lower in breast cancer patients (post treatment Ca Breast, pre-treatment CaBreast) compared to control [($p=0.001$) ($p=0.003$)] respectively, No significant difference has been shown between Pre-treatment CaBreast and fibrocystic disease patients and control ($P>0.05$) There were non-significant differences between Breast disease groups when compared together ($P>0.05$). The serum levels of FT3 which were elevated significantly in breast diseases patients (post treatment Ca Breast, pre-treatment CaBreast and fibrocystic disease) compared to control [(P=0.018),(0.046),(0.007)] respectively. There were non-significant differences between Breast disease groups when compared together ($P>0.05$). T3 it was significantly higher in breast cancer post treatment patients compared to controls ($p=0.009$), No significant difference between respective groups in present study when compared together ($p>0.05$). Serum levels of TSH, T4, Anti-Tpo and Anti-Tg showed. No difference between breast cancer patients, fibrocystic patients and controls. **Conclusion:** Subclinical hypothyroidism more frequent in breast cancer patients, change level of thyroid hormones suggested deregulation in breast cancer patients, these data must be confirmed in large patients prospective study.

## A-015

### MALAT1 Is Associated Q1 with Poor Response to Oxaliplatin-Based Chemotherapy in Colorectal Cancer Patients and Promotes Chemoresistance through EZH2

P. Li[1], C. Wang[2]. *[1]Qilu Hospital of Shandong University, Jinan, China, [2]The Second Hospital of Shandong University, Jinan, China*

Chemoresistance to oxaliplatin based therapy has been a key barrier to the efficacy of colorectal cancer (CRC) treatment. One major reason for oxaliplatin chemoresistance in CRC is the acquisition of epithelial-mesenchymal transition (EMT) in cancer cells. Recently, long non-coding RNAs (lncRNA) have emerged as new gene regulators and prognostic markers in several cancers including CRC. LncRNA MALAT1 is a highly conserved nuclear ncRNA and a key regulator of metastasis development in several cancers. However, its role in oxaliplatin-induced metastasis and chemo-resistance is not well known.

In this study, we investigated the prognostic and therapeutic role of lncRNA MALAT1 in CRC patients receiving oxaliplatin-based therapy, and further explore the potential transcriptional regulation through interaction with EZH2 based on the established HT29 oxaliplatin-resistant cells. We found that MALAT1 expression was higher in human CRC tissues where it was associated with reduced patient survival. Moreover, MALAT1 expression level was much higher in patients who did not respond to treatment than those who experienced response to chemotherapy. And the proportion of patients not responding to chemotherapy was significantly higher in the high MALAT1 expression group than in the low expression group. The Kaplan-Meier survival analysis indicated that high MALAT1 expression was associated with shorter overall survival and disease-free survival in CRC patients receiving oxaliplatin treatment. Subsequently, oxaliplatin-resistant CRC cells were established, and the cells exhibited epithelial-mesenchymal transition (EMT) and high MALAT1 expression level. MALAT1 knockdown significantly increased E-cadherin expression in both HT29 parental and oxaliplatin-resistant cells. To verify the potential pathway, we determined EZH2 expression in CRC tissues and found that EZH2 was upregulated in CRC tissues compared to normal colon tissues. RNA immunoprecipitation assay showed a significant enrichment of MALAT1 with EZH2 antibody compared with the non-specific IgG antibody. Furthermore, EZH2 knockdown significantly increased E-cadherin level and impaired cell migratory and invasive capacity in CRC cells. Chromatin immunoprecipitation date showed that the binding level of EZH2 and H3K27-me3 with E-cadherin promoter was significantly decreased by MALAT1 knockdown compared to control cells. More importantly, targeted inhibition of MALAT1 or EZH2 dramatically reversed EMT and chemoresistance induced by oxaliplatin. Finally, the interaction between lncRNA MALAT1 and miR-218 was observed. Knockdown of MALAT1 significantly increased miR-218 expression; however, after overexpression of miR-218, MALAT1 expression was significantly decreased in CRC cells, which suggested that the interaction between MALAT1 and miRNA-218 has reciprocal effects. Clinically, the number of patients not responding to standard FOLFOX treatment was significantly higher in the high MALAT1 and low miR-218 expression group compared with other groups, further indicating that the biomarker panel of MALAT1 combined with miR-218 could be a potential novel chemotherapeutic indicator for CRC patients receiving standard FOLFOX treatment.

In conclusion, the present work has identified that lncRNA MALAT1 was correlated with tumor metastasis and associated with poor response to oxaliplatin-based chemotherapy in CRC patients. MALAT1 promotes oxaliplatin-induced EMT through EZH2 and interacts with miR-218. These indicated that overexpression of MALAT1 confers a potently poor therapeutic efficacy. Thus, lncRNA MALAT1 may be a potential functional biomarker and therapeutic target in CRC patients.

## A-016

### RAC1 Induces Monocyte Infiltration and Breast Cancer Progression by Elevating ROS Production

W. Xu[1], L. Li[1], G. Hu[1], L. Qu[1], H. Zhang[1], R. Xu[2]. *[1]Department of Laboratory Medicine, The First Hospital of Jilin University, Changchun, China, [2]Markey Cancer Center, University of Kentucky, Lexington, KY*

**BACKGROUND:**

The tumor microenvironment plays an important role in breast cancer and the role intratumoral immune cells play in the pathology of lymphoma has been significantly understated. Inflammation is one of the hallmarks of cancer development. Macrophages are the most abundant immune-related stromal cells in tumor microenvironment, and infiltration of monocytes is associated with disruption of polarized acinar structure and correlates with poor prognosis in breast cancer patients.

**OBJECTIVE:**

Dominant activated RAC1 (RAC1 L61) in breast cancer cells significantly enhances monocyte infiltration in the 3D co-culture assay.

**METHODS:**

Cell polarity was authenticated using the basal marker α6-integrin and cell proliferation was assessed by quantification of proportion of cells with Ki-67 positive staining. To evaluate ROS levels, cells were cultured in 3D Matrigel and stained with CellROX Deep Red, followed by live cell imaging and quantification. Disruption of acinar structure during cancer development is accompanied by loss of tissue polarity and increased cell proliferation, so we introduced constitutively active RAC1 L61 and dominantly active Akt to decouple cell proliferation from polarity establishment/ disruption.

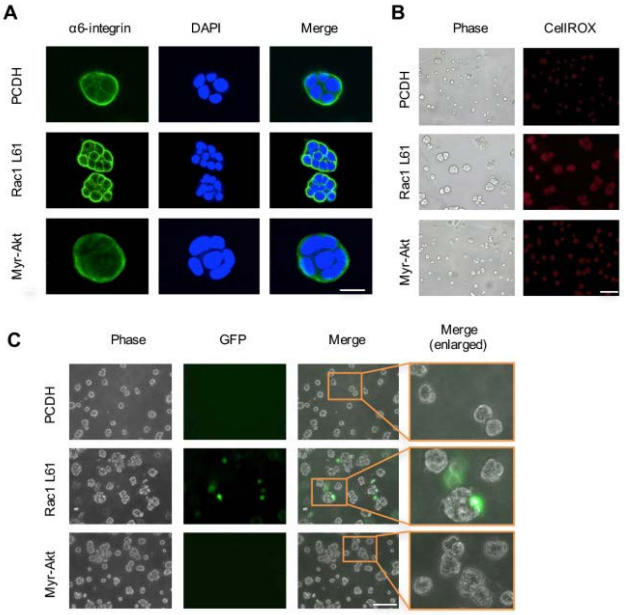
**RESULTS:**

**Introduction of constitutively activate RAC1 disrupts basal polarity of tyrphostin treated T4-2 cells in 3D culture:** Introduction of RAC1 L61, a constitutively active RAC1, into T4-2 cells inhibited restoration of polarity in response to TYR (tyrphostin), but had little effect on cell proliferation, and expression of RAC1 L61 significantly elevated ROS levels in TYR-treated T4-2 cells, indicating increased proliferation and loss of tissue polarity are functionally separable consequences of increased ROS production.

**Disruption of polarized acinar structure promotes monocyte infiltration by elevating ROS levels:** Constitutively activated RAC1 L61 but not Myr-Akt in TYR treated T4-2 cells resulted in a significant fold increase in THP-1 infiltration using the 3D co-culture assay.

**CONCLUSION:**

RAC1 is a potent inducer of monocyte infiltration and cancer metastasis.




## A-017

### The differential analysis and functional verification of miRNAs expression profile in breast cancer

J. Xu, D. Liu, D. Liu, J. Xu, L. Lin, F. Qiu, Y. Dai. *The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China*

**Background:** Breast cancer is one of malignant tumor which causes serious damage to the women's health. MiRNA is a fascinating kind of biomolecule due to their vital functions in gene regulation and potential value as biomarkers for serious diseases including cancers. Genome-wide miRNA expression may be useful for predicting risk and/or for the early detection of breast cancer.

**Methods:** 10 cases diagnosed breast cancer patients and 10 cases of normal controls were selected. By high-throughput miRNA sequencing from 10 pairs breast cancer tissues, adjacent normal tissues, serum and serum from normal persons, and using Target Scan and Clip-seq database, we selected miRNAs specifically expressed in breast cancer. Then we used real-time fluorescence quantitative PCR test the level of miRNA expression from serum of large samples. We picked the miRNA with statistical difference. For the miRNA function, we designed and synthesised the miRNAs inhibitors, and transfected to breast cancer cells MDA-MB-435, MDA-MB-468 to detected cell vitality by CCK8.

**Results:** After the analysing of miRNA high-throughput sequencing from breast cancer pathology organization, adjacent tissues, serum and serum from normal persons, we picked out 28 cases difference expression of miRNAs in 986 cases.More than 2 times different expression and less than 30 CT value of RT-PCR as the standard, we selected 4 miRNAs: miR-374a-5p, miR-223-3p, miR-423-5p and miR-320a. To RT-PCR results showed the stable and different expression of miRNA in 113 cases of breast cancer patients from 104 cases of healthy control group. The miRNA expression of miR-374a-5p, miR-223-3p, miR-423-5p and miR-320a in the breast cancer group was up-regulated with diffenence respectively($P < 0.05$). Besides, the expression of miRNA had significant correlation with clinical pathological characteristics. MiR-374a-5P had higher expression in lymph node metastasis group ($P = 0.001$); MiR-223-3P expressed differently between the different molecular classification ($P = 0.040$), the higher expression of miR-223-3P associated with ER negative estrogen receptor status ($P = 0.035$); The expression of miR-423-5p, miR-320a are positive correlation with clinical stages($P = 0.001$,0.014) and Ki-67 ($P = 0.001$,0.015). Functionally, we designed and synthesised the miRNAs inhibitors, and transfected to breast cancer cells MDA-MB-435, MDA-MB-468 to detected cell vitality by CCK8. We found that miR-223-3p inhibitor could inhibit miR-223-3p in MDA-MB-468, result in the inhibition of MDA-MB-468 energy. At the same time, miR-423-5p could inhibit in MDA-MB-468 and MDA-MB-435,suggesting that miR-223-3p, miR-423-5p could be inhibitors of tumor suppressor genes and regulate activity of breast cancer cells.

**Conclusion:** With help of the new generation of high throughput sequencing method of breast cancer, we have picked out specific differentially expressed miRNAs, including miR-374a-5p, miR-223-3p, miR-423-5p, miR-320a. The expression of the miRNAs were stable in serum and tissue. Meanwhile, The miR-223-3p inhibitor and miR-423-5p inhibitor can effectively inhibit breast cancer cell activity, which lay a foundation for subsequent function study. So we hypothesized they can be used as potential biomarkers of breast cancer for early diagnosis, prognosis and therapy target.

## A-018

### Development of Multiplexed Mass Spectrometry-based Assays for Urine Biomarkers of Aggressive Prostate Cancer

C. E. Knezevic, W. Yang, S. Thomas, D. Chan, H. Zhang. *Johns Hopkins Hospital, Baltimore, MD*

**Background:** Currently, biochemical testing for prostate cancer utilizes serum total, free, and p2PSA to screen males over 50 years of age for the presence of disease. Follow-up of a positive serological result is a multi-core biopsy and pathological grading using a Gleason score. Measurement of biomarkers in urine would enable less invasive disease monitoring and prognosis determination. We have generated a workflow for developing mass spectrometric multiplexed selected reaction monitoring (SRM)-based assays for the detection of urine protein biomarkers for aggressive prostate cancer. This strategy exploits prostate cancer proteins previously found both in tumor tissue and in the urine of prostate cancer patients. To generate a multiplexed MS assay, urine PSA will be monitored, along with urine proteins zinc-alpha-2-glycoprotein (AZGP1) and inactive tyrosine protein kinase 7 (PTK7). PTK7 has been reported to have higher expression levels in aggressive prostate tumor tissues while the opposite trend has been found for AZGP1. **Methods:** A purified extract or recombinant form of each target protein was denatured with urea, reduced,

alkylated, and digested with chymotrypsin. The resulting peptides were analyzed via LC-MS/MS on a Thermo Velos linear ion trap MS to identify candidate precursor peptides. MaxQuant searches of the human proteome positively identified peptides and confirmed that each peptide was unique to its corresponding protein. Following the identification of candidate peptides, selected ion monitoring and product ion scan experiments were carried out on a Shimadzu 8040 triple quadrupole to select appropriate transitions. Target peptides eluted between 20-35 minutes using a 50 minute acetonitrile/water LC gradient on a Restek C18 column. Urine specimens were concentrated with a 10K filter before processing proteins as above, then analyzed using the previously selected transitions in a multiplexed SRM assay. **Results:** This workflow enabled the identification of peptides and transitions for PSA, PTK7, and AZGP1. Of note, 19 candidate PSA peptides were identified by MS/MS and 11 were further selected based on high peak intensity and amino acid composition (excluding methionine and asparagine-containing peptides). For each peptide, product ion scans were performed to identify specific transitions. Four PSA peptides were chosen for use in SRM measurement of total urine PSA levels: ASGWGSIEPEEF (mass 1307.57, charge +2, 654.79 *m/z*, product ions at 521.2, 788.3, and 1014.4 *m/z*), DLPTQEPALGTTCY (mass 1564.71, charge +2, 783.36 *m/z*, product ions at 882.2, 965.5, 714.3 *m/z*), GSEPCALPERPSLY (mass 1574.73, charge +2, 788.38 *m/z*, product ions at 861.4, 715.3, 974.5 *m/z*), and TGGKSTCSGDSGGPL (mass 1379.59, charge +2, 286.1, 344.1, 692.3 *m/z*). . These transitions were recapitulated in patient samples and the chosen peptides were detected using multiplexed SRM. These peptides are detectable in urine from prostate cancer patients ranging from stage T1c to T4. **Conclusion:** This workflow enables the systematic development of multiplexed mass spectrometric SRM methods for the relative quantification of protein biomarkers from the urine of prostate cancer patients. The overall benefit of this work is the ability to generate assays for the assessment of protein biomarkers in urine, which is of particular interest for the non-invasive identification of aggressive prostate cancers.

## A-019

### Validation of the Ella™ chromogranin A (CgA) immunoassay

P. Erdman, A. Matthew, B. Hurtado, M. Fleisher, L. Ramanathan, K. Thoren. *Memorial Sloan Kettering Cancer Center, New York, NY*

**Background:** Chromogranin A (CgA) is a 48 kDa acidic glycoprotein comprised of 439 amino acids belonging to the granin family. It is considered a highly sensitive, nonspecific marker for the presence of neuroendocrine tumors due to its role in the formation of secretory granules within the neuroendocrine system. In addition CgA is used as a marker for neuroendocrine differentiation in prostate cancer and is used to monitor for disease progression. Currently, there is no established FDA-approved assay for the analysis of CgA and there is no gold standard reference method. Existing methods yield results that can vary significantly due to the lack of standardization and utilization of different antibodies between assay methods. Simple Plex (ProteinSimple, San Jose, CA) is an attractive immunoassay platform as its novel microfluidic design with separate channels for antigen/antibody reactions prevents cross-reactivity and allows the process to be automated. The objective of this study was to evaluate the performance of the Ella™ single-plexed CgA immunoassay and compare its results with those of the Quest Diagnostics™ (Madison, NJ) assay.

**Methods:** Quality control (QC) material, sample diluent, running buffer, Simple Plex cartridges and the Ella instrument were obtained from ProteinSimple. Concentrations were calculated using vendor-determined calibration curves. High and low QC material was used to determine assay precision. The limit of detection was established by running blank samples (n=12) and calculating the mean + 2 SD. Analytical linearity was determined by serially diluting high quality control material and 1 patient sample. Reference ranges were determined by measuring CgA levels in 120 apparently healthy volunteers. Accuracy was determined by splitting 120 patient samples that were sent to a reference laboratory and run on the Ella for comparison. Both serum and plasma was measured as part of this study.

**Results:** Within-run precision ranged from 2.8 - 5.2%; between-run precision ranged from 2.9 - 6.1%. The limit of detection was 0.017 ng/mL. Overall, the linearity of the assay was acceptable; the slopes ranged from 0.910 - 0.9348, R2 values ranged from 0.9959 - 0.9976. The reference range among healthy volunteers showed a non-parametric distribution. The calculated reference range was 10.82-15.70 ng/mL. Method comparison to the Quest method showed poor correlation (R2 value of 0.3961). Plasma samples showed consistently higher values compared to serum. Age range varied from 22-87 years and the mean age was 66 years. 108 samples were from male patients and 12 patients were from female patients. Samples were obtained from patients with various disease states including: neuroendocrine (10.8%), carcinoid (7.5%), pheochromocytoma/paraganglioma (1.67%), other (5.83%) and prostate adenocarcinoma (74.2%). **Conclusion:** The Simple Plex has acceptable precision, limits of detection and linearity for measurement of chromogranin A. Method comparison with Quest Diagnostics™ showed poor correlation and may be due to the fact that different immunoassays recognize different epitopes and because chromogranin A is highly heterogeneous. We will investigate this further and evaluate the clinical performance of the Simple Plex assay. The Ella instrument is easy to use and may be a good alternative to traditional ELISA assays.

## A-020

### Genetic variants of the aryl hydrocarbon receptor-interacting protein gene (*AIP*) in patients with clinical features of familiar isolated pituitary adenomas(FIPA): First series in Argentina

M. Viale[1], M. Serra[2], A. Kozak[2], A. Stigliano[2], R. Garraza[2], P. Fainstein Day[2]. *[1]Hospital Italiano de Buenos Aires, CABA, Argentina, [2]Hospita Italiano de Buenos Aires, CABA, Argentina*

**Background:** Familiar isolated pituitary adenomas (FIPA) encompasses the familiar occurrence of isolated pituitary adenomas in at least two members of the same family outside the setting of syndromic conditions such as MEN1 and Carney's complex, and comprise about 2-3% of pituitary adenomas. About 20% of FIPA have germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene (*AIP*), usually associated with a worse outcome because these tumors are large, occur at a young age and also demonstrate features of aggressiveness and treatment resistance. It is an autosomal dominantly inherited disease with a penetrance of *AIP* mutation between 15-30%.Objective:The aim of this study was to study the prevalence of germinal mutations of *AIP* gene and polymorphisms(SNPs) in a cohort of patients with FIPA or with diagnosis of pituitary macroadenomas under the age of 36

**Methods:** We studied 29 potential carriers with a family history of pituitary tumor or clinical features of FIPA and 66 healthy subjects (mean age 34,3 +/- 0,7 years, 50 women and 16 men) was analyzed as control group. In controls, age, gender and medical history were recorded and we also exclude subjects with personal or family history of MEN1 or Carney complex. Genomic DNA was prepared from blood samples.The promoter and exons 1 to 6 and intronic flanking regions were amplified by PCR using specific primers. The DNA fragments were analyzed by direct sequencing. All genetic alterations were confirmed by a second PCR and direct sequencing. All subjects gave informed consent to genetic studies

**Results:**We found the following SNPs: c.682C> A (Q228K) in 29 of the 29 patients studied, c.920A > G(Q307R) in 26 of 29, IVS 3 + 111 C> T in 17 of 29, c.-810T> G in 1 of 29, c.993+60G> C 3' UTR in 1of 29 and , c.- 941 A> G in 1 of 29 this variant has not been previously described in the literature.We did not find any mutations in the patients. Due to the high frequency of the Q228K and Q307R in the patients group we decided to study them in the control group and we found the Q228K in heterozygous form in 1 of 66 and 65 of 66 in homozygous form and the Q307R all in homozygous form.

**Conclusion:** There are limited data on AIP SNPs with potential functional consequences. In the literature Q228K and Q307R have been found significantly different between FIPA patients and healthy controls however in our population are equally distributed.Our results suggest that these variants do not have pathological implications. The functional consequences should be evaluated and synergism between the SNPs founded cannot be excluded. For genetic counseling, genetic testing could be proposed to evaluate relatives as a high-risk population for developing pituitary tumors. The investigation for mutations in the AIP gene in families with pituitary adenomas is necessary, since it is associated with poor outcome and resistance to treatment.

## A-021

### High detection rate of CRC-related mutants in stool samples of patients with polyps by MassARRAY

P. Chang, J. Chen, S. Chang, M. Wang, N. Chang, H. Liu, J. Lu. *Chang Gung Momorial Hospital, Tauyuan county, Taiwan*

**Background:** Fecal occult blood test (FOBT) is adapted globally as a colorectal cancer (CRC) screening tool to identify high risk individuals. However, FOBT has some pitfalls, only 20% of the adenomas can be detected and at least half of FOBT(+) individuals undergo unnecessary colonoscopy procedures. In the present study, we aim to establish a stool DNA (sDNA) test by using a Single Allele Base Extension Reaction (SABER) MassARRAY platform to increase the detection accuracy. The sDNA panel contains 30 hot spots on 5 CRC-associated genes.

**Methods:** 2-fold serially diluted LoVo cell line DNAs were tested for analytic sensitivity. 10 FOBT (+) stools from individuals with negative colonoscopy finding

were collected for specificity assessment. 21 CRC and 15 polyps patients whose neoplastic tissues had been pre-screened by Thermal NGS Cancer Panel provided pre-operational stool samples for representativeness evaluation. Albumin housekeeping gene is included in the panel for internal control.

**Results:** The SABER test can detect as low as 1.4% of the variants. 52.4% of CRC and 40% of adenoma patients have positive signals on any one of five genes in their stool samples with 100% specificity of negative reaction in 10 control samples. By comparison of mutations found in their matched tissue samples, 44.4% (16/36) of patients have concordant mutation pattern in stool samples and 19.4% (7/36) of patients appear unexpected variants which were not present in tissue samples. The sDNA detection rate is affected by lesion size (25% polyps <1cm, 45.5% polyps≧1 cm, 52.4% tumor ~3.8cm) and location (33% right, 50% left, 56% rectum).

**Conclusion:** SABER MassARRAY is a cost-effective and sensitive method with only 100 ng stool DNA input. Although the neoplasm size and anatomic site affected the detection rate, sDNA test demonstrates better sensitivity in detection of large adenomas and excellent specificity than FOBT. High mutation concordance rate found between stool and original lesions reveal the trueness of sDNA mutations. Further studies are warranted to validate the findings in a larger population and optimize the sDNA test.

## A-022

### Accurate Sequencing and Enrichment-Based qPCR as a Combinatorial Approach for Liquid Biopsy Detection and Monitoring

C. Kasbek, Y. Song, Q. Song, D. Quintanilha, S. Chen, K. Tang, J. Huang.
*Admera Health, South Plainfield, NJ*

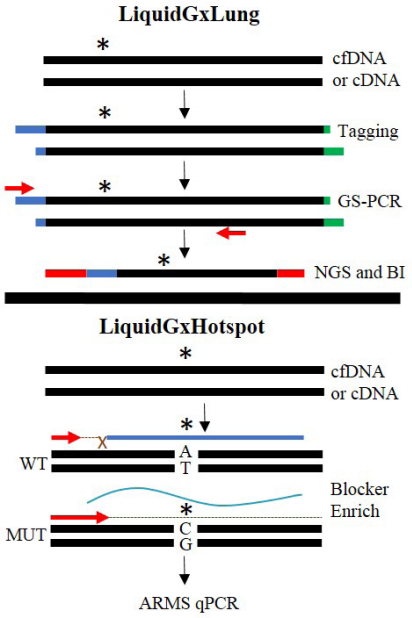
*Clinical relevance*: Liquid biopsy as a non-invasive method for detection and monitoring of rare variants is a rapidly emerging field. Lung cancer is the leading cause of cancer death and lung biopsies are invasive and prone to result in insufficient material for diagnosis.

*Objective*: To develop a non-invasive test to identify DNA and RNA variants present at low frequencies in lung cancer patients, we utilize a combinatorial approach of accurate sequencing and enrichment-based qPCR.

*Methodology*: Our accurate sequencing uses gene-specific PCR of individually tagged cfDNA or cDNA molecules. Our current NGS panel (LiquidGxLung) detects ≈75 variants in 10 genes (AKT1, ALK, BRAF, EGFR, ERBB2, KRAS, MET, PIK3CA, RET, and ROS1). Sensitivities are 94% and 90% for 0.1% SNPs and indels, respectively, while fusions are detected as low as 0.1%. Our CLIA-approved qPCR tests (LiquidGxHotspot) utilize blocker-based enrichment of mutant DNA followed by qPCR detection. These seven separate tests (ALK, BRAF, EGFR, KRAS, MET, RET, ROS1) have a limit of detection of as low as 0.01%. LiquidGxLung and LiquidGxHotspot display ≈100% specificity with TAT's of 4-6 and 1-2 days, respectively.

*Validation*: To assess the performance in a clinical setting, accurate sequencing was performed on 103 lung cancer patients while qPCR was performed on 71 of these patients. The mutations identified closely mirrored published lung cancer tissue biopsy data, while the two assays exhibited 90% concordance with each other. In 5 out of 7 non-concordant instances, the qPCR enriched a mutation that was below the 0.1% detection threshold of the accurate sequencing.

*Conclusions*: This NGS panel can be performed for the identification of variants in late-stage lung tumors, with monitoring of treatment response using the more targeted enrichment-based qPCR. This technology is easily adaptable to the diagnosis and monitoring of other cancer types.




## A-023

### Alpha-Fetoprotein (AFP) in Peritoneal, Pleural and Pericardial Fluids: A Body Fluid Matrix Evaluation

W. E. Owen[1], J. J. H. Hunsaker[1], J. R. Genzen[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT*

**Background:** Alpha-fetoprotein (AFP) is glycoprotein produced during fetal development. It is measured clinically as a tumor marker for malignancies such as hepatocellular carcinoma and germ cell tumors. It is also used for the assessment of fetal neural tube defects during pregnancy. In association with imaging and cytology, AFP may provide supportive evidence for malignancy when measured in body fluids. A College of American Pathologists (CAP) Laboratory Accreditation Program checklist item for body fluid analysis (COM.40620) permits the use of method performance specifications from blood specimens for body fluids if the laboratory can reasonably exclude the possibility of matrix interference. The objective of this study was therefore to conduct a matrix evaluation using the Access AFP assay (Beckman Coulter; Brea, CA) with three body fluids - peritoneal (ascites), pleural, and pericardial.

**Methods:** The UniCel DxI 800 immunoassay platform (Beckman Coulter) was used for AFP testing. Previously collected clinical body fluid specimens were retrieved from frozen storage (-20°C) and de-identified according to an IRB-approved protocol. For spiked recovery studies, 20 low AFP pools (near zero) for each fluid type were created from patient body fluid specimens. These were spiked with patient serum samples containing elevated AFP concentrations (spiked volume 2.5 to 10%). Spiked body fluid samples were analyzed in duplicate for AFP. Percent recovery was calculated for each specimen. Mixed recovery and linearity studies were conducted covering the analytic measurement range (AMR) of the assay (0.5–3,000 ng/mL). For these studies, admixtures of high and low (near zero) AFP pools for each fluid were created and tested for AFP in duplicate. Percent recovery was calculated and linearity was assessed. For evaluation of precision, high and low AFP pools for each fluid were prepared to provide 5 aliquots each. These aliquots were frozen at -70$^0$C. Aliquots were thawed, mixed, centrifuged, and tested for AFP in four replicates each day over five days of testing. Results were analyzed using Excel 2010 (Microsoft; Redmond, WA) and EP Evaluator 11 (Data Innovations; Burlington, VT).

**Results:** In spiking and mixed recovery studies, the average percent recovery was within predefined acceptable limits (less than ±15%) for all three body fluids. Linearity was observed over the AMR for all three body fluids (slope, intercept, % total error): peritoneal, 0.986, 0.0, 4.1%; pleural, 1.016, 0.0, 1.6%; pericardial, 0.988, -0.1, 6.1%. Imprecision was acceptable (%CV, <10%) for all three body fluids at both the high and low AFP concentrations.

**Conclusion:** Exclusion of matrix interference should be conducted prior to performing body fluid testing on assays whose performance specifications were originally derived using blood. The present studies provide evidence against the presence of any systematic matrix interference for AFP in peritoneal, pleural, and pericardial fluids on the Access AFP assay.

## A-024

### Serum Concentrations of Open Reading Frame 1 (ORF1) protein Produced by Activation of Long Interspersed Nuclear Element 1 (L1) In Subject Being Screened for Lung Cancer with CT scan

K. Hosseinnejad[1], M. Bousamra[2], M. Burch[2], A. Elliot[2], D. Eichenherger[2], S. A. Jortani[1]. *[1]University of Louisville, Louisville, KY, [2]Baptist Health Floyd Hospital, New Albany, IN*

**Background**. Retrotransposons are repetitive DNA sequences capable of copying and moving themselves and other sequences to new locations throughout the genome. One of the most abundant and active groups of them is the Long interspersed nuclear element 1 which becomes activated by hypomethylation of its promoter region. The major causes of L1 activation include environmental stressors such as particulate air pollutions, chemical carcinogens and inducers of oxidative stress. We investigated the serum ORF1 protein concentrations in a group of smokers presenting for CT scan for lung cancer at Baptist Health Floyd hospital (New Albany, IN). **Methods:** Subjects (>50 years of age) with the history of smoking for more than 30 pack/year and presenting to the radiology department for routine lung cancer screening by chest CT scan were screened, consented and enrolled in this study. Upon completion of chest CT scan, a non-fasting blood sample was collected for measuring ORF1 protein concentration. Using the CT scan report, 72 subjects were initially grouped into two major categories. Those with negative chest CT result (45) and others who were considered to be positive (27) and were asked to return for further work up as a part of their routine clinical care. In the chest CT negative group, 5 were found to have nodules or cysts in kidneys, liver or thyroid. Ultimately, 40 subjects were entirely negative for any nodules or cysts in the organs or tissues assessed on the CT scan. In order to measure ORF1 protein concentration in serum of each subject, we used an in-house competitive ELISA assay. It involved a custom-made antibody against the select amino acid sequence of ORF1 protein. The same peptide sequence, coupled with biotin was used as an anchor in streptavidin coated 96-well plates. Then, a secondary antibody (GAR-HRP) and colorimetric substrate were used to generate a blue color. Absorbance values at 450 nm were measured and patient sample ORF1 concentrations were calculated based on a logistic 4-parameter standard curve generated from calibrators of known ORF1 concentrations. **Results:** The mean ORF-1 protein concentration in subjects with suspicious nodule(s) based on CT scan who were recommended for further follow up (n=27) was 13.6 ng/ml (SD=13.5). In 45 subjects with negative lung CT scans, the mean ORF1 was 8.08 ng/ml (SD=8.2). The difference between these groups was significant (P=0.039, Mann-Whitney Rank Sum). The mean concentration of ORF1 in subjects with no nodules or cysts found in any of the studied tissues (lung, kidney, liver and thyroid) was 15.4 ng/ml (SD=8.3). The difference between the means of positive lung CT versus a completely negative CT results was also significant (P=0.016, Mann-Whitney Rank Sum). **Conclusion:** Measurement of serum concentration of ORF1 protein generated by L1 activation shows promise as a non-invasive and potentially widely available risk assessment tool for lung cancer screening in smokers.

## A-025

### Suppression of the non-involved heavy/light chains pair isotype as new biomarker of poor prognosis in Multiple Myeloma

J. Garcia de Veas Silva[1], M. López Vélez[1], A. Espuch Oliver[1], C. Bermudo Guitarte[2], N. Barbosa de Carvalho[3], J. García Lario[1], T. De Haro Muñoz[1]. *[1]Complejo Hospitalario Universitario de Granada, Granada, Spain, [2]Hospital Universitario Virgen Macarena, Sevilla, Spain, [3]The Binding Site, Barcelona, Spain*

**Background**: The outcome for patients with Multiple Myeloma (MM) is highly variable. Understanding the prognosis for a particular patient can help when selecting the intensity of treatment to be used and the frequency of reviews. The quantification of heavy/light chains pairs by the immunoassay Hevylite (HLC) allows us a precise measurement of monoclonal and non-monoclonal immunoglobulins of the same isotype. In this study we evaluate i) the impact of the "HLC ratio" defined as monoclonal immunoglobulin over isotype matched non-monoclonal immunoglobulin (involved/uninvolved HLC ratio or i/u HLC ratio), ii) the suppression on non-monoclonal pair denominated "HLC-matched pair suppression" and III) the effect of "systemic immunoparesis" at diagnosis and at +100 days after autologous stem cell transplantation (ASCT).

**Material and methods:** 85 patients (50 Male:35 Female) with a median age of 70 years (56-78) were followed (35 IgGK, 18 IgGL, 17 IgAK and 15 IgAL). The median follow-up of the patients was 19 (5-30) months. Sixteen patients (18%) presented ISS stage I, 15 (28%) with stage II and 54 (64%) with stage III disease. Thirty patients that reached ASCT were evaluated at +100 days after ASCT. Immunoglobulin heavy/light chain pairs (HLC) were assessed by Hevylite assays (The Binding Site). Overall survival (OS) and progression-free survival (PFS) were evaluated by Kaplan-Meier method. Statistical analysis was made with Prism 6.0.

**Results:** The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.71). **At diagnosis**, a i/u HLC ratio>80 was significantly associated with worse OS (48 vs. 61%, p=0,005) and shorter PFS (23% vs. 42%, p=0,006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35% vs. 81%, p=0,004) and PFS (21% vs. 50%, p=0,013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32% vs. 81%, p=0,030) but not with shorter PFS (26% vs. 44%, p=0,306).

**In the post-ASCT evaluation of the patients (n=30)**, normalization of HLC ratio was observed in 22 patients (73%). An altered HLC ratio was significantly associated with shorter PFS after ASCT (25% vs. 70%, HR: 3,42, 95%CI 1,12-11,97, p=0,039) and with a trend towards a worse OS (p=0,072). Severe HLC-matched pair suppression was found in 12 patients (40%) and was predictive of worse OS (0% vs 70%, HR: 10,63, 95%CI: 1,11-114,11, p=0,023) and shorter PFS (35% vs. 71%, HR: 8,87, 95%CI: 1,72-45,92, p=0,002). On the other hand, the severe systemic immunoparesis observed in 17 patients (57%) was not associated with OS (p=0,644) and PFS (p=0,750).

**Conclusions:** Severe HLC-matched pair suppression and i/u HLC>80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

## A-026

### Screening and Diagnosis of Monoclonal Gammopathies: An International Survey of Laboratory Practice

J. R. Genzen[1], D. L. Murray[2], G. Abel[3], Q. H. Meng[4], R. J. Baltaro[5], D. D. Rhoads[6], J. C. Delgado[1], R. J. Souers[7], C. Bashleben[7], D. F. Keren[8], M. Q. Ansari[9]. *[1]University of Utah / ARUP Laboratories, Salt Lake City, UT, [2]Mayo Clinic, Rochester, MN, [3]Lahey Hospital & Medical Center, Burlington, MA, [4]University of Texas MD Anderson Cancer Center, Houston, TX, [5]East Carolina University, Greenville, NC, [6]Case Western Reserve University, Cleveland, OH, [7]College of American Pathologists, Northfield, IL, [8]University of Michigan, Ann Arbor, MI, [9]Cleveland Clinic, Cleveland, OH*

**Objectives:** Serum tests used for the screening and diagnosis of monoclonal gammopathies include serum protein electrophoresis (SPE; either agarose gel [AGE] or capillary zone [CZE]), immunofixation (IFE) and immunosubtraction capillary electrophoresis (IS-CE), serum free light chains (sFLCs), quantitative immunoglobulins, and heavy / light chain combinations. Urine protein electrophoresis (UPE) and urine IFE may also be used to identify Bence Jones proteinuria. International Myeloma Working Group (IMWG) guidelines recommend the combination of SPE, serum IFE, and sFLC when screening for new monoclonal gammopathies. The objective of this study was to assess the current state of laboratory practice for monoclonal gammopathy testing.

**Methods:** In April 2016, a voluntary questionnaire was distributed to 923 laboratories participating in a College of American Pathologists (CAP) protein electrophoresis proficiency testing survey.

**Results:** 774 laboratories from 38 countries and regions completed the questionnaire (84% response rate). The majority of participants (68.6%) use AGE as their SPE method, while 31.4% use CZE. The most common test combinations used in screening were: SPE w/ reflex to IFE/IS-CE (39.3%); SPE only (19.1%); SPE and IFE or IS-CE (13.9%); and SPE w/ IFE, sFLC, and quantitative immunoglobulins (11.8%). Only 39.8% of laboratories offered panel testing for ordering convenience. While SPE was used by most laboratories in diagnosing new cases of myeloma, when laboratories had to select only one test used to follow patients with monoclonal gammopathy,

55.7% of laboratories chose SPE, with the next most common selections being IFE (18.9%), sFLC (11.7%), and IS-CE (2.1%). Few laboratories (13.4%) cancel IFE if an M-protein has previously been characterized. A slightly higher percent (21.8%) cancel IFE if the M-protein has the same migration as the original specimen. Less than half of laboratories (40.4%) comment in their reports on whether monoclonal proteins increased, decreased, or did not change from a prior specimen.

**Conclusions:** Current practices vary widely across laboratories and often do not reflect IMWG guidelines. Efforts on improving utilization management and report content, as well as further recognition and development of lab-directed testing guidelines, may serve to enhance the clinical value of testing while decreasing cost-of-care.

## A-027

### Novel reference materials for *EML4-ALK* testing

R. Peng[1], J. Li[2]. *[1]National Center for Clinical Laboratories, Beijing Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, [2]National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:**

In the emerging clinical paradigm of precision medicine, accurate detection of the rearrangements of echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene is important to select the subgroup patients for crizotinib therapy. Despite many attempts have been done to improve the *EML4-ALK* detecting in clinical practice, the inaccuracy of testing is still an important yet to be solved problem within all the methodologies, including fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), reverse transcription real-time PCR (RT-PCR) and next-generation sequencing (NGS). For the clinical laboratories, in order to improve accuracy and reliability of detection, a proper reference material for validation of laboratory-developed tests, verification of commercial detection kits, internal quality control and proficiency testing is of prime important. Therefore, to ensure the accuracy and reproducibility of detection, here we developed a kind of well-characterized candidate reference materials for *EML4-ALK* testing.

**Methods:**

In this study, clustered regularly interspaced short palindromic repeat/CRISPR associated protein 9 (CRISPR/Cas9) technique was used to edited three types of cell lines containing *EML4-ALK* rearrangements variant 1, 2, and 3a/b. Single guide RNAs (sgRNAs) were designed in silico to target specific *EML4* locus and *ALK* locus. Px330 vector expressing Cas9 and specific sgRNA was transfected into HEK293T cells. The edited individual positive clones were verified by PCR, RT-PCR, and western blotting. Followed by subcutaneous inoculation, the formalin fixed paraffin embedded (FFPE) samples based on CRISPR/Cas9 and xenograft were prepared and tested for suitability as candidate reference materials by FISH, IHC, RT-qPCR and NGS. All the results were compared with the authentic clinical specimens to assess the commutability. In addition, homogeneity and stability assessments were also performed.

**Results:**

By editing HEK293T cells by CRISPR/Cas9 system, three kinds of cells containing *EML4-ALK* variant 1, 2, and 3a/b have been constructed and named as 1-F8-G9, 9-E11-G5, 14-F9-E8, respectively. The edited cells were all verified on DNA, RNA and protein levels, and can be observed a high frequency of exact fusion events. Via subcutaneously injection, the corresponding xenograft tumors were obtained in their fourth week and embedded as FFPE blocks. All kind of FFPE samples derived from xenograft tumors were found with typical histological structures by using HE staining, such as tumor infiltrating, inflammation, and partial necrosis. In addition, by FISH, IHC, RT-qPCR, and NGS, all the materials were verified to be *EML4-ALK* rearrangements-positive. Among four methodologies for *EML4-ALK* detection, the validation test showed 100% concordance. Meanwhile, compared with clinical ALK positive-NSCLC specimens, the novel FFPE samples showed great commutability. Furthermore, the materials were also completely homogeneous and stable for at least 2 months.

**Conclusion:**

Without limitations on variant types and production, our novel FFPE samples based on CRISPR/Cas9 editing and xenograft are suitable for all the methodologies as candidate reference materials in the validation, verification, internal quality control and proficiency testing of *EML4-ALK* detection.

## A-028

### LacdiNAc-PSA improves differential diagnosis and predicting aggressiveness of prostate cancer

S. Kojima[1], T. Kaneko[1], N. Noro[1], K. Nagae[1], Y. Tobisawa[2], T. Yoneyama[2], S. Hatakeyama[2], Y. Hashimoto[2], T. Koie[2], C. Ohyama[2], Y. Suda[1], T. Kaya[1]. *[1]Konica Minolta, Inc., Tokyo, Japan, [2]Hirosaki University, Hirosaki, Japan*

**Background:** Prostate cancer (PCa) is the second most common cancer among men worldwide. Prostate specific antigen (PSA) is a widely used biomarker for screening and monitoring patients of PCa, although the lack of specificity causes overdiagnosis and overtreatment. It is known that the *N*-glycan structure on PSA changes according to carcinogenesis. LacdiNAc-PSA is an aberrant glycosylation isoform of PSA which has LacdiNAc structure on its *N*-linked glycan terminal. We have demonstrated a pilot study of LacdiNAc-PSA by automated immunoassay system which utilizes surface plasmon field-enhanced fluorescence spectroscopy (SPFS) as its detection principle. The aim of this study is to evaluate the clinical utility of serum LacdiNAc-PSA compared to serum total PSA and the ratio of free PSA to total PSA (%free PSA).

**Methods:** A total of 407 patients with PCa (n=223) and benign prostatic hyperplasia (BPH, n=184) were investigated. 92 PCa patients who underwent radical prostatectomy (RP) were investigated before prostate biopsies (Pbx) to evaluate the pre-operative prognostic performance. The final diagnosis of PCa or BPH were confirmed using the histopathological findings of Pbx. The grade group (GG) of PCa specimens were evaluated according to the International Society of Urological Pathology guidelines. The serum LacdiNAc-PSA was measured by SPFS-based automated two step sandwich immunoassay system [Kaya, T. *et al.* Anal. Chem. 2015;87:1797-1803.]. Serum total PSA and free PSA was tested on automated immunoassay analyzer Architect i1000 (Abbott Japan). Diagnostic accuracy was assessed for LacdiNAc-PSA, total PSA, and %free PSA.

**Results:** Among PCa and BPH patients, serum LacdiNAc-PSA levels in the both range of total PSA <20 ng/mL and <10 ng/mL were significantly higher in patients with PCa (median: 0.1700 U/mL and median: 0.1140 U/mL, respectively) than BPH (median: 0.0715 U/mL and median: 0.0670 U/mL, respectively), $p<0.0001$. At the cutoff LacdiNAc-PSA level (0.0495 U/mL) for the prediction of PCa, the specificity was 40.8% at its 90% sensitivity, which was much higher than that of %free PSA (32.5 %) and total PSA (13.4%). The AUC of LacdiNAc-PSA showed quite better performance in each total PSA range (0.807 and 0.755, respectively) than that of total PSA (0.641 and 0.542, respectively) and %free PSA (0.716 and 0.689, respectively). Serum LacdiNAc-PSA levels of PCa patients were much higher at Pbx GG 3 (median: 0.2500 U/mL) than Pbx GG 2 (median: 0.1280 U/mL), $p=0.0118$, while total PSA and %free PSA could not discriminate between Pbx GG 2 and 3. This trend was also found for PCa patients with GG after RP (ope GG) ≥3 (median: 0.1885 U/mL) and ope GG ≤2 (median: 0.0985), $p=0.0068$.

**Conclusion:** These data suggest that serum LacdiNAc-PSA improves diagnostic accuracy of PCa against BPH, which lead to large reduction of unnecessary biopsies. Predicting performance of LacdiNAc-PSA could be used as a clinical index of patients under active surveillance. Moreover, correlations between LacdiNAc-PSA level and histopathological evaluation indicate the potential use for stratification of patients according to the aggressiveness of PCa.

## A-029

### Ligand Binding Assay Development for Full Length KRT19 Measurement in Serum

S. Patil[1], V. R. Akmaev[1], M. A. Kiebish[1], M. Kellogg[2], W. Wu[1]. *[1]Berg LLC, Framingham, MA, [2]Boston Children's Hospital, Boston, MA*

**Background:** Keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Keratin 19 is one of the three main keratins besides CK8 and CK18 expressed in simple or stratified epithelium and in various carcinomas including lung cancer, bladder cancer, breast cancer, and other cancer types. KRT19 is cleaved by caspase 3, and the soluble fragments are released and detected in cancer patients' serum. CYFRA21-1 assay which measures the c-terminal fragment of KRT19 has been cleared by FDA for aiding in monitoring disease progression during the course of disease and treatment in lung cancer patients.

Though CYFRA21-1 is the predominant assay used for clinical studies on various cancer types, literature showed that cancer cells actually release full length KRT19 (FL KRT19) actively. The observation based on cancer cell lines was extended to breast cancer patients with existence of FL KRT19 releasing- bone marrow cancer cells linked to metastatic progression. The theory is that CYFRA21-1 is passively

released from tumor cells undergoing apoptosis or necrosis, while active release of full length KRT19 could promote tumor metastasis.

**Results:** Assay was developed to specifically measure FL KRT19 but not CYFRA21-1, with two antibodies' binding epitopes flanking the KRT19 caspase 3 cleavage site. Electrochemiluminescence technology was used to enhance assay analytical sensitivity. The assay has an analytical range of 6.9 pg/ml to 5 ng/ml. Spike and recovery accuracy study showed average of 71.5%, 75%, and 68% of recovery efficiency for high, middle, and low levels of analyte spiked. The limit of quantification of the assay was 22.6 pg/ml. With FL KRT19 above 22.6 pg/ml, samples demonstrate intra-assay variation of <20.8% and inter-assay variation of <20.0%. Specificity and interferences test included two keratin family members KRT13 and KRT17 and four common interferents: unconjugated bilirubin, hemolysates, lipids (triglyceride-rich lipoproteins) and biotin. The only interference observed was from high concentration of hemolysates (positive bias of 41% at 500 mg/dL of hemoglobin). Sample stability was also studied, FL KRT19 was sensitive to temperature change, showing significant deterioration under 37°C and room temperature, or for prolonged time in 4°C (> 2hrs). Sample incubation condition (time and temperature) is critical for reproducible quantification. In contrast, up to 5 freeze/thaw cycles do not affect the FL KRT19 stability significantly.

## A-030

### Development and Characterization of Multiplex Immunoassays for Ovarian Cancer Serum Biomarkers: An NCI Early Detection Research Network Study

J. Song[1], L. J. Sokoll[1], W. Xu[1], J. J. Pasay[1], S. J. Skates[2], K. D. Rodland[3], T. Liu[3], C. Patriotis[4], D. W. Chan[1], Z. Zhang[1]. *[1]Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD, [2]Massachusetts General Hospital, Boston, MA, [3]Pacific Northwest National Laboratory, Richland, WA, [4]Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Bethesda, MD*

**Background:** Ovarian cancer is the fifth leading cause of cancer death among U.S. women and has the highest mortality rate of all gynecologic cancers. Due to the lack of effective screening tools and therapy, the mortality of ovarian cancer has not declined significantly in the past three decades. Most cases of ovarian cancer (~75%) are diagnosed at an advanced stage of the disease. Whereas patients with early-stage disease will have a 5-year survival of >74%, those with advanced-stage cancer will have overall survival rates of only 19% to 30%. Although serum CA-125 followed by ultrasound for elevated tests has the necessary specificity, there remains concern about its sensitivity for early stage disease. The U-PLEX platform enables flexible multiplexing of immunoassays using Meso Scale Discovery (MSD)'s MULTI-ARRAY technology. Multiplex immunoassay simultaneously measuring multiple analytes in the same patient sample using minimum volume allows us to evaluate serum biomarker panels that can potentially complement CA-125 in the early detection of ovarian cancer.

**Objective:** To develop and characterize multiplex immunoassays for a panel of serum biomarkers for ovarian cancer as part of the NCI's Early Detection Research Network (EDRN) program.

**Method:** Existing candidate serum biomarkers identified by multiple EDRN sites were triaged first through *in silico* mRNA analysis and then by a Mass Spectrometry-based approach. U-PLEX assays were developed for 8 selected biomarkers using a MESO QuickPlex SQ 120 system (MSD). Briefly, antibodies were conjugated with biotin and/or SULFO-TAG and antibody pairs targeting the specific isoform of the MS-identified peptides were screened. Immunoassays for the individual candidates were first developed on small spot streptavidin plates followed by development and optimization of multiplex assays on U-PLEX plates. Spike & recovery, linearity, and cross-reactivity studies were performed. Patient samples including 10 healthy control sera and 11 ovarian cancer sera were analyzed.

**Results:** The U-PLEX assays of 4 candidate biomarkers or 2 candidate biomarkers including two FDA approved markers (CA-125 & HE4) had negligible cross-reactivity among analytes, recovery of 72-124%, intra-assay precision of 1.0-10.3% for 21 patient samples, and wide dynamic ranges for target measurements. The sensitivity of the U-PLEX assay was at 1.95 pg/ml for CA-125 assay. The U-PLEX assay significantly correlated with its respective monoplex assay using 21 patient sera (CA-125: Pearson *R*/*p* value = 0.9967/<0.00001). Among 11 ovarian cancer sera with available CA-125 values analyzed on the Tosoh Bioscience AIA-2000, the U-PLEX assay also significantly correlated with their clinical measurements (Pearson *R*/*p* value = 0.9986/<0.00001).

**Conclusion:** The utility of the developed multiplex assays was demonstrated with sufficient analytical performance. The developed multiplex assays can be used to evaluate serum biomarker panels of ovarian cancer and other cancers.

## A-031

### Improved detection of hidden Beta-2 monoclonal proteins by capillary electrophoresis using the Beta-2 to Beta-1 ratio

T. Morrison, P. Catomeris. *LifeLabs, Toronto, ON, Canada*

**Background:** Serum protein electrophoresis (SPE) is used for the diagnosis and monitoring of multiple myeloma and its related disorders by detecting monoclonal paraproteins. Capillary electrophoresis separates serum protein into 6 fractions: Albumin, Alpha-1, Alpha-2, Beta-1 (B1), Beta-2 (B2), and Gamma. While paraproteins are often detected in the gamma and beta fractions as an extra peak on the electropherogram, paraproteins have also been identified hidden in the B2 fraction. Identification of these paraproteins by inspection of the electropherogram alone is difficult. However, an elevated ratio of the B2 fraction over the B1 fraction increases suspicion of a hidden monoclonal protein in the B2 fraction. The manufacturer recommends flagging electropherograms with a B2/B1 ratio of 1.00 or higher for further investigation. However, in the setting of a community population, we questioned the value of a 1.00 threshold which, in our hands, was producing excessive false negative results. This study investigated the threshold necessary to achieve the most efficient positive detection rate of hidden B2 monoclonal proteins using the B2/B1 ratio.

**Methods:** Patient SPE data was collected retrospectively across 3 months for a total of 18,376 data points. The B2/B1 ratios were compared between cases with B2 monoclonal proteins confirmed by immunofixation electrophoresis, and cases without B2 monoclonal proteins. The data were used to predict the positive detection rate of B2 monoclonal proteins when the threshold for B2/B1 was set between 1.00 and 1.20.

**Results:** In this data set, 0.9% (n=165) of all cases had a B2 monoclonal protein and their B2/B1 ratios ranged from 0.58 to 19.42. When the B2/B1 ratio was set to a threshold of 1.00, 90.9% of all B2 monoclonal protein cases (n=150) had a ratio greater than the threshold. However, 1,947 cases negative for any monoclonal protein also had a ratio greater than the threshold. This produced a positive detection rate for B2 monoclonal proteins of 7.2%. Setting the threshold to 1.20 flagged 85.5% of all B2 monoclonal protein cases (n=141) as well as 156 negative cases. This produced a positive detection rate of 46.5%. Increasing the threshold to 1.20 considerably improved the likelihood of finding a B2 monoclonal protein by reducing the number of false negative cases flagged for further investigation. However, 24 B2 monoclonal protein cases had a B2/B1 ratio less than 1.20 and would be missed for further investigation. Similarly, 15 B2 monoclonal protein cases had a ratio less than 1.00 (the manufacturer recommended threshold). To detect 100% of B2 monoclonal proteins by capillary electrophoresis only, the positive detection rate would be 1.2% which is not a sustainable approach to screening for monoclonal proteins.

**Conclusion:** In a community population, setting the B2/B1 ratio to 1.20 for further investigation of suspected hidden B2 monoclonal proteins will help identify B2 monoclonal proteins at a detection rate of 46.5% without compromising patient care. This is a considerable increase compared to a threshold of 1.00 where the detection rate was 7.2%, flagging an excessive amount of false negative cases.

## A-032

### Performance Evaluation of an Automated Assay for the Measurement of Calcitonin on the Siemens ADVIA Centaur® Immunoassay Systems

L. Ferguson[1], N. McIntosh[1], A. Rybacka[1], A. Andrioae[1], P. Kelly[1], D. Hovanec-Burns[2]. *[1]Axis-Shield Diagnostics Ltd, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

**Background:** Calcitonin, a 32-amino acid polypeptide secreted by the C cells of the thyroid gland, reduces calcium levels in the blood. Calcitonin is used as an aid in the diagnosis and treatment of diseases involving the thyroid and parathyroid glands, including carcinoma and hyperparathyroidism. The objective of this study was to develop and evaluate the initial analytical performance of a calcitonin assay* on the ADVIA Centaur® XP Immunoassay System (Siemens Healthcare Diagnostics Inc.).

**Methods:** A sandwich immunoassay is being developed using direct chemiluminescent technology for the quantitative measurement of calcitonin in human serum and plasma. Calcitonin is bound to mouse monoclonal anti-calcitonin antibody-coated particles and is then detected by an acridinium-labeled anti-calcitonin mouse monoclonal

Fab. Following a wash stage and magnetic separation, acidic and basic reagents are added to the reaction mixture and the resulting chemiluminescence is measured. Assay performance was evaluated for precision, linearity, limit of quantitation (LoQ), and method comparison to the cobas e 411 assay (Roche Diagnostics). The method comparison study was performed per CLSI EP-09-A3 using 107 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively.

**Results:** Observed reportable range of the prototype Calcitonin assay was up to 2000 pg/mL without dilution or up to 200,000 pg/mL with automated or manual dilution. The assay demonstrated linearity up to 2000 pg/mL. The limit of quantitation was observed at 1.74 pg/mL, with a total error of 20%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 2.6-4.4%. Passing-Bablok procedure comparison of the Calcitonin assay to the cobas e 411 assay using 107 patient samples gave a slope and intercept of 0.98 and 0.50 pg/mL, respectively, with a correlation coefficient (r) value of 0.99.

**Conclusions:** The feasibility of an automated Calcitonin assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of calcitonin.

*Assay under development by Axis Shield Diagnostics Inc for Siemens Healthcare Diagnostics Inc. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

## A-033

### A biomarker discovery platform for non-serous ovarian cancers using integrative proteomics

F. Leung[1], M. Q. Bernardini[1], K. Liang[2], M. Rouzbahman[1], E. P. Diamandis[1], V. Kulasingam[1]. *[1]University of Toronto, Toronto, ON, Canada, [2]University of Waterloo, Waterloo, ON, Canada*

**Background:** Ovarian cancer (OvCa) is not a single disease but rather, several subtypes including serous, endometrioid (EC), clear cell (CC) and mucinous (MC) carcinoma. In fact, each subtype represents a distinct disease that differs in etiology, progression and treatment response. While timely and accurate diagnoses are critical to patient management, the current markers (CA125 and HE4) are specific for serous OvCa and perform poorly for the non-serous subtypes. As such, biomarkers with the ability to definitively diagnose the non-serous subtypes are sorely needed. **Methods:** Tissues from EC (n=7), CC (n=6), and MC (n=7) were compared to their respective controls - endometriosis and benign endometrium (n=8) for EC and CC; mucinous cystadenoma (n=6) for MC - using 2D-offline liquid chromatography tandem mass spectrometry on the Q Exactive Plus. MaxQuant was used for protein identification and quantification while statistical and bioinformatics analyses were performed using Perseus and various proteomic and transcriptomic databases. To identify high-priority candidates, a scoring algorithm was developed based on five criteria: (1) method of identification; (2) statistical metrics; (3) tissue specificity; (4) transcriptomic annotation; and (5) cellular localization. **Results:** Approximately 10,000 unique proteins were identified in total, with roughly 7000 proteins being identified within each patient cohort. Overall, the expression profiles of EC and CC were associated with endometriosis and benign endometrium while those of MC were associated with mucinous cystadenoma. Using a scoring algorithm (Figure 1), high-priority candidates were identified for each subtype. Several high-priority candidates have shown strong associations to their respective subtypes, such as napsin A for CC and meprin A for MC. **Conclusions:** We have identified high-priority biomarker candidates for non-serous subtypes with the use of a novel scoring algorithm. Several of the candidates have shown strong relevance to their respective subtypes, demonstrating the utility of the biomarker discovery platform for subtype-specific biomarkers.

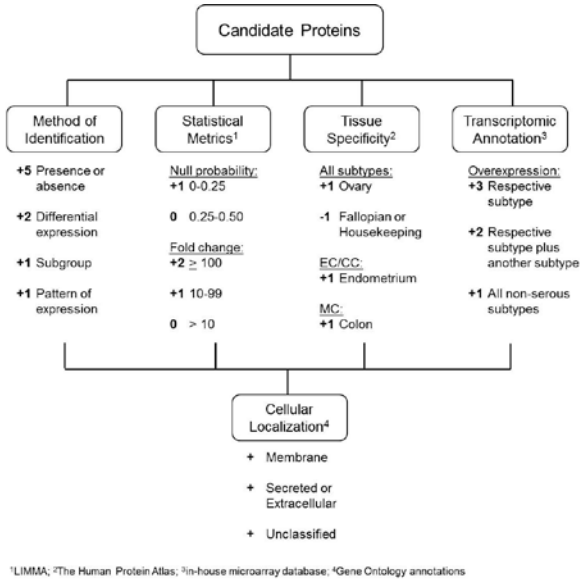



**Figure 1 –** Scoring algorithm for candidate proteins.

## A-034

### Diagnostic Performance of Serum Dickkopf-1 in Egyptian Patients with HCV Related Hepatocellular Carcinoma

R. N. Bedair[1], G. M. H. Magour[1], M. M. K. Eldeeb[1], M. Shamsia[2], M. A. Hammouda[3]. *[1]Chemical Pathology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt, [2]Department of Internal Medicine, Medical Research Institute, Alexandria University, Alexandria, Egypt, [3]Department of Pharmacology and Toxicology, Pharos University, Alexandria, Egypt*

**Background:** Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most common cause of cancer deaths worldwide. In Egypt, HCC was reported to develop in about 5% of patients with chronic liver disease. Early detection of HCC is critical as poor prognosis is correlated with diagnostic delay. Serum α-fetoprotein (AFP) is the tumor marker universally utilized for HCC, however studies revealed its poor sensitivity and specificity for proper surveillance and diagnosis. Therefore, additional and more sensitive diagnostic tools are needed. Dickkopf-1 (DKK-1) was considered a diagnostic and prognostic biomarker in multiple cancers. DKK-1 is a secretory antagonist of canonical Wnt signalling pathway which is hardly expressed in normal tissues except in placental and embryonic. DKK-1 could serve as a tumor suppressor or metastasis promoter. Recently, it was reported to be involved in HCC migration and invasion.

The aim of the present work was to evaluate the serum level of DKK-1 in patients with chronic HCV related liver cirrhosis with HCC and to compare it with its level in chronic HCV related liver cirrhosis without HCC. The diagnostic performance of DKK-1 was also evaluated.

**Methods:** 80 subjects were included in the present study and were divided into 3 groups: Group I (Control group) included 20 apparently healthy volunteers, Group II included 30 patients with HCV related liver cirrhosis and Group III included 30 patients with HCC on top of HCV related liver cirrhosis. Child-Pugh classification, Abdominal US, Triphasic CT and determination of serum levels of AFP and DKK-1 were done.

**Results:** The median value of AFP was significantly higher in patients with chronic HCV related liver cirrhosis with HCC compared to the other two groups (p=0.001). DKK-1 showed a significant increase in chronic HCV related liver cirrhosis with HCC when compared to chronic HCV related liver cirrhosis without HCC (p=0.001). There was a significant decrease in DKK-1 in chronic HCV related liver cirrhosis with HCC when compared to the control group (p=0.001). Receiver operating characteristics (ROC) curve showed that AFP surpassed that of DKK-1 in the diagnosis of chronic HCV related liver cirrhosis with HCC when compared to the control group with diagnostic sensitivity 83.33%, specificity 90%, positive predictive value 92.6% and negative predictive value 78.3%. While DKK-1 showed diagnostic sensitivity 60%, specificity 95%, positive predictive value 94.7% and negative predictive value 61.3%.

Also, when comparing the diagnostic performance in chronic HCV related liver cirrhosis without HCC with chronic HCV related liver cirrhosis with HCC, there was superior performance for AFP with diagnostic sensitivity 73.33%, specificity 76.67%, positive predictive value 75.9% and negative predictive value 74.2 %. While DKK-1 showed diagnostic sensitivity 56.67%, specificity 70.0%, positive predictive value 65.4% and negative predictive value 61.8%.

**Conclusion:** Serum AFP is better than serum DKK-1 in the diagnosis of HCC in patients with chronic HCV related liver cirrhosis due to its higher diagnostic sensitivity and specificity. Further studies are needed to indicate if serum DKK-1 can be used as tumor biomarker and to address its role in diagnosis and prognosis.

## A-035

### A Novel Bioluminescence/Fluorescence Platform for Rapid Detection and Post-Detection Analysis of Circulating Tumor Cells

Y. Yang, R. Cote, S. Daunert. *University of Miami, MIAMI, FL*

**Background:** Circulating tumor cells (CTCs) have shown a significant prognostic value as a surrogate marker in various cancers. To employ point-of-care CTC assay into clinical practice, an assay requires rapid CTC detection in blood with high sensitivity and specificity. Characterization of CTCs also provides valuable information on patient-specific cancer-omics profiles for the guidance of treatment decision. It will be beneficial for patients if an assay is able to accurately identify individual CTCs and select them for single cell analysis after the detection process. Therefore, the objective of this study was to develop the assay for rapid detection of circulating tumor cells in blood with high sensitivity and subsequently post-detection analysis of those cells at the single-cell level.

**Methods:** Gaussia luciferases were genetically fused with three antibody mimetics respectively, which serves as targeting molecules that bind specifically to tumor surface antigens (EpCAM, HER2, and EGFR). The antibody-mimetic_gaussia luciferase fusion proteins were expressed in the E. coli expression system, and purified using affinity chromatography. The cytotoxicity assay was used to determine the safety of the fusion proteins for viable cells. The fusion proteins were conjugated with a fluorescent dye that allows for identifying individual tumor cells for single-cell analysis.

**Results:** The fusion proteins were able to efficiently bind to the target tumor cells but not leukocytes. Due to the heterogeneous nature of CTCs, the multi-marker combination strategy showed the improvement of detection sensitivity in the comparison of a single marker (EpCAM)-based detection. In the model spiking experiments, our approach showed high specificity and sensitivity in detection of tumor cells (10 cells/mL blood) with no observed cell toxicity. Subsequently, those spiked tumor cells were able to grow in culture over 6 days. Moreover, incorporation of fluorescent dyes with the fusion proteins was able to efficiently identify individual tumor cells spiked in blood after bioluminescent detection, and those cells can be picked up by CellCelector, a single-cell isolation device, for single-cell analysis.

**Conclusion:** Our study shows that the dual detection method is capable of rapidly detecting viable CTCs and permits further downstream analysis of these cells via ex vivo culture; this viably labeled population can also be utilized for single-cell analysis. Our assay will have the potential point-of-care application as a diagnostic and predictive tool for CTC detection in clinical settings.

## A-036

### Expression of miRNAs Dysregulated by Human Papilloma Virus 16 E5, E6, E7 Oncoproteins in Cervical Carcinogenesis

M. HAN[1], Y. Park[2], H. Kim[2]. *[1]U2 Bio Co. Ltd., Seoul, Korea, Republic of, [2]Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of*

**Background**: Cervical cancer is the third most common malignancy in women worldwide. Almost all cervical cancers are associated with human papilloma viruses (HPV) but the majority of women with HPV do not develop cervical cancer. This study is aimed to find the microRNAs (miRNAs) as an explanatory variable and early tumor marker in cervical carcinogenesis.

**Methods**: We analyzed the miRNAs expression profiles in 12 cervical tissues by NanoString nCounter system miRNA Assay (813 miRNAs panel) using the digital multiplexed counting method. Significant miRNAs were selected based on arbitrary |fold change| $\geq 2$ and p-value $\leq 0.01$ using R software (v3.1.1). To validate selected significant miRNAs, thirty-four cervical tissues performed real-time RT-PCR using the miScript II RT Kit (Qiagen, Hilden, Germany) and the StepOnePlusTM Real Time PCR System (Applied Biosystems, CA, USA) using 2X QuantiTect SYBR Green PCR Master Mix (Qiagen). The data were analyzed using the StepOne software v2.2.2 (Applied Biosystems). The expression levels of each miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with endogenous control small RNA, U6.

**Results**: Six genes (miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p) were expressed significantly higher and two genes (miR-597-5p, -655-3p) were expressed lower in HPV16-positive cervical cancer group than the HPV16-positive normal group and pooled normal group (HPV-negative and HPV16-positive) in clinical tissues experiments.

**Conclusion**: Eight dysregulated miRNAs in cervical cancer will be possible early diagnostic biomarkers for women with HPV16 to predict cancer development. Especially, the miR-9-5p and miR-199b-5p might be expected most significant among them considering with previous reports consistent with our results.

## A-037

### Variations of Trace Elements Associated with Oxidative Stress in Breast Cancer Patients

S. A. K. Saleh, H. M. Adly. *Biochemistry Department, Faculty of Medicine, Umm AlQura University, Makkah, Saudi Arabia*

**Backgrounds:** Breast cancer is the most common female malignant tumor worldwide, representing 30% of female new cancer cases diagnosed annually in the USA and 33.5% in most Arab countries. Improvements of early detection, screening and treatment translating to decline the death rates of the disease. Variations of trace elements levels can induce metabolic disorders and cellular growth disturbance, even mutation and carcinogenesis. Reliable associations were determined between micronutrient alterations and risk of many cancers. Oxidative stress occurred as a result of excessive formation of reactive oxygen species (ROS) which frustrates and overpower the antioxidant defense mechanisms and free radical formation leading to oxidative damage of biomolecules, mutagenesis and tumorigenesis. **Objective:** This study explored the alterations of some trace elements [selenium (Se), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), iron (Fe)] and their relation to oxidative stress index (OSI), total oxidative capacity (TOC) and total anti-oxidative capacity (TAC) in female Saudi patients with breast cancer. **Patients and Methods:** The study included 92 newly diagnosed female breast cancer patients, 24 benign breast conditions and 48 apparently healthy women with no history of any benign or malignant breast diseases who attended routine health examination. Participants were recruited from volunteers of matched age and socioeconomic conditions. Patients with renal or liver diseases, smokers, and individuals on trace element supplementation for the past 3 months were preclude from the study. Clinical data and medical history were recorded for all subjects. Patients with breast cancer and benign breast conditions were undergone screening and diagnostic mammogram, Fine needle aspiration biopsy, CT scan, breast MRI scan and histopathological examination, accordingly breast cancer stages and metastatic disease were confirmed. Blood samples were collected from all subjects and serum concentrations of trace elements Se, Zn, Cu, Mn, Ni and Fe were analysed by ICP-MS. Serum TOC and TAC levels were analysed using ELISA technique. The OSI was calculated as the TOC / TAC ratio. **Results:** Mean serum Se, Zn, Mn and Ni concentrations of breast cancer group were significantly decreased compared to both benign and healthy groups ($p<0.05$) whereas the mean serum levels of Cu and Fe were significantly higher in breast cancer than benign and healthy groups ($p<0.05$). A marked disruption of OSI was observed in patients with breast cancer as a result of significant decrease of TAC and increase of TOC levels in breast cancer compared to benign and healthy groups ($p<0.005$). **Conclusion:** Breast cancer may be associated with trace element metabolic disorders. Low levels of Se, Ni and Zn and elevated levels of Cu and Fe appeared to be associated with the risk of breast cancer. Variation of trace elements status and disruption of metal ion homeostasis could induce oxidative stress, by formation of free radicals and/or other ROS leading to DNA mutation, biomolecules peroxidation and carcinogenesis. Trace elements status may be useful for breast cancer prediction; However, larger prospective studies are warranted to validate their role in prognosis and screening of breast cancer.

## A-038

### Genome-Based Discovery of Novel CpG Biomarkers for Early Diagnosis and Prognosis of Leukemia

D. D. Zhang, K. Zhang. *UCSD Institute for Genomic Medicine, La Jolla, CA*

**Background:**

Clinical cancer diagnosis faces many challenges, particularly in its low sensitivity and accuracy for early tumor detection. The goal of this project is to develop a noninvasive early cancer diagnostic platform using methylated CpG biomarkers. Methylation pattern of CpG islands is an epigenetic regulator of gene expression, and extensive alterations of CpG methylation are well documented in cancer. Importantly, the presence of CpG hypermethylation is commonly observed in precancerous lesions. As a key initiating event, CpG methylation at the DNA level occurs before histomorphological changes and biomarker expression. In addition, this pattern of DNA methylation is stably inherited from one cell generation to the next. Therefore, we hypothesized that genome-wide screening of CpG methylation sites can identify possible diagnostic CpG markers involved in early cancer development. For this study, we focused our efforts to identify unique CpG biomarkers of two common types of leukemia, acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL).

**Methods:**

An experimental approach was designed with use of genome-wide methylation profiling in combination with computational analysis, which allowed us to perform CpG biomarker analysis on a blood sample set of 194 AML patients, 136 ALL patients and 754 normal controls. For methylation sequencing data of AML patients, we used the public database TCGA (https://tcga-data.nci.nih.gov/tcga/). For ALL patients and normal controls, we performed genomic DNA extraction, bisulfite conversion, genome-wide CpG site specific capture, PCR and sequencing. The CpG methylation data were then analyzed with software bis-ReadMapper for determination of methylation levels of corresponding sequences. To classify the genome-wide methylation data, a supervised machine learning technique "nearest shrunken centroids" was adopted. We first mixed AML, ALL and normal blood samples together. 70% of these combined samples were put into the training set while 30% were put into the validation set. The PAM procedure was then performed with 10-round cross-validations on the training data set, and obtained robust classifiers for each AML-normal, ALL-normal and AML-ALL comparison. These classifiers were then used to classify the validation data.

**Results:**

Our classification results are of 97% accuracy. We discovered four and seven novel CpG biomarkers for early diagnosis of AML and ALL, respectively. Importantly, these CpG islands control genes like MNDA, RBP5, TCF25, GDF15, etc. Expression of these genes are down-regulated or up-regulated in leukemia, which is consistent with their CpG methylation profiles. For diagnostic tests with clinical relevance, a simple methylation specific PCR (MPCR) test was designed with a pair primers targeting methylated or unmethylated DNA sequence of confirmed CpG biomarkers. Our results of MPCR reactions were excellent, matching to the expected methylation specific PCR reaction profiling of AML patients or ALL patients with 100% accuracy. Therefore, MPCR can serve as a simple test for early cancer leukemia screening.

**Conclusion:**

A methylation-based survival classifier was also developed which successfully divided patients into high-risk and low-risk groups, with significant differences of clinical outcome in leukemia subtypes. Together, these findings demonstrated that our CpG panels can be highly sensitive and specific in the accurate diagnosis of AML and ALL with implications for prognosis and treatment selection.

## A-039

### Normal reference range of PIVKA-II in Chinese population: preliminary data from the Asian multiple-centers study

C. Yan[1], Z. Fan[2], J. Hu[3], W. Chen[3], X. Wang[3], R. Han[1], W. Zhang[4], A. Tran[5], A. Beshiri[6], A. Soh[7], Y. Zheng[8]. *[1]Department of Clinical Laboratory, Peking University First Hospital, Beijing, China, [2]DMedical Examination Center and Department of Gastroenterology, Renji Hospital, Shanghai, China, [3]Department of Clinical Laboratory, Xi'an Jiaotong University First Hospital, Xi'an, China, [4]Department of Mathematics & Statistics, University of Arkansas at Little Rock, RA, AK, [5]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Ho Chi Minh City, Viet Nam, [6]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Chicago, IL, [7]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Singapore, Singapore, [8]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Shanghai, China*

**Background**

Clinical application of protein induced by vitamin K absence or antagonist-II (PIVKA-II) has been widely used in Japan for a couple of years while it's still new to other Asian countries and needs more evidence supports in other population. From the preliminary reports, the normal reference ranges vary between Europe (95% confidence interval, CI: 11.12-32.01 mAU/ml) and Japan(95% confidence interval, CI: 17.36-50.90 mAU/ml). It indicated the ethical variation in different regions. Previously, we initiated an Asian study on the application of PIVKA-II in HCC diagnosis and differential diagnosis, including China, Singapore, Vietnam and Thailand. Here we report the preliminary results of the normal reference range from three sites in China.

**Methods**

A total of 309 cases of healthy subjects were recruited from routine health check subjects with a complete health examination results from three sites (Peking University First Hospital, Renji Hospital and Xi'an Jiaotong University First hospital) in China. Participants (aged 18-85 years) were with normal liver/kidney function and normal results for routine blood tests/urinalysis, and were excluded with liver disease history, kidney disease history or cancer history of any organ system. The study received ethical approval from the sites.

PIVKA-II levels were examined by the ARCHITECT PIVKA-II assay (Abbott, USA).

**Results**

of the PIVKA-II distribution is concentrated between 15.41 and 40.01mAU/ml. Among the 309 subjects, PIVKA-II mean level is 25.46 mAU/ml. All 95% reference ranges are estimated by nonparametric method. The 95% reference range of PIVKA-II is 14.26-40.44 mAU/ml. While those of individual centers are 13.68-38.77 mAU/ml (Beijing), 15.96-41.71 mAU/ml (Shanghai) and 14.07-41.76 mAU/ml(Xi'an ) respectively .

**Conclusions**

This is the first multiple-centers study that released the normal reference range of PIVKA-II (14.26-40.44 mAU/ml) in Chinese population to support the local application. However, this is only the preliminary data and more information is still under investigation.

Table.1 Reference Range in Chinese population.

| | 95% Reference Range |
|---|---|
| **All Centers (n=309)** | 14.26-40.44 |
| **Beijing (n=120)** | 13.68-38.77 |
| **Shanghai(n=112)** | 15.96-41.71 |
| **Xi'an(n=77)** | 14.07-41.76 |

## A-040

### A new panel of SNPs to assess thyroid carcinomas risk: a pilot study in a brazilian admixture population

I. Santos[1], D. Marques[1], J. Genre[1], A. Silva[1], J. Santos[1], J. Araújo[1], V. Duarte[1], A. Carracedo[2], M. Torres[2], G. Bastos[3], C. Ramos[4], V. N. Silbiger[5], A. LUCHESSI[1]. *[1]Universidade Federal do Rio Grande do Norte, NATAL, Brazil, [2]Centro Nacional de Genotipagem, SANTIAGO DE COMPOSTELA, Spain, [3]Department of Clinical Analysis and Toxicology of São Paulo University, São Paulo, Brazil, [4]Liga Norte Riograndense Contra o Câncer, NATAL, Brazil, [5]Universidade Federal do Rio Grande do Norte, PARNAMIRIM, Brazil*

Thyroid cancer (TC) is the most common endocrine malignancy, and its incidence rate has been appreciably increasing over the last few years. Hence, if the observed trends are maintained, TC will replace colorectal cancer as the fourth leading cancer diagnosis by 2030 in the United States. According to the Brazilian National Cancer Institute, about 6.960 new cases of this disease were expected in Brazil in 2016 [4]. In addition, the state of Rio Grande do Norte has an estimated incidence of 2.270 new cases, second only to the Southeast region of Brazil. In this study, we aimed to analyze the possible association of 45 single-nucleotide polymorphisms (SNPs) with this cancer in a population from Rio Grande do Norte, Brazil. Considering that the population of this region is characterized by a marked ethnic mixture, this study may significantly contribute to elucidation of molecular basis underlying both predisposition to TC and the effect of interbreed populations on SNP-Based Association Studies.

To this end, 90 thyroid carcinoma samples were collected from the biobank at the Laboratory of Pathology of Liga Norte Riograndense Contra o Câncer. Then, patient's samples were genotyped using the MassARRAY platform (Sequenon, Inc) followed by statistical analysis employing the SNPassoc package of R program. In addition, the genotypic frequencies of all 45 SNPs obtained from the International HapMap Project database and based on data from ancestral populations of European and African origin, were used to compose the control study group. In our study, eleven SNPs were excluded from the analysis because they were not in Hardy-Weinberg equilibrium ($p<0.05$). Another eleven SNPs showed significant differences in their frequency when compared the study and control groups: rs3744962, rs258107, rs1461855, rs9993140, rs4075022, rs9943744, rs4075570, rs2356508, rs17485896 and rs2651339. Furthermore, polymorphisms rs374492 C/T, rs258107 C/T and rs4075022 C/T were associated with a relative risk for thyroid carcinoma of 3.78 ($p= 0.0000627$), 2.91 ($p= 0.00008272$) and 2.35 ($p= 0.002011$), respectively. Thus, these three polymorphisms could be potential biomarkers of predisposition to thyroid carcinoma in the population of Rio Grande do Norte. Furthermore, they may be considered suitable molecular marker for early diagnosis of the disease. In addition, SNPs rs1461855 and rs2356508 might be possibly associated with a protective effect against TC development. However, complementary studies with a control group constitute by samples obtain from healthy subjects from Rio Grande do Norte state should be conducted to confirm these results.

## A-041

### Identification of a Portuguese founder mutation in *BRCA2* gene in two Brazilian women

N. P. Lopes[1], M. A. Pereira[2], P. G. P. Couto[3], F. C. A. Brito[1], E. Mateo[2], M. C. M. Freire[1], M. G. Zalis[1]. *[1]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil, [2]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [3]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil*

**Background:** *BRCA1* and *BRCA2* are tumor suppression genes associated with a large number of DNA repair pathways. Alterations in these genes can lead to an improper DNA repair, increasing the probability to develop several cancers types, e.g. breast, ovarian, prostate, pancreatic cancers and melanoma. Hereditary breast and ovarian cancer (HBOC) diagnosis is performed mainly by the identification of heterozygous germline pathogenic variants in *BRCA* genes. Different methodologies can be used for variant screening such as next-generation sequencing, Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). MLPA assays are used for the detection of deletions and duplications, and some specific rearrangements. **Objective:** This report describes two independent cases of a *BRCA2* founder mutation identified in Brazilian women by MLPA. **Methodology:** Genomic DNA was extracted from blood samples of the patients using QIAsymphony (QIAGEN). Gene deletions and duplications analysis were carried out using SALSA MLPA probemixes P002 BRCA1 and P045 BRCA2/CHEK2 (MRC-Holland). **Results:** A pathogenic rearrangement in *BRCA2* was identified in both patients: c.156_157insAlu. This mutation has been first described in a Portuguese family as an Alu insertion at codon 52 of *BRCA2* and is considered as a founder mutation of the Portuguese population, mostly originated from central/southern Portugal. This insertion leads to exon 3 skipping and an in-frame deletion of amino acids 23 to 105 of BRCA2 protein. **Discussion:** Alu elements are considered pathogenic because of their capacity to alter the genome. The exon 3 skipping due to an Alu insertion is important since this exon was reported to encode a transcriptional domain that interacts with the EMSY protein which is involved in chromatin remodeling. The c.156_157insAlu BRCA2 mutation is one of the most common variant identified in Portuguese HBOC families, responsible for more than half of all germline deleterious *BRCA2* mutations observed. However, it is still rare in Brazilian population. Investigation of this rearrangement in suspected HBOC families from countries with Portuguese ancestry (e.g., Brazil, Angola and Mozambique) or with a large community of Portuguese immigrants is strongly recommended. **Conclusions:** Screening of founder mutations in other populations allows evaluation of whether or not it is a population-specific mutation. Furthermore, analysis of founder mutations decreases genetic testing cost since it is more cost-effective to test only for some variants prior the screening of whole gene, making it possible to use more specific approaches for molecular testing.

## A-042

### Impact of NGS four-gene panel in screening genes with potentially therapies in NSCLC

C. Bustamante[1], M. C. M. Freire[1], N. P. Lopes[1], P. G. P. Couto[2], E. Mateo[3], M. G. Zalis[1]. *[1]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil, [2]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil, [3]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

**Background:** Targetable genomic mutation detection has innovated personalized medicine in non-small cell lung cancer (NSCLC) mainly through detection of *EGFR* activation mutations, which predicts susceptibility to tyrosine kinase inhibitors (TKIs). Although patients render positive response to TKIs initially, resistance could eventually arise, due to the secondary mutation T790M, therefore decreasing patient's survival and limiting treatment options. NSCLC patients harbor a variety of mutated genes, that could potentially be treated with drugs already approved, or in ongoing clinical trials for other types of tumor. *KRAS* is one of the most frequently mutated genes in NSCLC and could act as a new target for lung cancer treatment. Although no targeted therapy has yet been approved for mutated-*KRAS*, there are multiple potential treatment strategies under investigation. *BRAF* is another gene that offers a rational therapeutic strategy, since its pathway plays a role in the carcinogenesis of NSCLC. **Objectives:** Determine the prevalence of *KRAS*, *BRAF* and *EGFR* among patients with next generation sequence technology and reveal the importance of multi-gene panel screening. **Methodology:** We used a custom multigene panel for Ion Platform to investigate *EGFR*, *KRAS* and *BRAF* mutations in 491 NSCLC Brazilian patients. Genomic DNA libraries were prepared from FFPE derived DNA and sequenced in the Ion PGM. **Results:** *KRAS* was the most frequently mutated gene, found in 27.6% of all samples, thus could be an appealing target for new therapy strategies as described in other studies. 111 (82.2%) samples harbored mutation in codon 12, being G12C mutation the most common one, present in 37.8%. The second most prevalent mutated gene was *EGFR* with 119 (24.3%) mutated samples. The most common mutations were exon 19 deletions (most frequently E746-A750), found in 42% of the samples and L858R, found in 32% of the samples. *BRAF* displayed prevalence rates of 3.7%. V600E mutation was present in 55.5% of the *BRAF* mutated samples, followed by mutations in codon G469 (22%). We identified 17 double and coexisting mutations. *EGFR* T790M was the most common secondary mutation, found in six (12%) samples concomitantly with p.E746_A750del, indicating that these patients might have a reduced response to TKIs therapy. Interesting is the fact that three of these double mutant samples had prior cobas® *EGFR* Test results from 2-3 previous years with absence of T790M mutation, indicating that resistance may have arrived in a 2 to 3 years life span. Although, *EGFR* gene mutations are reported as mutually exclusive with *KRAS* or *BRAF* mutations in lung cancer, we found three samples harboring concomitant *EGFR* and *KRAS* mutations. Previous studies also reported that mutations in *KRAS* and *BRAF* are mutually exclusive, but we found one sample harboring mutations in both genes, indicating that this is a rare event. **Conclusions:** This study shows that development of future therapies, targeting *KRAS* should be prioritized. Although *BRAF*-mutated samples displayed short prevalence, it could be a potential target among wild-type *EGFR* and *KRAS* cancer patients. This study shows the importance of multi-gene panel screening to reduce overall turnaround time generating results in a single test.

## A-043

### Conventional Cytogenetics Analysis in Multiple Myeloma in a Routine Laboratory

L. M. E. Wingist, J. M. Fernandes, R. Kuhbauche, J. R. Alves, M. P. Migliavacca, G. A. Campana, M. Conchon. *DASA, Barueri, Brazil*

**Background:** Multiple myeloma (MM) is characterized by the accumulation and proliferation of malignant plasma cells, secreting monoclonal immunoglobulins. Genetic abnormalities are powerful prognostic factors in MM for risk stratification and therapy strategies. The standard diagnostic tests to detect genetic abnormalities in MM include Conventional Cytogenetic Analysis (CCA) and Interphase Fluorescence *In Situ* Hybridization (FISH). The interpretation of cytogenetic abnormalities is complicated by the number and complexity of the abnormalities and the methods used to detect them.

**Objective:** In the present study, we investigated the frequency of chromosomal abnormalities (CA) in samples of patients with MM in a private laboratory on CCA.

**Methods:** We performed CCA in 474 samples of patients with diagnostic hypothesis that included multiple myeloma, monoclonal gammopathy and/or monoclonal peak, between 2015-2016 in a private laboratory. Bone marrow samples were cultured according to cytogenetics protocols. The karyotype analysis was reported according to ISCN 2016.

**Results:** Among 474 samples of MM patients, 451 (95%) had normal karyotype and 24 (5%) had cytogenetic abnormalities. The abnormalities were: Complex karyotype, t(1;19)(p22;p13), trisomy 8, t(7;13)(p15;q14), t(6;7)(p21;q22), del 9q, del 11q23, trisomy 12, del 20q, del 13q, monosomy Y and X. Of the 474 samples, the percentage of plasma cells could be done in 292 samples by myelogram. 87 samples (30%) presented more than 10% of plasma cells and 86 (almost 30%) presented less than 1% of plasma cells.

**Conclusion:** Cytogenetic abnormalities in MM affect every aspect of the disease, from evolution of the malignancy to clinical presentation, response to therapy and prognosis. For clinical purposes, cytogenetics in MM can be assessed by metaphase karyotyping through CCA or by FISH. Metaphase cytogenetics requires proliferating cells and is not sensitive for the detection of either primary or secondary cytogenetic abnormalities in MM. Further, any prognostic impact that is seen with a metaphase detected abnormality is probably not due to that abnormality per se but simply a reflection of the fact that patient had a more aggressive disease. In general, metaphase cytogenetics are mainly useful to determine the presence of myelodysplastic syndrome that may occur during the course of the disease secondary to therapy. In fact, from twenty cytogenetics abnormalities we found seven samples with complex karyotype, two samples with deletion 11q23 and one with 20q deletion. Most of our karyotypes (95%) did not presented any abnormality. In fact, in newly diagnosed MM, the abnormal plasma cells have a low proliferative activity, and the analyzable metaphase spreads from CCA are derived from normal hematopoietic cells, thus resulting in a normal karyotype. In our analysis, 30% of our samples showed more than 10% of plasma cells and 30% had less than 1%. Cytogenetics assessment of MM is essential for clinical practice, and the importance of this evaluation is indicated by recent incorporation of high-risk cytogenetics abnormalities into Revised International Staging System for MM. However, the conventional technique have limitations which can be overcome partly by the incorporation of FISH analysis of interphase nuclei.

## A-044

### Alteration of Serum CA125 and HE4 levels in Response to Chemotherapy in Patients with Different Histological Subtypes of Ovarian Cancer

Y. Wang[1], S. Narla[1], E. Champion-Lyons[1], W. Graybill[1], Y. Zhu[2]. *[1]Medical University of South Carolina, Charleston, SC, [2]Penn State University Hershey Medical Center, Hershey, PA*

**Background:** Serum CA125 is used to monitor response to chemotherapy, relapse, and progression in ovarian cancer patients. Recently, HE4 has been used in combination with CA125 to assess the risk of ovarian malignancy. This study aims to evaluate the change of these biomarkers in response to chemotherapy in patients with different histological subtypes of ovarian cancer.

**Methods:** 11 ovarian cancer patients with subtypes of serous (5), clear cell (2), mucinous (1) carcinoma, and carcinosarcoma (3) were enrolled in this study. All patients received postoperative adjuvant paclitaxel/carboplatin chemotherapy for up to 6 cycles. No recurrence of ovarian cancer was identified during the course of this study. Serum CA125 and HE4 collected at pre-surgery (baseline) and after each cycle of chemotherapy were concurrently measured using immunochemiluminometric assay (Abbott Architect). The reduction% was calculated as change of CA125 and HE4 from basal level. The overall %reduction was the mean of the reduction% of all cycles for each patient.

**Results:** Abnormally elevated CA125 and/or HE4 baselines were observed in all patients, except that two normal CA125 baselines were found in one patient with clear cell carcinoma and another with carcinosarcoma, and four normal HE4 baselines were seen in one patient with serous carcinoma, two with clear cell carcinoma, and one with mucinous carcinoma. The overall %reduction of CA125 is significantly higher than that of HE4 in all ovarian cancer patients ($P<0.05$), regardless of their subtypes. Over 50% reduction of CA125 after 2nd cycle of chemotherapy was observed in all subtypes and was stable for up to 6th cycle. The similar reduction pattern for HE4 was only found in serous carcinoma and carcinosarcoma. In contrast, patients with either clear cell or mucinous carcinoma and normal HE4 baseline displayed an increase or a bidirectional alteration in HE4 value, which was inconsistent with the unidirectional decrease of CA125 during the chemotherapy, and might be due to biological variations.

**Conclusion:** Our data demonstrate consistently large reduction (>50%) of CA125 after successful chemotherapy in all subtypes of ovarian cancer, and indicate that serum CA125 might outperform HE4 as biomarker to monitor the response to chemotherapy in ovarian cancer patients, especially for serous and clear cell carcinoma. However, additional studies with a larger sample size are needed to achieve a definite conclusion.

## A-045

### Association of INDEL polymorphisms with Colorectal Cancer risk and prognostic follow-up in Brazilian population

D. Marques[1], L. R. Ferreira-Costa[1], L. L. Ferreira-Costa[1], R. S. Correa[2], A. M. P. Borges[2], C. C. O. Ramos[2], R. H. Bortolin[1], A. D. Luchessi[1], Â. Ribeiro-dos-Santos[3], S. Santos[3], V. N. Silbiger[1]. *[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Liga Norte Riograndense Contra o Câncer, Natal, Brazil, [3]Universidade Federal do Pará, Belém, Brazil*

**Background:** Colorectal cancer (CRC) is the third most common cancer type in men and the second in women. Despite the effective strategies for prevention, early detection, and treatment, there are ethnic differences in CRC incidence and survival. These variances occur specifically in African Americans, who have higher CRC incidence and lower survival rates than other ethnic groups. Thus, in this study was to perform the association between 16 INDEL polymorphisms with CRC risk and prognostic follow-up in an admixture population.

**Methods:** A total of 280 participants were enrolled in the study, which 140 were diagnostic with CRC and 140 were free-cancer subjects. The polymorphisms and ancestry distribution were genotyped by Multiplex-PCR reaction, separated by capillary electrophoresis on the ABI 3130 Genetic Analyzer instrument and analyzed in GeneMapper ID v3.2. Furthermore, considering that ancestry distribution in an admixed population might influence cancer development susceptibility and affect the polymorphism distribution, all polymorphism analysis were adjusted according the individual proportions of European, African, and Amerindian genetic ancestries.

**Results:** The logistic regression analysis showed that INDEL polymorphism variations in *ACE* (rs4646994), *IL4* (rs79071878), and *TYMS* (rs151264360) genes were associated with CRC risk. Reference to anatomic localization of tumor Del allele of *NFKB1* (rs28362491) and *CASP8* (rs3834129) were associated with more incidents to colon than rectosigmoid. In relation to the INDEL association with TNM stage risk, the Ins allele of *ACE* (rs4646994), *HLAG* (rs371194629) and *TP53* (rs17880560) were associated with higher TNM stage. Furthermore, regarding INDEL association with relapse risk, the Ins allele of *ACE* (rs4646994), *HLAG* (rs371194629), and *UGT1A1* (rs8175347) were associated with relapse risk, as well as the Del allele of *TYMS* (rs151264360). About INDEL association with death risk, the Ins allele of *SGSM3* (rs56228771) and *UGT1A1* (rs8175347) were associated with death risk.

**Conclusion:** In summary, in this study, we showed that INDEL polymorphisms in ACE, TYMS, IL4, NFKB1, CASP8, TP53, HLAG, UGT1A1, and SGSM3 might be useful as cancer panel to determine CRC risk and prognostic follow-up, even as auxiliary to the better clinical management.

## A-046

### ARCHITECT NSE: AN ASSAY FOR MEASUREMENT OF HUMAN NEURON-SPECIFIC ENOLASE

E. Riedel, M. Davison, B. Williams, Z. Li, T. Kettlety, G. Thorne. *Fujirebio Disgnostics, Inc., Malvern, PA*

**Background:** Neuron-specific enolase (NSE), a glycolytic enzyme (2-phospho-D-Glycerate hydrolase), can be found in a variety of non-neuroendocrine cells (European Group on Tumor Markers, Lung Cancer Guidelines, 2012). NSE is a marker in the management of small cell bronchial carcinoma (Larmerz, 1998). An assay for NSE on ARCHITECT *i* System (ARCHITECT NSE) is being developed, and analytical performance is being presented. **Methods:** The ARCHITECT NSE (in development) is a one-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of NSE in human serum. Determination of NSE is used for monitoring response to therapy and detection of recurrent disease, such as SCLC and neuroendocrine tumors. The specimen is incubated with paramagnetic microparticles coated with the monoclonal antibody (mAb) NSE21, and acridinium-labeled mAb NSE17 conjugate on the ARCHITECT® *i* System. After incubation and washing, Pre-trigger and Trigger are added. The resulting relative light units (RLUs) are directly proportional to the amount of NSE in the sample, allowing for the quantitative determination of NSE in serum. **Results:** The calibration range for the assay is 0.0 to 400.0 ng/mL. The limit of blank (LoB) and limit of detection (LoD) were 0.04 and 0.16, ng/mL, respectively. The limit of quantitation (LoQ) at 20% CV was 0.35 ng/mL. Linearity was demonstrated for a range of 0.8 through 650.8 ng/mL. A Precision study of 3 controls and 4 panels spanning the range of the assay demonstrated a total %CV ≤ 3% at all levels. In the sample tube type study, a matrix comparison of 30 matched serum and SST samples were evaluated. A Passing-Bablock slope of 1.01 and r of 0.99 was observed when evaluating samples within the measurement range for serum separator tubes compared to the Red Top serum samples. Six (6) endogenous substances were evaluated for interference in the ARCHITECT NSE assay. The average percent difference between test and control samples for all endogenous interferents was < 10%. Percent difference in the presence of twenty (20) potentially interfering drugs was ≤ 4.0%. The potential cross-reactant alpha-enolase was evaluated at a concentration of 900 ng/mL and the percent cross-reactivity was ≤ 2%. **Conclusion:** The ARCHITECT NSE assay under development demonstrates a sensitive and precise assay for the quantitative determination of NSE in human serum.

## A-047

### ARCHITECT Tg: AN ASSAY FOR MEASUREMENT OF HUMAN THYROGLOBULIN

A. Vasko, E. Cawley, A. Motchenbacher, Z. Li, T. Kettlety, G. Thorne. *Fujirebio Disgnostics, Inc., Malvern, PA*

**Background:** Circulating levels of thyroglobulin (Tg) are critical in management of patients with differentiated thyroid cancer (DTC), and are measured following thyroidectomy or radioactive iodine ablation (NACB, 2002). ARCHITECT Thyroglobulin (ARCHITECT Tg) is an immunoassay that determines circulating levels of thyroglobulin. **Methods:** The ARCHITECT Tg assay (in development) is a delayed one-step chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of thyroglobulin in human serum or plasma on the ARCHITECT *i* System. The assay is intended to aid in monitoring DTC patients in the absence of anti-Thyroglobulin autoantibodies. The method utilizes paramagnetic microparticles coated with a highly specific monoclonal antibody (mAb) which capture the Tg in the specimen. After incubation, the acridinium-labeled anti-Tg mAb conjugate is added to complete sandwich format. After another incubation and wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of Tg in the specimen and the RLUs detected by the ARCHITECT *i* System optics. **Results:** The ARCHITECT Tg assay has been standardized against the European Community Bureau of Reference (BCR) CRM 457. The calibration range for the assay is 0 to 500 ng/mL. The limit of blank (LoB) and limit of detection (LoD) were estimated to be 0.026 and 0.048 ng/mL, based on guidance from CLSI EP17-A2. The limit of quantitation (LoQ) defined as the lowest concentration observed at ≤ 20%CV was determined to be 0.072 ng/mL. The assay demonstrated linearity from 0.08 to 521.6 ng/mL. The 20-day precision study tested with 8 samples (3 controls and 5 panels) showed a total within-laboratory imprecision of ≤ 0.013 ng/mL for samples with Tg ranging from 0.1 to 0.8 ng/mL, and ≤ 5%CV for samples above 0.8 ng/mL. Method Comparison to the Roche Cobas Tg II assay demonstrated a linear correlation with a slope of 0.99 and a correlation coefficient of 1.00 (n = 208). In a tube type equivalency study, serum tube was compared to six other tube types including serum separator tube, EDTA and heparin plasma tubes. Passing Bablock slopes ranged from 1.00 to 1.05, and r values were all 0.99. Seven endogenous substances and 15 potentially interfering drugs were evaluated for interference in the ARCHITECT Tg assay. The average percent difference between test samples and control ones for all interferents evaluated was ≤ 10%. The potential cross-reactants TSH and TBG were evaluated at concentrations of 1000 mIU/L and 200,000 ng/mL, respectively, with the percent cross-reactivity ≤ 5%. **Conclusion:** The ARCHITECT Tg assay under development demonstrates a sensitive and precise assay for the quantitative determination of Tg in human serum and plasma that meets the American Thyroid Association guidance for management of Thyroid Nodules and differentiated thyroid cancer.

## A-048

### Clinical Significance of Pancreatic Cyst Fluid CEA Level

J. F. Annunziata[1], S. Jackson[2], C. Narick[2], J. F. Silverman[1], S. Finkelstein[2]. *[1]Allegheny Health Network, Pittsburgh, PA, [2]Interpace Diagnostics, Pittsburgh, PA*

**Background:** Measurement of pancreatic cyst fluid (PCF) CEA level is an important first line test to determine mucinous cysts and risk for pancreatic cancer. We have reviewed our large PCF experience (n=11,222) to better understand the predictive value of CEA determination.

**Methods:** PCFs grouped according to CEA (ng/ml) as follows: very low (VL), 0-4.99 (n=1914), low (L), 5.0-191.9 (n=4326), high (H), 192.0-999.9 (n=2524), very high (VH), over 1000.0 (n=2458). Each group was assessed for mucinous cyst formation based on the presence or absence of 1) gross fluid viscosity, 2) cytologic evidence of stainable mucin, and KRAS/GNAS oncogene point mutational change. Each group was assessed for malignancy risk in each group was evaluated using an established four tier risk classifier based on integrated molecular pathology (IMP) characteristics (Endoscopy, 2015:Feb;47(2):136-42. doi: 10.1055/s-0034-1390742. Epub 2014 Oct 14.).

| | Benign | stat indolent | Low risk | stat higher risk | aggressive | High risk | cyto mucin | grossly viscous | KRAS/GNAS |
|---|---|---|---|---|---|---|---|---|---|
| CEA<5 (n=1914) | 1713 | 132 | 1845 (96.4%) | 67 | 2 | 69 (3.6%) | 194/1424 (13.60%) | 276/1728 (16.00%) | 98 (5.10%) |
| CEA 5-192 (n=4326) | 3309 | 777 | 4086 (94.5%) | 234 | 3 | 237 (5.5%) | 718/3140 (22.90%) | 1219/4014 (30.40%) | 1570 (36.30%) |
| CEA 192-1000(n=2524) | 1642 | 638 | 2280 (90.3%) | 235 | 9 | 244 (9.7%) | 510/1745 (29.20%) | 857/2373 (36.10%) | 1250 (49.50%) |
| CEA>1000 (n=2458) | 1026 | 750 | 1776 (72.2%) | 627 | 55 | 683 (27.8%) | 632/1759 (35.90%) | 949/2279 (41.60%) | 1263 (51.40%) |
| Low risk combines benign and stat indolent categories, high risk combines higher risk and aggressive categories | | | | | | | | | |
| Assume n is same as in column A unless otherwise stated (see columns H and I) | | | | | | | | | |

**Results:** Cytologic mucin ranged from 13.6%-VL to 35.9%-VH. Grossly viscous fluid ranged from 16.0%-VL to 41.6%-VH. Positive oncogene mutations ranged from 5.1%-VL to 51.4%-VH. Each of the three methods to affirm mucinous cyst formation was negative in majority of CEA subsets. High risk for malignancy was detected in 3.6%-VL, 5.5%-L, 9.7%-H and 27.8%-VH. Low risk, benign disease was seen in the majority of all CEA subsets, including patient with highly elevated levels. **Conclusion:** While progressive levels of PCF CEA are more likely to be associated with mucinous cyst formation, the majority of mucinous cysts prove to be negative. More than half of cases with PCF-VH are negative for mucin by cytology or gross fluid viscosity. Similarly, while malignancy risk increased with CEA elevation, the vast majority of PCF samples were low risk notwithstanding elevated CEA levels. However, the risk for malignancy nearly triples from PCF-H to PCF-VH, whereas incidence of KRAS/GNAS is nearly the same in both categories.

## A-049

### Personna Onco: a multi-cancer panel for mutational screening in a Brazilian cohort

M. A. Pereira[1], M. C. M. Freire[2], P. D. R. Cirillo[1], N. P. Lopes[2], C. Bustamante[2], R. L. M. Guedes[1], F. C. A. Brito[2], L. C. Almeida[2], E. Mateo[1], M. G. Zalis[2]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil*

**Background:** Next-Generation Sequencing (NGS) technology revolutionized the medical diagnosis field through concomitant analysis of multiple regions/genes and several individuals in a single experiment. This is currently allowing the transition from traditional to precision medicine, leading to a more accurate cancer diagnosis and enabling the best selection of molecular target drugs for individual treatments. **Objective:** The aim of this study was to survey the spectrum and prevalence of variants in solid tumor samples using Oncomine™ Focus Assay (Thermo Fisher Scientific), a multi-biomarker NGS assay for the detection of relevant alterations (hotspots, SNVs, indels, CNVs and gene fusions) in 52 oncogenes associated with different solid

tumors. **Methodology:** Formalin-Fixed Paraffin-Embedded (FFPE) samples were obtained from 362 patients (186 males and 176 females) and clinical-pathological data were obtained from pathologist/physician responsible for each patient. Genomic DNA and total RNA were extracted from all samples. Libraries were prepared and sequenced on Ion Torrent PGM sequencer. Sequencing data was analyzed using Ion Reporter software integrated with Oncomine® Knowledgebase. **Results and Discussion:** A total of 106 patients (29.3%) did not present any variants and only 13 patients (3.6%) showed inconclusive results due to poor DNA/RNA quality and/or quantity. These patients were excluded from further analysis. In general, 68 CNVs, 36 fusion genes and 245 point mutations/indels were observed for the remaining patients (n=243). The most frequent tumor type observed was lung cancer (46.5%) showing *EGFR* (34.5%) and *KRAS* (31%) as the most frequently altered genes. These mutations are. *KRAS* G12 hotspot (22.1%: p.G12C - 8.0%, p.G12D - 7.1%, p.G12V - 4.4%, p.G12A - 1.8%, p.G12F - 0,9%), *EGFR* p.E746_A750del (6.2%) and *EGFR* p.L858R (6.2%). Other observed tumors were colon, breast, bladder, ovary, liver, uterus, brain, skin and bone. Interestingly, this panel allowed the identification of not commonly observed variants in these tumors such as ovary adenocarcinoma *PIK3CA* p.M1043I, moderately differentiated colon adenocarcinoma *FGFR4* amplification, lung adenocarcinoma *BRAF* p.G469A and *ERBB2* amplification. All these alterations were previously associated with drug response for other cancer types. **Conclusions:** This study demonstrated that a multi-cancer panel plays an important role for clinicians and patients allowing the identification of multiple druggable variants with approved therapies, potentially improving patient outcomes and increasing the chances of getting the best treatment. In addition, the generation of large-scale results of variants in tumors contributes to the description of mutations classified as non-pathogenic and that may eventually become driver mutations.

## A-050

### Driver mutations in *EGFR* gene identified in tumor cell-free DNA: application of liquid biopsy for use tyrosine kinase inhibitors in non-small cell lung cancer patients

P. D. R. Cirillo[1], N. P. Lopes[2], C. Bustamante[2], A. A. Morais[2], N. L. Viana[2], C. S. Portella[2], E. Mateo[1], M. C. M. Freire[2], M. G. Zalis[2]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil*

**Background:** Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers in world wide. The 5-year survival rate in NSCLC patients is less than 5% thus development of strategies to improve the treatment and survival of these patients is necessary. Activating mutations of epidermal growth factor receptor (*EGFR)* occur in approximately 15% in Caucasian and approximately 30% in Brazilian NSCLC. The EGFR tyrosine kinase inhibitors (TKIs) have been widely used for advanced NSCLC patients and liquid biopsies using plasma-derived cell-free DNA (cfDNA) is a non-invasive test allowing a better selection and monitoring of them. **Objective:** The aim of this study was evaluate the frequency of *EGFR* mutations in cfDNA before and after TKIs treatment in a cohort of NSCLC Brazilian patients submitted to liquid biopsy. **Methods:** Mutations were investigated by Real Time qPCR Cobas® EGFR Mutation Test v2. Clinical data were obtained prior blood collection. Samples were collected at Progenetica Laboratory or sent from other laboratories, centrifuged and plasma were immediately frozen. Fisher´s exact test was calculated to compare frequency distribution between two groups (GraphPad Prism version 6.00). **Results and Discussion:** This study enrolled 535 NSCLC Brazilian patients from Southeast+South (74.9%), North+Northeast (8.8%), and Midwest+Federal District (4.7%). Regional information was missing for 11.3% of cases. NSCLC were more frequent among women (56.4%) compared to men (43.6%). Four-hundred cases (74.8%) not showed any *EGFR* mutations, however 135 cases presented 19Del (57.8%), L858R (16.3%), double 19Del; T790M (11.1%), double T790M; L858R (8.15%), G719X (2.9%), L861Q (1.48%), 20Ins, triple L858R; T790M; 20Ins and S768I; G719X (0.74% each one) mutations spanning 18, 19, 20, and 21 *EGFR* exons. Previous results from liquid biopsy for *EGFR* mutation were obtained for 75/535 cases (14%). Among them, 10/75 cases (13.3%) presented non-detected mutations. Deletions at exon 19 (19Del) and mutations at exon 21 (L858R) were frequently observed accounting for 61.5% and 23%, respectively. Since 19Del and L858R are drug-sensitive mutations we compared the frequency of *EGFR* mutations in patients before and after TKIs treatment. Interestingly, patients harboring these mutations before the treatment were negative for the presence of 19Del (37.5%) and L858R (66.7%) after the treatment, respectively ($p<0.0001$). In addition, *EGFR* T790M mutation already associated with drug-resistance was identified in 3/65 (4.6%) of cases in double with 19Del before the TKIs treatment and one patient was negative and two patients remaining positive for both mutations after treatment, respectively. **Conclusion:** These results showed that liquid biopsy is a powerful tool for personalized medicine for early detection, stratification and monitoring of *EGFR* druggable mutations in cfDNA from NSCLC patients. To our knowledge this is the first report showing the application of liquid biopsies for management of NSCLC patients in Brazil. Clinical data collection is undergoing for a better reliability results. cfDNA has been related to aggressiveness and poor prognosis of NSCLC patients and not all cases present tumor cfDNA. High index of non-detected results was observed at this study however new Next-Generation Sequencing and digital PCR protocols are under development to improve the sensitivity of this test.

## A-051

### A case of the invisible IgM

A. K. Tsui[1], M. Curran[2], P. Rowe[2], M. Savoy[2], T. Higgins[2]. *[1]University of Alberta, Edmonton, AB, Canada, [2]DynaLIFE Medical Labs, Edmonton, AB, Canada*

**Background:** Protein electrophoresis is performed to detect and quantify monoclonal proteins (M-protein) used for diagnosis and monitoring of patients with monoclonal gammopathies. In rare cases, the M-protein can be missed due to the properties of the different types of immunoglobulins, specifically IgM. The objective of this project is to present a case in which M-protein (IgM of 6g/L) using gel electrophoresis was not observed in a 71 years old male patient previously diagnosed with monoclonal gammopathy of undetermined significance.

**Methods & Results:** Serum and urine protein gel electrophoresis (Sebia Hyadrasys; Hydragel) were requested by the physician. Initial serum protein electrophoresis (SPE) result was unremarkable other than a low background in the gamma region. Serum IgG (2.37g/L; RI: 6.94-16.18g/L) and IgA (0.19 g/L; RI: 0.7-4.0g/L) concentrations were low, whereas IgM was increased (11.57g/L; RI: 0.6 - 3.0 g/L) using immunoturbidimetry (Siemens ADVIA). Urine protein electrophoresis (UPE) (Sebia Hyadrasys; Hydragel) demonstrated a large band in the near gamma region, which was confirmed by urine immunofixation electrophoresis (IFE) as a free kappa light chain. In addition, free light chain (FLC) assay demonstrated abnormally elevated free kappa light chain (182.5 mg/L; RI: 3.3 - 19.4 mg/L). Due to inconsistent results between SPE and UPE, other tests (immunoglobulin quantitation, FLC assay and a serum IFE) were ordered by the laboratory. Similar to the initial SPE results, no distinct band was visible in IFE. After treating the samples with beta-mercaptoethanol or Fluidil to break the disulfide bonds, a distinct band of M-protein of IgM-kappa quantifying at 6 and 5 g/L, respectively was noted. **Conclusion:** The characteristic large size and viscous nature of the pentameric IgM poses challenges for the laboratory to accurately detect the presence of IgM M-protein. In the testing laboratory, mechanisms were in place to alert for any potential false results from SPE, including keeping a laboratory record of all myeloma patients, recognizing the common ordering physicians (i.e. hematologist), and determining any inconsistencies between the related test results. The falsely normal SPE result was initially missed because of the fact that a normal globulin level did not trigger further investigation. However, the recognition that a normal total globulin measurement was inconsistent with a suppressed SPE background prompted additional exploration. Due to the potential of false SPE results in IgM M-protein, additional methods such as UPE, FLC assay, capillary electrophoresis, immunoglobulin quantitation, total protein and globulin should be used in conjunction to interpret monoclonal protein.

## A-052

### Extended Stability of Free and Total PSA and f/t PSA Ratio% in Frozen Male Serum

T. F. Soriano[1], H. A. Fritsche[2], R. P. Thiel[3], W. A. Smith[1], J. E. Loebel[1], R. Dema-ala[1]. *[1]DOCRO, Inc., Oxford, CT, [2]Fritsche Consulting Services, Houston, TX, [3]Thiel Statistical Consulting, Oxford, CT*

**Background:** The long term stability of free and total PSA and the f/t PSA Ratio% in male serum stored continuously at -80ºC beyond 9 years has yet to be proven. This study, conducted in 2017, retesting never thawed frozen serum aliquots from the same subjects first collected and tested in 2001 demonstrates stability at -80ºC for each analyte and the ratio for at least 15.5 years. This extended stability allows the performance of important clinical and analytical studies using existing specimens collected around the turn of the last century

**Methods:** Blood was collected (1999 - 2001) from 174 subjects (50 - 75 years of age) prior to DRE/biopsy during prostate cancer screening. Serum was prepared and kept refrigerated until frozen in 0.5 mL aliquots at -80ºC within 36 hours of collection. In 2001, free and total PSA assays were performed on the Siemens Dimension RxL. Aliquots were thawed immediately prior to testing. The f/t PSA ratio% calculated for

each specimen. 15.5 years later, the same instrument was used to measure free and total PSA and calculate f/t PSA Ratio% on freshly thawed aliquots of the same 174 specimens.

**Results:** y = tPSA 2017 and x = tPSA 2001, y = 0.9685 + 0.9299 x, $R^2$ = 0.9929; y = fPSA 2017 and x = fPSA 2001, y = 0.0388 + 0.9908 x, $R^2$ = 0.9931; y = f/t PSA% 2017 and x = f/t PSA% 2001, y = 0.0075 + 0.9885 x, $R^2$ = 0.9714.

**Conclusion:** Free and total PSA concentrations, as well as f/t PSA Ratio%, in serum are not affected significantly by continuous storage at -80ºC for at least 15.5 years.

| Free and Total PSA Stability Over 15.5 Years at -80 Degrees C | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Statistic | **tPSA 2001 ng/ mL** | **tPSA 2017 ng/ mL** | **Ratio tPSA 2017/ 2001** | **fPSA 2001 ng/ mL** | **fPSA 2017 ng/ mL** | **Ratio fPSA 2017/ 2001** | **f/t PSA% 2001** | **f/t PSA% 2017** | **Ratio f/t PSA% 2017/ 2001** |
| Mean | 16.32 | 16.15 | **1.03** | 1.76 | 1.70 | **0.96** | 0.13 | 0.12 | **0.94** |
| Standard Deviation | 14.58 | 13.61 | **0.09** | 2.66 | 2.64 | **0.10** | 0.10 | 0.10 | **0.10** |
| Minimum | 0.42 | 0.38 | **0.79** | 0.05 | 0.04 | **0.65** | 0.02 | 0.02 | **0.64** |
| Maximum | 75.04 | 70.07 | **1.29** | 30.18 | 29.98 | **1.36** | 0.76 | 0.84 | **1.36** |
| Range | 74.62 | 69.69 | **0.51** | 30.13 | 29.94 | **0.71** | 0.74 | 0.82 | **0.72** |

## A-053

### Vitamin D deficiency and tumor markers of ovarian cancer

J. D. Santotoribio, C. Cañavate-Solano, J. F. Cuadros-Muñoz, M. Mayor-Reyes, S. Perez-Ramos. *Puerto Real University Hospital, Cadiz, Spain*

**Introduction:** 25-OH-vitamin D or calcidiol is the best marker for measuring vitamin D levels. Most experts define vitamin D deficiency as calcidiol values <20ng/ml (<50nmol/L). There are studies that find an association between low levels of vitamin D and an increased incidence and mortality of ovarian cancer. Some clinical guidelines recommend the use of serum cancer antigen 125 (CA 125) levels for diagnosis of epithelial ovarian cancer. Serum cancer antigen 19.9 (CA 19.9) is used for the diagnosis of mucinous ovarian cancer. The aim of this study was to determine the relationship between vitamin D deficiency and the tumor markers of ovarian cancer.

**Method:** We studied asymptomatic women without history of cancer, in which we determined serum calcidiol, CA 19.9 and CA 125 levels in the same sample. Calcidiol, CA 19.9 and CA 125 were measured by electrochemiluminescence immunoassay in MODULAR E-170 autoanalyzer (Roche-Diagnostic®). The patients were classified into two groups: with vitamin D deficiency (calcidiol<20ng/ml) and without vitamin D deficiency (calcidiol>20ng/ml). The statistical analysis was performed using the MedCalc® software.

**Results:** 72 women aged 40-86 years (median=62 years); 26 with vitamin D deficiency (group 1) and 46 without vitamin D deficiency (group 0) were included in this study. The median and the range of the variables are shown in the following table:

| Group | n | Age | Calcidiol | CA 19.9 | CA 125 |
|---|---|---|---|---|---|
| **0** | 46 | 62 years (40-84) | 29.9 ng/mL (21.1-60.5) | 8.21 U/L (0.66-28.41) | 10.73 U/L (5.58-26.30) |
| **1** | 26 | 67 years (48-86) | 16.1 ng/mL (4.8-19.6) | 9.94 U/L (0.60-27.41) | 16.07 U/L (7.44-28.25) |

Mann-Whitney U test showed statistically significant differences between the two groups with CA 125 levels (p=0.0016). No statistically significant was found with CA 19.9 (p>0.05). There was an inversely proportional correlation between serum levels of CA 125 and calcidiol, Spearman's coefficient of rank correlation (rho) was -0.346 (p=0.0036). No correlation was found between CA 19.9 and calcidiol (p>0.05).

**Conclusions:** Women with vitamin D deficiency have higher serum levels of CA 125 than women without vitamin D deficiency. Vitamin D deficiency could be an important data for the diagnosis and management of ovarian cancer.

## A-054

### Pleural fluid homocysteine and carcinoembryonic antigen for diagnosis of malignant pleural effusion

J. D. Santotoribio[1], L. del Valle-Vazquez[2], C. Cañavate-Solano[1], A. Garcia-de la Torre[3], J. F. Cuadros-Muñoz[1], S. Perez-Ramos[1], M. J. Sanchez del Pino[4]. *[1]Puerto Real University Hospital, Cadiz, Spain, [2]Health Center Las Beatas, Sevilla, Spain, [3]Virgen de la Victoria University Hospital, Malaga, Spain, [4]University of Cadiz, Cadiz, Spain*

**Background:** Analysis of tumor markers (TM) in pleural fluid is a non-invasive technique for identify of malignant pleural effusion (MPE). TM commonly used in pleural fluid are Carcinoembryonic Antigen (CEA), Cancer Antigen (CA) 15.3, CA 125 and CA 19.9. Pleural fluid homocysteine (HCY) has been proposed as a TM with high accuracy for diagnosis of MPE. The aim of this study was to develop a multivariate model with pleural fluid TM for the diagnosis of MPE.

**Methods:** We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. Pleural fluid CEA, CA 15.3, CA 19.9 and CA 125 were measured by electrochemiluminescence immunoassay in modular E-170 (Roche Diagnostic®) and pleural fluid HCY by nephelometry in BNII (Siemens Diagnostic®). Patients were classified into two groups according to the aetiology of pleural effusion: benign pleural effusions (BPE) and MPE. Pleural effusion was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. All variables were included in a multivariate regression analysis to identify variables independently associated with MPE.

**Results:** We studied 60 patients with ages between 1 and 96 years (median = 65.5 years), 28 women and 32 men. Thirty-one were BPE (12 trasudates, 7 parapneumonic, 3 tuberculosis and 9 other benign aetiology), and 29 were MPE (11 lung cancer, 4 breast cancer, 2 lymphomas, 2 mesotheliomas and 10 other tumors). The multiple regression analysis identified HCY and CEA as variables independently to develop the multivariate model, excluding the others TM. The multivariate model was:

HCY+CEA = -7.8646 + 0.5154 x HCY + 0.3927 x CEA

AUC, optimal cut-off value, sensitivity and specificity of pleural fluid TM and multivariate model are shown in the following table (CI: confidence interval):

| | AUC (95% CI) | Cut-off | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|
| **HCY+CEA** | 0.953 (0.865-0.990) (p<0.0001) | 0.3352 | 82.8 % (64.2-94.1) | 93.5% (78.5-99.0) |
| **HCY** | 0.859 (0.745-0.935) (p<0.0001) | 13.60 µmol/L | 62.1% (42.3-79.3) | 90.3% (74.2-97.8) |
| **CA 15.3** | 0.819 (0.698-0.906) (p<0.0001) | 31.00 U/L | 55.2% (35.7-73.5) | 100% (88.7-100) |
| **CEA** | 0.766 (0.639-0.865) (p<0.0001) | 4.38 ng/mL | 58.6% (38.9-76.5) | 93.5% (78.5-99.0) |
| **CA 19.9** | 0.687 (0.555-0.801) (p=0.0066) | 4.77 U/L | 48.3% (29.5-67.5) | 90.3% (74.2-97.8) |
| **CA 125** | p>0.05 | - | - | - |

**Conclusions:** The combination of pleural fluid HCY and CEA was the best model to predict whether a pleural effusion is benign or malignant.

## A-055

### Sensitive and Specific Detection of Variants in Circulating Tumor DNA by Anchored Multiplex PCR and Next-Generation Sequencing

J. E. Lee[1], N. Manoj[1], J. Haimes[1], S. J. Mishkin[1], P. G. Roberts[1], E. M. Davis[1], I. McKittrick[1], S. Elmore[2], L. M. Griffin[1], R. D. Walters[1], B. A. Kudlow[1], M. L. Gulley[2], B. P. Culver[1]. *[1]ArcherDX, Boulder, CO, [2]University of North Carolina School of Medicine, Chapel Hill, NC*

**Introduction:** Liquid biopsies are a promising, minimally invasive alternative to tissue biopsies that have potential cost, time and safety benefits, as well as a greater ability to interrogate heterogeneous tumors. However, except in advanced disease states, cell free DNA (cfDNA) is typically of low abundance and only a small portion of cfDNA originates from tumor cells as circulating tumor DNA (ctDNA), which tends to be highly fragmented (100-300bp). Therefore, NGS-based assays to detect variants in ctDNA must be sensitive enough to detect mutations at allele frequencies (AF) <2% from <100ng of highly fragmented DNA.

**Methods:** We developed the Archer® Reveal ctDNA™ 28 assay based on Anchored Multiplex PCR (AMP™), a target enrichment method for NGS that uses unidirectional gene-specific primers and molecular barcoded (MBC) adapters for amplification. AMP is well suited to amplify small cfDNA fragments, as it only requires one intact

primer-binding site within a fragment. Single primers capture target regions from both strands independently, increasing the sensitivity of variant detection from low-input samples. MBC adapters ligated prior to amplification permit post-sequencing error correction, reducing background noise and increasing analytical sensitivity of ultra low-allele frequency variant detection. Finally, variant filtering in the Archer Analysis pipeline further increases the specificity of variant calls.

**Results:** Using commercially available reference ctDNA standards, we demonstrate that genomic DNA present in plasma does not significantly impact amplification of small, fragmented ctDNA with the AMP-based Reveal ctDNA 28 assay. Based on sequenced reads, AMP enabled interrogation of more than 65% of the input molecules from 50ng starting material. As a result, we show 100% detection sensitivity for 1% AF variants using 10ng DNA input and 71.9% detection sensitivity for 0.1% AF variants using 50ng DNA input. MBC-enabled post-sequencing error correction and variant filtering reduced the number of false positives by 98%, resulting in 91.7% specificity. Finally, mutations detected from liquid biopsy-derived ctDNA showed cancer type-dependent concordance with tissue biopsy findings, and revealed additional oncogenic driver mutations.

**Conclusions:** The Archer Reveal ctDNA 28 assay is a powerful tool for sensitive and specific NGS-based detection of variants in ctDNA, demonstrating accurate allele frequency quantification of synthetic reference standards. This assay is a promising approach to characterize solid tumors from liquid biopsies, showing cancer type-dependent concordance of tissue and plasma mutation profiles, as well as identification of additional oncogenic driver mutations in ctDNA.

## A-056

### Development of ctDNA Reference Materials in a Human Plasma-EDTA Matrix with low interfering endogenous DNA

L. Liu, S. Dasari, K. L. Norman. *Thermo Fisher Scientific, Fremont, CA*

**Background:** Liquid biopsy is a novel, non-invasive method for identifying biomarkers present in circulating tumor DNA (ctDNA). Recent ctDNA detection technologies could potentially revolutionize early cancer detection and therapeutic monitoring in clinical laboratory medicine. However, with the advent of a breakthrough technology comes an equally important need for novel reference materials and quality assessment schemes to ensure quality test implementation. Development of ctDNA reference materials is challenging due to the presence of endogenous, uncharacterized DNA normally present in pooled human plasma. In this study, we developed ctDNA reference materials based on the highly characterized NIST Genome in a Bottle GM24385 in low interfering endogenous DNA human plasma-EDTA.

**Methods:** Plasma was collected from long term donors and screened for the presence of blood borne pathogens and several cancer mutations. Single or double EGFR/KRAS/NRAS cancer hotspot mutations at 10%, 5%, 1% and 0% allelic frequencies in a background of the NIST genomic DNA was fragmented and spiked into the plasma. The allelic frequency was determined using BioRad® droplet digital PCR and fragment size distribution was analyzed on Bioanalyzer and TapeStation; all tests were performed in triplicate, and the mean and standard deviation were calculated.

**Results:** Plasma screened negative for all infectious agents tested as well as for cancer mutants described above. Allelic frequencies showed no difference between pre- and post-spiking into plasma, and the observed frequencies were at 10% error of the target frequencies. These data suggest that interfering endogenous plasma DNA levels were negligible, which enabled precise control of allelic frequencies and controlled definition of gDNA background. Notebly, fragmented ctDNA demonstrated a length of 162 ± 2.5 bp on Bioanalyzer, but was 205 ± 2.6 bp on TapeStation, suggesting a need for further nucleic acid sizing technology standardization.

**Conclusion:** A novel method for producing ctDNA controls has been developed that enables preparation of a multitude of target mutations at a wide range of frequencies with a known gDNA background. Simpler QC materials mimicking patient samples will enable simpler ctDNA test method development and analytical validation, which will be critical for laboratories to introduce ctDNA testing into the field.

## A-057

### Analytical validation of a blood-based colorectal cancer and advanced adenoma risk assessment LDT

R. Dillon, M. Blimline, J. You, A. Yee, J. Levy, S. Beasley, D. Cuevas, G. F. Mina, V. Chan, J. Diggs, R. B. Carino, S. N. Kairs, J. Bucci, L. J. Croner, B. E. Wilcox. *Applied proteomics, San Diego, CA*

Background: Colorectal cancer (CRC) is the second leading cause of cancer-related death in men in the United States and the third leading cause in women. With a 5-year survival rate of 90% for early-stage detection versus 13% for late-stage detection, early detection of CRC is critical to reducing associated mortality. Despite the importance of early detection, only ~60% of individuals who would benefit from screening are actually tested. The low compliance rate to colonoscopy and fecal-based screening suggests the need for an alternate blood-based test as a means to improve uptake. We describe here the analytical performance validation of a 15-analyte multiplexed assay that measures protein levels in plasma as a CRC and advanced adenoma (AA) risk assessment to symptomatic patients.

Objective: This poster describes the analytical validation of a Lead Development Test (LDT) multiplexed immunoassay as required by CAP/CLIA and performed in accordance with CLSI guidelines.

Methods: A 5-panel electrochemiluminescent (ECL) multiplexed immunoassay measuring 27 CRC-related proteins was developed on the Meso Scale Discovery (MSD) U-PLEX platform. A discovery and validation study using this 5-panel assay and a large patient population (n=4,435) with symptoms of colorectal neoplasia, revealed a 15-analyte CRC and AA classifier with CRC clinical sensitivity and specificity of 80% and 83%, respectively. The analytical performance of the 15-analyte (4-panel) classifier was further validated in accordance with CAP/CLIA requirements to demonstrate analytical performance and clinical accuracy following CLSI guidelines. A minimum of 74 plasma samples spanning the clinical reportable range (CRR) for each analyte were used to demonstrate assay ruggedness, sample stability, intra-day precision and inter-day relative accuracy. The specificity of each analyte was evaluated using plasma samples spiked with common endogenous interferants. Both the reportable and reference range for each analyte was established, prior to demonstrating clinical accuracy of the 4-panel multiplexed assay using a symptomatic patient cohort.

Results: Deming regression and Pearson correlation coefficients were used to assess the precision and accuracy of each sample measurement across assay runs. Analysis of intra-day precision and inter-day accuracy were within acceptable limits (Correlation coefficient 0.82 - 0.92) for all analytes. Assay specificity was acceptable for all analytes in the presence of a range of bilirubin and intralipid levels and only one analyte displayed interference in grossly hemolytic samples. The dynamic range of each assay spanned 2-3 logs with the LOD 1 to 4-fold below the CRR. Ruggedness was found to be acceptable across all instrument systems tested as measured by Deming regression and Pearson correlation. Sample stability was validated for up to six freeze-thaw cycles for 3 panels and up to five freeze-thaw cycles for the 4$^{th}$ panel. A Clinical accuracy study using the analytically validated multiplexed assay demonstrated greater than 80% concordance with clinical calls.

Conclusion: Analytical validation demonstrates a high quality, low-burden LDT for evaluating CRC and AA risk in a symptomatic patient population. The ability to evaluate CRC risk with the ease of a blood draw provides an important tool in helping to improve CRC screening compliance rates.

## A-058

### Troponin I levels as an early biomarker for cardiotoxicity after chemotherapy with doxorubicin in women with breast cancer

R. Simões[1], L. M. Silva[2], H. H. M. Oliveira[2], A. P. Sabino[1], K. B. Gomes[1]. *[1]Pharmacy Faculty, Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Ezequiel Dias Foundation, Belo Horizonte, Brazil*

**Background:** Cardiovascular diseases in cancer patients are frequent events due to chemotherapy, which has resulted in increased survival of patients but exposes them to cardiotoxicity. The aim of this study was to evaluate the use of Troponin I (TnI) as a marker to identify early cardiotoxicity in women with breast cancer under chemotherapy with anthracycline doxorubicin.

**Methods:** This study included 76 women (49.8 ± 11.3 years old) with breast cancer that received neoadjuvant chemotherapy with 372 mg/m$^2$ mean cumulative dose of doxorubicin, divided in 3-4 cycles. The serum TnI levels were evaluated before the chemotherapy and immediately after the treatment. We used immunometric

immunoassay kit. The sensitivity was 95% and the specificity was 93%, intra and inter-assay coefficient of variation were 4.2 and 8.3%, respectively.

**Results:** No subject presented significant decline in left ventricular ejection fraction evaluated trough echocardiogram. However, the median (interquartile-range) levels of TnI before treatment [0.012 (0.002) ng/mL] was significantly lower than TnI levels observed after chemotherapy [0.028 (0.013) ng/mL] ($p<0.0001$). **Conclusion:** The elevation in TnI levels observed in this group suggest increased risk of myocardium injury after chemotherapy with doxorubicin. The use of TnI may be important as a biomarker for early intervention for cardiotoxicity progression in patients with breast cancer receiving anthracycline therapy.

## A-059

### Genetic variants in the vascular endothelial growth factor pathway as potential markers of ovarian cancer risk, therapeutic response, and clinical outcome

L. Cao, Z. Ma, Q. H. Meng. *MD Anderson Cancer Center, Houston, TX*

**Background:** As a key regulator of angiogenesis, vascular endothelial growth factor (VEGF) plays an important role in physiology of normal ovaries and pathogenesis of ovarian cancer, such as tumorigenesis and metastasis. The objective of this study was to assess the association of genetic variants in the VEGF pathway with ovarian cancer risk, therapeutic response, and survival. **Patients and methods:** A cohort of 339 ovarian cancer patients matched with 349 healthy controls by age, gender, and ethnicity were tested for single nucleotide polymorphisms (SNPs) in VEGF and VEGF receptor (VEGFR) genes using the Illumina iSelect platform. The statistical analyses were performed using Intercooled STATA software. P-value $\leq 0.05$ was considered significant. The overall risk of ovarian cancer and likelihood of poor treatment response were estimated as odds ratios (OR) with 95% confidence intervals (95% CI) for each SNP using unconditional multivariate logistic regression. The overall risk of death was estimated as hazard ratios (HR) and 95% CIs for each SNP using the Cox proportional hazards model. **Results:** 16 SNPs from 5 genes in the VEGF-VEGFR axis were identified as significantly associated with an increased risk of ovarian cancer. Among which, VEGFR-3 rs6877011 showed the highest risk of ovarian cancer (OR, 1.66; 95% CI, 1.09-2.53), while VEGFR-1 rs11149523 showed the lowest risk (OR, 0.51; 95% CI, 0.32-0.83). 10 SNPs from 4 genes were identified as significantly associated with platinum-based chemotherapeutic response. Among which, VEGFR-2 rs1531289 was associated with a favorable treatment response (OR, 0.64; 95% CI, 0.42-0.98), and all the other variants showed a significant association with a poor chemotherapeutic response. In particular, VEGFR-1 rs8000288 showed the highest risk of poor chemotherapeutic response (OR, 4.25; 95% CI, 1.10-16.43). 12 SNPs from 4 genes were identified as significantly associated with the overall survival of ovarian cancer patients. Among which, fms like tyrosine kinase 3 (Flt3) rs3003955 was the most significant variant associated with improved prognosis (HR, 0.59; 95% CI, 0.41-0.84), while VEGFR-1 rs17626553 was the most significant one associated with poor prognosis (HR, 4.07; 95% CI, 1.86-8.92). **Conclusions:** Multiple genetic variants in the VEGF pathway significantly associated with ovarian cancer risk, therapeutic response, and survival are identified in our study. These findings may provide a potential molecular approach for ovarian cancer risk assessment, patient management and clinical outcome prediction.

## A-060

### Multi-organ Tumor Secretome Panel has Utility for Cancer Risk, Treatment and Prognosis.

M. Obrenovich, S. Emancipator, D. Anthony. *Case Western Reserve University, Cleveland, OH*

**Background:** A recognized function of the immune system is surveillance providing the host with the ability to recognize and destroy foreign or altered self, e.g., cells that undergo malignant transformation or recognized as "non-self". Hypofunctioning immune surveillance is associated with a propensity for development of malignant disease. Immunologic surveillance theory is supported by both evidence from the experimental animal as well the human and has led to an intensive research effort to develop practical modalities to prevent or treat cancer in the human by exploitation of the immune system. Significant advances in the field of tumor immunotherapy demonstrate that the immune system can selectively recognize and subsequently destroy cancer cells. Normal immune mechanisms directed against cancer cells fail due to a possible survival mechanisms used by the cancer cell to evade the immune system. Tumors are known to shed or secrete soluble factors and cytokines into the microenvironment, in particular sTNFRs, which can bind surface TNF and suppress TNF activity. **Methods**: Antibody based Elisa kits and Peptide MRM LCMS methods were used to provide data and proof of principal for our observations. Approach: The purified serum protein product (50 µg) is incubated with cleavage enzymes. Multiple reaction monitoring mode in which high signal intensity parent peptide and daughter ion pair are selected for quantification. The cleavage products were identified using purified protein by a series of experiments done by core mass spectrometry facility using HPLC-electrospray-tandem mass spectrometry (LC-MS/MS) as proof of principle and de-identified patient samples were quantitated during routine analyses and confirmed by sandwich ELISA at Louis Stokes and elsewhere. **Results:** We have preliminary data finding that sTNFR1 values are generally 1/3 the value of sTNFR2 and that sTNFR2 Marker is best associated with cancer and apoptotic events and that sTNFR 1 and 2 both increase with INFLAMMATION, with AGE and during UREMIA and that sTNFRs are a predictor of morbidity and mortality and finally, when both sTNFR 1 and 2 are above 4,000 ug/ml the patient is at serious risk for adverse outcomes including death. Table 1. The average and normal ranges for the respective sTNFR isotypes under various collection conditions and biologic fluid type. *The urine values (n=33) for sTNFR I and (n=27) for sTNFR II are not normalized for creatinine content and medical histories for the "normal" range are not known for this data set. **Conclusion:** The data for two of our panel markers can be concluded to demonstrate that the responses exhibited linear function. Standard curves are plotted to show the level of detection as a function of the Spiked-in amount in fmol with $R^2$ of 0.997, 0.999, and 0.998, respectively and the linearity of the standard curves along with tissue samples for all three MRM peptides. Preliminary data suggest the limits of quantifications (LOQ) for all peptides was 50 amole/uL with a signal to noise ratio greater than 10.We have monitored sTNFR in deidentified cancer patients as compared to patients with ought know cancer and found a remarkable ability to detect various cancer types from any random population pool.

## A-061

### A new blood test for colorectal cancer in high-risk subjects

L. J. Croner, A. Kao, R. Benz, J. E. Blume, R. Dillon, B. Wilcox, S. N. Kairs. *Applied Proteomics, Inc, San Diego, CA*

**Background**

Colorectal cancer (CRC) is a broadly occurring and lethal cancer. CRC outcome dramatically improves with early detection and curative resection; thus CRC screening is recommended for U.S. patients over 50 years old. The gold standard screening test is colonoscopy, with some stool-based tests also having good performance. However, compliance with CRC screening recommendations is low; only about 60% of the over-50 population undergoes testing.

A low-burden CRC test for non-compliant patients, such as a blood-based test, has been widely sought. Signal may be stronger in patients with developed disease, such as those with symptoms of colorectal neoplasia, than in average risk patients. If so, the appearance of symptoms would offer an opportunity to provide low-burden CRC testing with higher performance; the results could be used to direct only the highest risk patients to colonoscopies.

**Objective**

The objective was to develop a blood-based CRC test with clinically useful performance in patients with CRC symptoms. This was achieved by building a classifier using samples from a truly representative intent-to-test (ITT) symptomatic population, without filtering based on sample type.

**Methods**

*Subjects.* 4,435 patient samples were drawn from the *Endoscopy II* sample set. Samples were collected at seven hospitals across Denmark between 2010 and 2012 from subjects with symptoms of colorectal neoplasia: abnormal bowel habits, abdominal pain, rectal bleeding, unexplained weight loss, meteorism, anemia, and/or palpable mass. Colonoscopies revealed the presence or absence of CRC.

*Candidate biomarkers.* 27 blood plasma proteins were selected as candidate biomarkers based on a previous targeted-mass spectrometry study. Multiplexed immunoassays were used to measure the concentration of these 27 proteins in all 4,435 samples. Age and gender were also considered biomarkers.

*Classifier discovery and validation.* 3,066 patients (340 CRC and 2,759 non-CRC) were randomly assigned to the classifier discovery set. Machine learning was used to build and test candidate classifiers in this set. A grid approach was used to examine many combinations of data pre-treatment, predictor selection methods, predictor numbers, and classifier algorithms. For each combination, 10-fold cross-validation was used to assess diagnostic performance. Some classifiers were refined by allowing up to a 25% indeterminate score range. The classifier that gave the best performance was selected for validation.

The remaining 1,336 samples (147 CRC and 1,189 non-CRC) were assigned to the validation set. The classifier selected during discovery was applied to these samples for final performance characterization.

**Results**

The final classifier was a logistic regression using 10 predictors : eight proteins, age, and gender. In validation, the indeterminate rate was 23.2%, sensitivity/specificity was 0.80/0.83, the PPV was 36.5%, and the NPV was 97.1%. This performance compares favorably to that from other CRC blood tests.

**Conclusions**

The validated classifier presented here can serve as the basis for a blood-based CRC test for symptomatic patients. Results from a test using this classifier can help assess symptomatic patients' CRC risk, increase their colonoscopy compliance, and manage next steps in their care.

## A-062

### The Potential for Ubiquitin Occupancy Profiles in Cancer Biomarker Screening

V. Hristova, H. Zhang, D. Chan. *Johns Hopkins University School of Medicine, Baltimore, MD*

**Background:** Post-translational modification (PMT) of oncoproteins can provide a detailed profile of abnormal proliferative signaling in cancer. The pattern of disease specific PTMs can serve as a biomarker for screening, diagnosis and treatment assessment. Ubiquitin PTM is primarily responsible for protein degradation by the 26S proteasome and impaired ubiquitin signaling has been implicated in numerous cancers. Ubiquitination also has non-degradation regulatory functions that contribute to the overall ubiquitin profile of cells and can be utilized in diagnostic screening. This discovery study examines ubiquitin occupancy, the presence or absence of ubiquitin modification, across the proteome of an ovarian carcinoma cell line. In addition to providing a unique ubiquitin occupancy profile this project has led to the discovery of novel ubiquitination sites in the HER2 oncoprotein. The primary objective of this project is to utilize stable isotope labeling by amino acids in cell culture (SILAC) and LC-MS/MS to characterize the ubiquitin occupancy profile of SKOV-2 ovarian carcinoma cells regardless if the PTM mediates protein degradation or is involved in other regulatory functions. The second aim of study focuses on the novel ubiquitination sites identified in HER2 and addresses their specificity to ovarian cancer.

**Methods:** SKOV3 cells are cultured in RPMI media with 10% FBS (Light) and SILAC RPMI media containing $^{13}C_6$-L-lysine and $^{13}C_6{}^{15}N_4$-L-arginine (Heavy) media supplemented with 10% FBS. Light cells are treated with MG132 proteasome inhibitor as well as DMSO (control). Both Light and Heavy cells are harvested 6 hours post-treatment and lysed in urea buffer. Protein concentration is determined for each sample and the Heavy and Light lysates are combined at a 1:1 ratio for each treatment. The samples then undergo reduction and alkylation, followed by trypsin digestion, and offline basic reversed phase (bRP) fractionation (Global samples). A sub-faction of the peptides undergoes further K-ε-GG ubiquitin remnant motif peptide enrichment, following the initial bRP fractionation step (Ubiquitin-enriched samples). Samples are subjected to LC-MS/MS using an Orbitrap mass spectrometer, and protein identification and quantification are conducted using MaxQuant. Additionally HER2 co-immunoprecipitation is used to identify changes in HER ubiquitination and examine HER binding-proteins under varying treatment conditions.

**Results:** Proteasome inhibition by MG132, shows overall enhanced protein up-regulation in Global and Ubiquitin-enriched samples compared to DMSO. Ubiquitin occupancy at all protein sites identified in the proteome is assessed and four HER2 ubiquitinated peptides exhibit a significant increase with proteasome inhibition. Close to 45% of all ubiquitinated peptides do not exhibit increased levels with MG132 inhibition, indicating ubiquitin modification at these lysine residues serves a non-degradation function. Ubiquitin occupancy is assessed by comparing ubiquitin-enriched and global peptides and provides a means of differentiation between protein targets modified by ubiquitin for turnover and those ubiquitinated for trafficking or other non-proteasome signaling.

**Conclusion:** Protein ubiquitination in SKOV3 ovarian carcinoma cells can be classified into degradation and non-degradation signaling functions. The ability to distinguish these proteins by SILAC-based quantitative proteomics allows

assessment of ubiquitin occupancy and generates a cell specific ubiquitin profile that can be utilized in assessing abnormal proliferative signaling.

## A-063

### Study of Tumor Markers in maintenance hemodialysis patients in Eastern India

A. Hazra[1], S. Mandal[2], J. Chakraborty[3], J. Datta[4]. *[1]Christian Medical College (CMC), Vellore, TN, India, [2]AIIMS Jodhpur, Jodhpur, Rajasthan, India, India, [3]ESI PGIMSR Manicktala, Jodhpur, Rajasthan, India, India, [4]ESI PGIMSR, Manicktala, Kolkata, WB, India*

BackgroundTumour markers are routinely used for screening, diagnosis and follow up of certain tumours. Renal failure is suspected to cause decreased excretion of some but not all tumor markers. Tumor markers reference ranges need to be thoroughly explored in renal failure patients.

Method 123 patients on maintenance hemodialysis in a hospital in eastern India were tested for the tumor markers CA19.9, PSA (Total & Free), CA125, AFP, CEA, CA15.3 by chemiluminescence immunoassay on Beckman Coulter Access 2 and qualitative Anti-HCV screen by ELISA. Patients had no known history or signs of malignancy. R version 3.1.3 was used for the statistical analysis.

Result A significant number of patients had a raised value for the tumor markers. About 66% patients had raised CEA, 25% raised CA125, 15% raised CA19.9, 9% raised CA15-3, 7% raised total PSA and only 2% raised AFP levels. These increase could not be attributed to hepatitis C or smoking. Only one of these patients had HCV and she did not have high levels of the markers. Free PSA was detectable in about 80% of all patients but in patients who had high total PSA the free to total PSA % ratio was not of the "high risk" type ie never below 10% and about half of them had the ratio >25%.

Conclusion Two third of hemodialysis patients have significantly high level of CEA whereas most of these patients do not smoke thus ruling out a confounding factor. Similarly the high CA125 and CA15.3 could not be attributed to any peritoneal or liver pathology eg hepatitis C. Thus majority of the traditional tumor markers we tested seemed to be raised in a significant proportion of hemodialysis patients except AFP, and thus all these markers should be interpreted with caution in these settings.

Tumor marker quantile distribution in hemodialysis patients

| | mean (sd) | IQR | 0% | 25% | 50% | 75% | 100% | Reference cut off | False positive rate |
|---|---|---|---|---|---|---|---|---|---|
| AFP | 3.26 (2.03) | 2.51 | 0.54 | 1.83 | 2.68 | 4.34 | 10.73 | 9 ng/ml | 2% |
| CA 19-9 | 22.51 (42.96) | 17.75 | 0.80 | 5.05 | 10.00 | 22.80 | 389.00 | 35 U/ml | 15% |
| CA 125 | 40.36 (83.16) | 27.28 | 0.70 | 5.50 | 12.45 | 32.78 | 518.30 | 35 U/ml | 25% |
| CA 15-3 | 16.41 (14.10) | 12.58 | 1.50 | 7.95 | 13.05 | 20.53 | 92.40 | 31.3 U/ml | 9% |
| CEA | 4.33 (3.14) | 3.31 | 0.23 | 2.10 | 3.76 | 5.41 | 20.80 | 3 ng/ml | 66% |
| tPSA | 0.71 (1.42) | 0.75 | 0.00 | 0.01 | 0.25 | 0.76 | 8.12 | * | 7% |
| fPSA | 0.24 (0.43) | 0.26 | 0.00 | 0.01 | 0.13 | 0.27 | 2.67 | ** | 7% |

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Cardiac Markers

## A-064

### The Effect of Heparin on Measurement of High Sensitivity Cardiac Troponin I: Influence on Diagnostic Misclassification at Decision Thresholds.

M. E. Lyon[1], P. A. Kavsak[2], A. W. Lyon[1]. *[1]Saskatoon Health Region, Saskatoon, SK, Canada, [2]McMaster University, Hamilton, ON, Canada*

BACKGROUND: The combined influence of anticoagulant bias and imprecision on hs-cTnI measurement on test interpretation is unknown. OBJECTIVES: 1) Determine the effect of heparin on the measurement of hs-cTnI. 2) Audit concentration of heparin in hs-cTni specimens. 3) Simulate the effect of heparin-bias on misclassification error at hs-cTnI thresholds. METHODS: Serum pools (9, 35, 77 ng/L) were prepared with patient specimens. Aliquots were dispensed into BD PST Lithium Heparin (n=5) and hs-cTnI was measured using the Abbott ARCHITECT i2000. Heparin concentrations were determined gravimetrically. Monte Carlo error simulation models were used to assess the misclassification rate at hs-cTnI thresholds with heparin-biases observed clinically and imprecision (0-20%). RESULTS: Heparin concentration demonstrated a negative troponin-dependent bias on measurement of hs-cTnI: (9ng/L cTnI, y= -0.006x + 9.32; 35 ng/L cTnI, y=-0.012x + 35.56; 77ng/L cTnI, y=-0.041x + 76.99). Whole blood heparin concentrations in hs-cTni specimens (n=167) ranged from 14.4 IU/ml to 96.6 IU/ml due to incomplete tube filling. Simulations predicted the combined influence of assay imprecision and heparin-bias on misclassification would be greatest at the 52 ng/L threshold: At CV=6%, a false-negative misclassification of 0.5% with 95 IU/mL heparin, and false-negative misclassification of 0.2% in the absence of heparin. CONCLUSION: Heparin potencies observed in clinical practice have a negative bias on hs-cTnI measurements. The influence of heparin is dependent upon troponin concentration and the predicted effect of heparin on hs-cTni misclassification is slight and unlikely to influence clinical decisions.

## A-065

### Measurement of intact fibroblast growth factor 23 in patients with heart failure with reduced ejection fraction

D. Gruson, B. Ferracin, S. Ahn, M. Rousseau. *Cliniques Universitaires St Luc, Bruxelles, Belgium*

**Background:** Biomarkers can contribute to the prognostication of patients with heart failure (HF) and to implementation of more tailored based approach. Fibroblast growth factor 23 (FGF-23) is the most potent phosphaturic hormone and also regulates bone and mineral metabolism. FGF-23 is a strong and independent factor of adverse cardiovascular events and death in HF patients. However, most of the studies were based on the measurement of C-terminal FGF23 and only few have investigated the concentrations of intact FGF-23 (iFGF-23) in HF. We determined the circulating levels of iFGF-23 in patients with HF with reduced ejection fraction (HFrEF) as well as its relation with cardiac biomarkers and adverse cardiovascular events. **Methods:** One hundred thirty three chronic HF patients (females n=31; males n=102; NYHA II-IV; mean age: 67 years; etiology: ischemic n=92, dilated cardiomyopathy n=41; mean EF: 23 %) were enrolled in the study. The primary outcome was CV death. Levels of iFGF-23 were measured at baseline with a recently released fully automated and sensitive immunoassay. The 95th percentile of the reference interval of this assay is 81 pg/mL. Levels of 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D (1,25(OH)2D), PTH(1-84), B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP), soluble ST2 (sST2) and Galectin-3 (Gal-3) were also determined. **Results:** The median plasma level of iFGF-23 was 73 pg/mL and 56 patients (42%) had values higher than the 95th of the reference interval. HF patients NYHA III-IV have significantly higher iFGF23 (81 pg/mL) than NYHA II (57 pg/mL). Concentrations of iFGF23 were not significantly different between dilated and ischemic cardiomyopathies (67 vs. 77 pg/mL). Intact FGF23 correlated with left ventricular ejection fraction (r = -0.18; P = .04), estimated glomerular filtration rate (eGFR; r = -0.43; P < .001), PTH(1-84) (r = 0.41; P < .001), (1,25(OH)2D) (r = -0.46; P < .001), Gal-3 (r = -0.39; P < .001) but not with age, (25OHD), BNP, NT-proBNP or sST2. After 8 years of follow-up, 106 patients reached the primary endpoint. Concentration of iFGF23 was significantly higher in HF patients who died in comparison to survivors (87 vs 57 pg/mL). In patients with eGFR >60 mL/min levels of iFGF23 remain associated to adverse cardiovascular events. **Conclusions:** Intact FGF23 is a strong and independent predictor of cardiovascular mortality in chronic HF.

## A-066

### Shorter Telomeres are Associated With Low Levels of Human Telomerase Reverse Transcriptase and Fetuin-A In Patients With Acute Myocardial Infarction

O. A. Mojiminiyi, R. Al Khaldi, N. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

**Background:** Telomeres, which are tandem repeats of DNA sequences at the end of eukaryotic chromosomes, have been linked to age related conditions and diseases including cardiovascular diseases (CVDs), Telomere lengths are maintained by telomerase and leukocyte telomere length (LTL) has been shown to be a marker of cell senescence and CVDs risk. Leukocyte telomerase activity has been associated with the presence and progression of calcified atherosclerotic coronary plaque in patients with short LTL. Fetuin-A, an anti-inflammatory glycoprotein synthesized in the liver, inhibits apoptosis of vascular smooth muscle cells and prevents heterotropic calcification. Low Fetuin-A concentration has been shown to be associated with overproduction of inflammatory cytokines, mitral and aortic calcification and cardiac fibrosis. As short LTL and low telomerase have been implicated in the development of atherosclerotic processes, this study explores potential associations between LTL, human Telomerase Reverse Transcriptase (hTERT) and Fetuin A as potential disease markers in patients with Acute Myocardial Infarction (AMI). **Materials and Methods:** LTL, hTERT, Fetuin A, Troponin I, and lipid profile were measured in 144 consecutive patients with increased Troponin I and symptoms suggestive of AMI and 192 age, gender and ethnicity matched healthy control subjects. AMI was diagnosed according to National Academy of Clinical Biochemistry (NACB) guidelines **Results:** AMI patients had significantly shorter (mean ± SD) LTL and lower (mean ± SD) hTERT levels compared to controls - [0.9±1.1 vs 4.5±3.9, $p$<0.0001] and [23.0±5.5 ng/ml vs 32.9±8.9 ng/ml, $p$<0.0001] respectively. Serum levels of Fetuin A were significantly ($p$<0.0001) lower in patients with AMI compared to control subjects - [31.9±9.5 ng/ml vs 38.5±12.9 ng/ml]. LTL correlated negatively with Troponin I and Fetuin -A levels (r = -0.13, $p$=0.02, r = -0.36, $p$=0.002). hTERT levels correlated negatively with Troponin I (r = -0.19, p=0.003) and Fetuin -A (r = -0.28, p=0.004). Binary logistic regression analysis showed that the odds ratio (OR) of having shorter LTL and lower hTERT were 2.1 (95%CI 1.5-6.2) and 1.6 (95%CI 1.2-8.8) respectively in patients with AMI compared to apparently healthy control subjects. **Conclusions:** We postulate that lower levels of hTERT and Fetuin A could predispose to heterotropic calcification in coronary-arteries. Short LTL and lower levels of hTERT and Fetuin A are risk factors that may play significant roles in the pathogenesis of AMI. Estimation of LTL, hTERT and Fetuin A could be useful adjuncts for the identification of patients with high risk of CVDs. .

## A-067

### Diagnosis based on admission or rapid serial measurements using the high sensitivity Abbott cardiac troponin I assay.

P. O. Collinson[1], D. Gaze[2], S. Goodacre[3]. *[1]St George's Hospital, London, United Kingdom, [2]Westminster University, London, United Kingdom, [3]University of Sheffield, Sheffield, United Kingdom*

Objectives: To examine diagnostic and prognostic efficiency of admission and rapid serial measurements of a high sensitivity cardiac troponin I (cTnI) assay.

Methods: Samples analysed were from the point of care arm of the RATPAC trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers), set in the emergency departments of six hospitals. Prospective admissions with chest pain and a non-diagnostic electrocardiogram were randomised to point of care assessment or conventional management. Blood samples were taken on admission and 90 minutes from admission.. Samples were initially analysed using the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30-50,000 ng/L 10% CV 60ng/L 99$^{th}$ percentile 70 ng/L. An additional blood sample was taken at admission and 90 minutes from admission, separated and the serum stored frozen until subsequent analysis for cTnI by using the Architect hsTnI (Abbott Diagnostics), range 1.1-50,000 ng/L 10% CV 4.7ng/L.

The universal definition of myocardial infarction (MI) utilising laboratory measurements of cardiac troponin performed at the participating sites together with

measurements performed in a core laboratory was used for diagnosis. Myocardial infarction was diagnosed by the combination of a delta troponin plus a value exceeding the 99th percentile. All patients were followed up to 30 days for major adverse cardiac events (MACE).The following diagnostic strategies were examined: the admission sample only using discriminants of 3 and 5 ng/L and the 99th percentile (26 ng/L); serial measurements using the peak value and the same discriminants plus additional use of an absolute delta value corresponding to a clinically significant change within the reference interval at 10% imprecision (8.7 ng/L).

Results: 290 patient samples were available (175 male, median age 54.3 years, range 23.7-90.6) with 180 serial samples. The incidence of MI was 26/290 (9%) and MACE 7/290 (2.4%). For admission measurement MI was excluded as follows: 3 ng/L 220/290 (75.9%); 5 ng/L 233/290 (80.3%) with no missed MI at either discriminants; 26 ng/L 261/290 (93.1%) with 9 missed MI. For patients below the diagnostic discriminant, there was no MACE at the 3 ng/L and 5 ng/L cut-offs and 3 MACE at the 26 ng/L cut-off. Serial sampling did not improve diagnostic sensitivity for rule out compared to admission sampling alone. Serial sampling detected 6 additional cases where the initial sample had a value between 3 and 26 ng/L. In patients with troponin above the low-level discriminants (3 ng/L or 5 ng/L) a positive delta value was seen in 4/6 patients with MI and 4/45 (>3ng/L) and 4/27 (>5ng/L) patients without MI. A delta value improved diagnostic specificity only when combined with use of the 99th percentile as discriminant. 2 cases with MI had a delta which failed to exceed the discriminant.

Conclusion: Rule out of MI using low-level discriminants with or without a delta change was reliable. Rule in requires the use of the 99th percentile plus judicious interpretation of a delta value. Delta values alone are unreliable to confirm or exclude MI.

## A-068

### Evaluation of the European Society of Cardiology recommended rapid diagnostic algorithms in a challenging low risk cohort.

P. O. Collinson[1], D. Gaze[2], S. Goodacre[3]. *[1]St George's Hospital, London, United Kingdom, [2]Westminster University, London, United Kingdom, [3]University of Sheffield, Sheffield, United Kingdom*

Objectives: To examine diagnostic efficiency of the proposed European Society of Cardiology rapid diagnostic algorithms in a challenging low risk cohort.

Methods: Samples analysed were from the point of care arm of the RATPAC trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers), set in the emergency departments of six hospitals. Prospective admissions with chest pain and a non-diagnostic electrocardiogram were randomised to point of care assessment or conventional management. Blood samples were taken on admission and 90 minutes from admission. Patients were admitted if the initial of 90 minute sample exceeded the 99th percentile for cardiac troponin I (cTnI) analysed using the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30-50,000 ng/L 10% CV 60ng/L 99th percentile 70 ng/L. An additional blood sample was taken at admission and 90 minutes from admission, separated and the serum stored frozen until subsequent analysis for cTnI by using the Architect hs cTnI (Abbott Diagnostics), range 1.1-50,000 ng/L 10% CV 4.7ng/L and high sensitivity cardiac troponin T (hs cTnT) by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Elecsys 2010, Roche diagnostics), range 3 - 10,000ng/L, 10% CV 13ng/L, 99th percentile 14 ng/L.

The universal definition of myocardial infarction (MI) utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. Myocardial infarction was diagnosed by the combination of a delta troponin plus a value exceeding the 99th percentile. The two proposed algorithms for ruling out and ruling in MI were then applied to the admission and serial samples to directly compare diagnostic efficiency of the two analytes.

Results: 276 patient samples were available (169 male, median age 54.5 years, range 23.7-90.6) with 165 serial samples. The incidence of MI was 276 (9.4%). A single measurement on admission excluded MI in 174/276 (63%) for hs cTnI with no missed cases, negative predictive value (NPV) 100% and in 219/276 (79.3%) for hs cTnT with 2 missed cases, NPV 99.1%. Serial sampling excluded 128/165 (77.6%) for hs cTnT with no missed cases, NPV 100% and 149/165 (90.3%) for hs cTnT with 1 missed case, NPV 99.3%. 27/165 (16.4%) were classed as indeterminate for hs cTnI and 8/165 (4.8%) for hs cTnT. Rule in sensitivity for hs cTnI was 100% (5/5) at 96.9% specificity with no indeterminate cases. For hs cTnT rule in sensitivity was 40% (2/5) at 96.3% specificity with 2 intermediate cases.

Conclusion: Both single measurement and serial measurement algorithms proved excellent rule out tools but the rule in algorithm was less reliable in this patient group. This probably reflects the difficulty of diagnoses in low risk patients with relatively small troponin changes.

## A-069

### Performance of body mass index and percentage of body fat to identify risk factors for cardiometabolic disease in Thai adults

P. Srisawasdi[1], S. Vanavanan[1], M. Rochanawutanon[1], B. Intachak[1], J. Kerdmongkol[1], K. Kruthkul[2], M. H. Kroll[3]. *[1]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [2]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [3]Quest Diagnostics, Madison, NJ*

**Background:** Body mass index (BMI) and percentage of body fat (PBF) are commonly accepted measures of obesity, with specified cut-offs for age, gender and race/ethnicities. Generally, Asian women have higher PBF and lower BMI than to men and other ethnic populations. We aim to compare the performance of BMI and PBF associate with obesity-related metabolic risks in the adult Thai population.

**Methods:** A total of 222 (64 men and 158 women), aged 21-79 years, outpatients were recruited. To define obesity, we used the criteria ⩾25.0 and ⩾27.0 kg/m$^2$ for BMI and ⩾35% and ⩾25.0% for PBF, for women and men, respectively. Blood samples were analyzed for total cholesterol, triglycerides, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, lipoprotein subclasses, apolipoprotein A-I, apolipoprotein B, glucose, HbA1c, insulin, adiponectin, leptin, high sensitive C-reactive protein and 25-hydroxyvitamin D.

**Results:** Among participants 42.3% were obese by PBF and 27.4% by BMI. Among those subjects normal by BMI, 25% were obese by PBF and among those normal by PBF, 7% were obese by BMI. Prevalence of metabolic syndrome was higher in subjects obese by PBF only (51.2%) than those obese by BMI only (33.3%). Obese by PBF showed greater association with the estimate of 10-year risk for coronary heart disease (Framingham risk score and The American College of Cardiology/American Heart Association guideline) than those obese by BMI. Using multivariable linear regression analysis adjusted for age and smoking, PBF showed significant associations with the lipid markers and adiponectin, while BMI associated with glucose metabolic markers in men (all $p < 0.005$). BMI was demonstrated a significant variable for lipid and glucose metabolic markers as well as adiponectin levels in women (all $p < 0.05$). There was no association between high sensitive C-reactive protein with BMI or PBF in men but there was with PBF in women. Both obesity indexes were significantly related with leptin concentration independent of gender. Cardiometabolic risk markers showed stronger association when both obesity indices were positive.

**Conclusion:** Obesity, in our Thai population, by PBF was more strongly associated with cardiometabolic risk markers than obesity by BMI; however, obese by BMI included those missed by PBF alone, thus both obesity indices provide information associated with risk factors. Because of the differences between PBF and BMI association with cardiometabolic risk markers and potentially cardiovascular disease, obesity by PBF should be included with that of BMI when screening populations for potential cardiovascular risk.

## A-070

### Characteristics of the new Beckman Coulter Access hsTnI Assay.

M. Thomas, P. Wynveen, C. Carollo-Neumann, M. Holland, S. Hausmann. *Beckman Coulter, Chaska, MN*

BACKGROUND: In order to meet new IFCC guidance new Troponin I assays should exhibit increased sensitivity (LoB, LoD and LoQ), precise measurement of cTnI concentrations in the range seen in healthy individuals and the capability to accurately detect changes in cTnI concentration within this range.

METHODS: The verification and clinical validation of the new Access hsTnI assay was performed in accordance with CLSI guidelines.

RESULTS: Beckman Coulter's new high sensitivity Access hsTnI assay exhibits superior sensitivity in comparison to other currently marketed devices with an estimated LoB of < 0.0005 ng/mL (0.5 pg/mL), LoD < 0.002 ng/mL (2 pg/mL) and 10% CV LoQ < 0.020 ng/mL (20 pg/mL). The estimated 99th percentile URL of a random healthy population is 0.032 ng/mL (32 pg/mL) determined with < 3% intra-assay and 8% Total imprecision. In addition, this new Access hsTnI assay is capable of accurately measuring 0.010 ng/mL (10 pg/mL) changes in cTnI concentrations. This Access hsTnI assay accurately measures cTnI in comparison to a currently validated device (correlation between Access AccuTnI+3 and hsTnI within 5%) and exhibits < 5% bias between sample types (serum, plasma). The assay does not exhibit cross reactivity to cardiac TnT, cardiac TnC, skeletal TnI or skeletal TnT and is robust against common interferences (400 mg/dL hemoglobin, 40 mg/dL bilirubin, 3000 mg/dL triglyceride 60 mg/mL albumin, 1000 mg/dL fibrinogen, 28.8 U/mL heparin).

CONCLUSIONS: Beckman Coulter's new high sensitivity Access hsTnI assay is highly sensitive and sufficiently accurate to precisely measure cTnI in > 90% of the normal population and meets new IFCC guidance to accurately detect changes in cTnI concentration within healthy subjects. This new assay is currently is in development, pending achievement of CE compliance and is not available for *in vitro* diagnostic use.

## A-071

### Combined troponin and CK-MB in early diagnosis of acute myocardial infarction

D. B. Laskar, J. Michl, G. Ilyas, A. Zuretti, M. R. Pincus. *Department of Pathology, State University of New York Downstate Medical Center, Brooklyn, NY*

**Background:** Despite the great advances in early diagnostics and rapid treatment, acute myocardial infarction (AMI) is the leading cause of death in the United States. Diagnostic work-up for ruling out AMI includes electrocardiogram (ECG) and serum biomarkers of cardiac injury testing. Two cardiac biomarkers of myocardial damage commonly employed are the MB isozyme of creatine kinase (CK-MB) and the inhibitory subunit of myocardial troponin (cTnI). When myocardial cells are infarcted, CK-MB and cTnI are released into the circulation at relatively high concentrations, which can be detected and quantified in patient's serum using immunoassays. Although both markers have been found to rise within the first six hours after the onset of AMI symptoms, there is some evidence that CK-MB may serve as a more reliable marker in the acute period. Our aim is to investigate the combined use of serum cTnI and CM-MB for the early diagnosis of AMI.

**Methods:** This is a retrospective, cross-sectional study looking at CK-MB and cTnI serum levels for all patients entering our emergency department (ED) for the month of January 2015 who presented for rule out AMI. We extracted CK-MB and cTnI data from our laboratory information system for which both tests were ordered and analyzed simultaneously. The records of these patients were also followed to determine the final diagnoses. We will determine the sensitivity and specificity of both tests.

**Results:** We've identified 148 patients (age range: 30 - 98 years; mean age: 64.5 ± 14.6 years; M/F: 1.3/1) who presented to our ED to rule out AMI where both CK-MB and cTnI were analyzed simultaneously. Twelve of the 148 patient cohort were found to have AMI. Of these either or both serum markers were found to be positive for eleven patients, giving a combined sensitivity of 91%. Significantly, three of the patients with negative troponins were found to be have positive CK-MB, and five other patients were found to be positive for troponin and negative for CK-MB. The remaining three patients were found to be positive for both markers. The specificity for troponin was 90% and that for CK-MB was 70%. The lower specificity for CK-MB derives from the occurrence of positive CK-MB values for patients with end-stage renal disease and/or acute exacerbations of congestive heart failure in patients who were subsequently found to be negative for AMI, findings which we are exploring further.

**Conclusion:** Our preliminary results suggest both tests should be performed on patients with rule out AMI. A positive result from either test increases the diagnostic accuracy and allows early diagnosis of this condition.

## A-072

### Red Cell Distribution Width and Cardiovascular Risk: Insights from de Longitudinal Study of Adult Health (ELSA-Brasil)

N. M. Carvalho, C. B. Maluf, S. M. Barreto, R. C. P. dos Reis, P. G. Vidigal. *Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

**Background**: Red Cell Distribution Width (RDW) is a quantitative laboratory test that measure the variability in the size of circulating erythrocytes. RDW is easily obtained with automated hematology analyzers, as part of complete blood count (CBC), and is generally used as an indicator of the differential diagnosis of microcytic anemia. Recent studies have shown that RDW is a predictive, diagnostic, and prognostic marker of mortality and cardiovascular events in general population as well as in patients with cardiovascular diseases (CVD). Although pathophysiological mechanisms are still unclear, the evidence obtained so far encourages further research on the RDW in different populations and clinical settings. The aim of this study was to investigate the relationship of the RDW with CVD risk factors included on the Framingham Risk Score (FRS), and the score itself, in participants of the Longitudinal Study of Adult Health (ELSA-Brasil). **Methods**: We used the baseline data (2008-2010) of 5136 civil servants (aged 35-74 year) enrolled in the ELSA-Brasil study. RDW were quantified by coefficient of variation of red blood cells volume (RDW-CV%) using XE 2100 D hematologic analyzers (Sysmex, Kobe, Japan), that use impedance technology to estimate particle count and volume. The population was distributed according to their exposure to different risk factors, and stratified for cardiovascular risk, based on FRS. The association of RDW with CVD risk factors was performed using the Spearman test. Multiple regression analysis was used to estimate the association of RDW measures with the FRS, after adjusting for variables that can increase the variation in the volume of red blood cells, and for variables that are not part of the FRS, but can increase CVD risk.

**Results**: RDW (adjusted $r^2$=0.517; p<0.001) was independently associated with the FRS after adjustment for age, education, skin color, body mass index, abdominal obesity, metabolic syndrom, bariatric surgery, hemoglobin concentration, mean corpuscular volume, platelets, C-reactive protein, estimated glomerular filtration rate, vitamin supplement use, dietary folate, iron and vitamin B12 intake, . It was observed that a one unit increase in RDW increases the FRS by 1.15%.

**Conclusion**: In this large cohort of free living Brazilians, ours results showed that increased RDW is independently associated with higher CVD risk based on the FRS. The investigation of inexpensive, easily obtained, and widely used markers, such as RDW, should be strongly encouraged in order to confirm its potential in predicting of adverse cardiovascular events. The prospective follow up of the participants will may help to clarify if RDW can add accuracy to the CVD risk stratification in this population.

## A-073

### Prognostic significance of Cystatin C in Acute coronary syndrome

S. Panakala[1], R. Kondreddy[1], J. R. Peela[2], A. K. Rawal[2], J. R. Kooriyil[2], M. Prasad[3], M. G. I. El Fituri[4], L. T. Peela[5], S. Shakila[6]. *[1]Department of Biochemistry,Mamatha Medical College, Khammam, India, [2]St Matthews University, Grand Cayman, Cayman Islands, [3]Narayana Medical College Hospital, Nellore,Andhra Pradesh, India, [4]Department of Global Health,College of Global Public Health,New York University, New York, NY, [5]Great Eastern Medical School, Srikakulam,Andhra Pradesh, India, [6]Department of Biochemistry,Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia*

**Back ground:** Coronary artery disease(CAD) is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium. The clinical spectrum of CAD is stable angina (SA), unstable angina (UA) and myocardial infarction (MI. ACS includes UA and MI. The frequency of ACS is extremely high among Indians; India has the highest burden of ACS in the world. The rising incidence of ACS in Indians may be associated with changes in the lifestyle, the westernization of the food practices, the growing prevalence of diabetes mellitus and probably genetic factors. In recent years cystatin C has emerged as a potential marker for cardiovascular risk and predicts the cardiovascular events. Cystatin C is a naturally occurring protease inhibitor that protects the host tissue from cysteine proteases, which is a proatherogenic factor. **Materials and Methods**:Study group comprised of 114 patients diagnosed as having ACS based on clinical and bio-chemical criteria. Control group included 66 age and sex matched subjects (non ACS cases) using the above mentioned criteria. **Results**:In this study, significant increase of mean serum cystatin C levels was observed in ACS cases than controls. Highest mean cystatin C values were observed in MI than UA.Highest mean cystatin C values were observed in ACS cases with risk factors. **Conclusion:** Cystatin C plays an important role in the development of ACS and serum cystatin C is a might have a role as a prognostic marker in patients with ACS.

## A-074

### Performance Evaluation of the Atellica® IM High Sensitivity Troponin I Assay

R. C. Payne[1], H. Zhang[1], L. Halik[1], J. Conklin[1], B. Valdivia[1], A. Chase[2], C. DiPasquale[1], F. Lu[1], J. Balderson[3], R. Gorman[3], J. Lei[1], H. Mindicino[1], C. Conarpe-Martinez[1], M. Anostario[1], R. Christenson[4], F. Apple[5], B. Plouffe[1], J. Freeman[1]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Siemens Healthcare Diagnostics, Walpole, MA, [3]Siemens Healthcare Diagnostics, Newark, DE, [4]Department of Pathology, University of Maryland School of Medicine, Baltimore, MD, [5]Hennepin County Medical Center, Minneapolis, MN*

**Introduction:** The 2015 European Society of Cardiology guidelines propose algorithms for faster rule-in or rule-out of AMI in patients and for the management of NSTEMI. High-sensitivity cardiac troponin I (cTnI) assays will more accurately and

precisely measure changes in cTnI concentrations in serial draws providing useful data to assist in identifying acute versus chronic cTnI elevations, and acceptable rule-in and rule-out performance within 1 to 3 -hours of presentation. This study evaluated the performance of Siemens High Sensitivity Troponin I (TNIH) assay[1] developed for use on the Atellica Immunoassay Analyzer[2] is presented. The assay is a dual-capture sandwich immunoassay using preformed magnetic latex particles, a proprietary acridinium ester for chemiluminescence detection, and three monoclonal antibodies.

**Methods:** The limit of blank (LoB) and limit of detection (LoD) assessments used three reagent lots on two Atellica immunoassay systems with both lithium heparin and serum matrixes and run according to CLSI EP-17A2. LoD studies collected 60 replicate measurements for each of 10 serum and 10 lithium heparin samples per lot and per system. CLSI protocol EP12-A2 was followed to compare the Atellica TNIH assay to the ADVIA Centaur TNIH assay with n = 144 AMI patient samples spanning the range of reportable results. The 99$^{th}$ percentile cutoff values were established using a well-characterized population of apparently healthy subjects (n=2007) in both lithium and serum matrixes. Clinical correlation of cTnI levels above the 99$^{th}$ percentile to adjudicated AMI diagnosis is assessed in all-comer emergency department (ED) subjects in both sample matrixes.

**Results:** The LoB was 0.58 ng/L across two Atellica Immunoassay analyzers and three reagent lots. LoD was determined to be 1.27 ng/mL. The cTnI concentration at 20% $CV_{Total}$ (LoQ) had a pooled value of 2.51 ng/L. 75% of the normal health population was above the LoD. The observed assay repeatability on the Atellica Immunoassay analyzer ranged from 4.0 to 5.4% CV, and within-lab precision ranged from 5.2 to 7.0% CV between 9 and 20 ng/L. Above 20 ng/L repeatability on the Atellica Immunoassay analyzer ranged from 0.9 to 3.2% CV, and within-lab precision ranged from 1.9 to 5.2% CV. The repeatability and within-lab precision at the pooled (female and male) 99$^{th}$ percentile (45.2 ng/L) were 2.8% CV and 3.7% CV, respectively. The 99$^{th}$ percentile observed for females is 34.11 ng//L and for males 53.48 ng/L. Method comparison between the Atellica IM TNIH and ADVIA Centaur TNIH assays yielded slopes of 104 to 109% across the three reagent lots. Clinical sensitivity and clinical specificity in pooled-genders at 1, 2, 3, and 6-9 hours post ED presentation ranged from 88 to 94% and 87 to 91% respectively.

**Conclusion:** The Siemens Atellica IM TNIH assay has a 10% Total CV at a cTnI concentration 10-fold lower than the 99th percentile. This new TNIH assay allows the establishment of gender specific 99$^{th}$ percentile cutoffs, and shows acceptable clinical utility in an all-comer ED population.

[1] Under development. Not available for sale in US. Not CE marked. The performance characteristics of this device have not been established. .

## A-075

### Diagnostic Performance of High Sensitivity Cardiac Troponin I for the Diagnosis of Myocardial Infarction

K. Schulz, y. sandoval, S. Smith, S. Love, A. Sexter, J. Nicholson, F. Apple. *Hennepin County Medical Center, Minneapolis, MN*

**Background:** The purpose of the present study was to compare the diagnostic performance of high sensitivity cardiac troponin I (hs-cTnI) vs. contemporary cTnI assays using the 99$^{th}$ percentile, alone or in combination with normal electrocardiogram (ECG), to rule-in and rule-out acute myocardial infarction (MI), including the integration of serial changes (delta) to improve the rule-in for acute MI. **Methods**: Our prospective, observational cohort enrolled consecutive patients presenting to the emergency department in whom serial cTnI measurements, using both a contemporary cTnI assay (clinically used; 99$^{th}$ percentile 30 ug/L) and a hs-cTnI (investigational; sex-specific 99$^{th}$ percentiles: male 34 ng/L, female 16 ng/L) assay (Abbott Diagnostics), were obtained on clinical indication at Hennepin County Medical Center (Minneapolis, MN, USA) (Use of TROPonin In Acute coronary syndromes [UTROPIA]; NCT02060760). Diagnostic performance for acute MI, including analyses by MI subtypes (types 1 and 2 MI), and 30-day risk stratification safety outcome of acute MI or cardiac death, were examined. **Results:** Among 1,631 patients, acute MI occurred in 12.9% using the contemporary cTnI assay and 10.4% using the hs-cTnI assay. For ruling-out acute MI, serial contemporary cTnI measurements at 0/3/6h with concentrations ≤99$^{th}$ percentile and a normal ECG had a NPV 99.5% (95% CI: 98.6-100) and sensitivity of 99.1% (95% CI: 97.4-100) for both the diagnostic and safety outcome. Conversely, serial hs-cTnI measurements at 0/3h with concentrations ≤99$^{th}$ percentile and a normal ECG had a NPV and sensitivity of 100% (95% CI: 100, 100) for both the diagnostic and safety outcome. For ruling-in acute MI, the contemporary cTnI assay had specificities of 84.4% (95% CI: 82.5-86.3) at presentation and 78.7% (95% CI: 75.4-82.0) with serial testing at 0/3/6h, improving to 89.2% (95% CI: 87.1-91.3) using a relative delta cTnI value >150%. In contrast the hs-cTnI assay had specificities of 86.9% (95% CI: 85.1-88.6) at presentation and 85.7% (95% CI: 83.5-87.9) with serial testing at 0/3h, improving to 89.3% (95% CI: 87.3-91.2) using an absolute delta hs-cTnI >5 ng/L. In early presenters (≤ 2h), using the hs-cTnI assay the sensitivity and NPV were 100% (95% CI: 100-100) at 0/3h, alone or in combination with a normal ECG for the diagnostic outcome of acute MI. **Conclusion**: Both the hs-cTnI and contemporary cTnI assays are excellent in ruling-out acute MI following consensus recommendations predicated on the use of a) serial testing and b) the 99$^{th}$ percentile upper reference limit, when used in combination with a normal ECG. Delta values improve the specificity for acute MI and varied according to whether the initial sample was normal or increased.

## A-076

### Dietary Biotin Causes a Negative Interference for Troponin in the Advia Centaur Assay

F. Fouladkou, A. Wu, K. Lynch. *San Francisco General Hospital, San Francisco, CA*

**Introduction:** Biotin, a common over-the-counter supplement, is involved as a coenzyme in several carboxylation reactions and its effects in strengthening hair and nails, controlling blood glucose levels, and easing peripheral neuropathy are well known. The recommended daily dose of biotin is 30 µg for adults. Biotin levels in the general population range from 0.3 to 1 ng/mL (1.2 to 4.3 nmol/L). Levels have been reported to reach 494.9 ± 161.0 ng/ml to 823.8 ± 303.1 ng/ml (Cmax) in 1.25 h to 1.5 h (tmax) after administration of a single dose of 100 to 300 mg in patients on high dose biotin supplementation respectively. Sufficient biotin concentrations in serum can lead to falsely increased (competitive assay) or falsely decreased (sandwich assay) results in immunoassays utilizing the biotin-streptavidin interaction.

**Objectives:** The aim of our study was to characterize the extent of biotin-mediated interference in ADVIA Centaur XP immunoassays. We studied the potential biotin interference in both competitive (free T4) and non-competitive immunoassays (troponin I and intact parathyroid hormone).

**Methods:** Biotin interference studies were performed by spiking increasing concentrations of biotin from 3-100 ng/mL (8 concentrations) into remnant serum patient pools. For troponin, 5 pools were evaluated at the following concentrations: 0.04, 0.09, 0.13, 6, and 8.5 ng/mL (99$^{th}$ percentile < 0.04 ng/mL). In addition, 18 individual remnant samples ranging from 0.05-0.27 ng/mL were evaluated after spiking in 100 ng/mL biotin. For iPTH and free T4, three and five serum pools, respectively, were evaluated as described for troponin. All measurements were performed on the ADVIA Centaur XP. A concentration differing by >10% was considered significant interference. MagnaBind™ streptavidin beads, which can be used for affinity purification of biotin labeled molecules, were evaluated in three serum pools in attempts to reverse the interferences observed. To remove the MagnaBind Beads from the suspension, an external magnetic field is used. The beads were washed three times with PBS and 70 µl of MagnaBind™ Streptavidin beads were added to serum samples and incubated on a shaker at room temperature for 1 hour. Four conditions were evaluated and compared, original sample with or without biotin and with or without Streptavidin bead treatment.

**Results:** No significant difference was observed for free T4 or iPTH after biotin treatment at all concentrations. However, significant differences (>10%) were observed for troponin with concentrations greater than 50 ng/mL in all 5 serum pools. At 100 ng/mL biotin, concentrations were decreased up to 90%. In the 18 individual patient samples with troponin levels right above the 99$^{th}$ percentile (0.05-27 ng/mL), 14 samples dropped to below the 99$^{th}$ percentile after addition of 100 ng/ml biotin. The MagnaBind™ streptavidin beads successfully reversed the biotin interference.

**Conclusions:** This study verified that the TRPI assay is sensitive to biotin interference. The magnitude of interference could have a potential clinical impact on patients taking biotin supplements. It highlights the importance of laboratory, physician, and patient awareness of potential biotin interference in immunoassays.

## A-077

### Plasma kynurenine predicts severity of heart failure and associates with established biochemical and clinical markers of disease

T. B. Dschietzig[1], K. Kellner[2], J. Ruppert[1], F. P. Armbruster[1], V. Mitrovic[3]. *[1]Immundiagnostik AG, Berlin, Germany, [2]Neuroimmun GmbH, Karlsruhe, Germany, [3]Kerckhoff Clinic, Bad Nauheim, Germany*

**Background:** Kynurenine (KYN) is a metabolite of tryptophan (TRP) produced by the enzyme indoleamine 2,3-dioxygenase. Apart from exerting effects on neuro-regulation and cancer immunology, KYN and its metabolites impact on vascular inflammation, endothelial integrity, and oxidative stress. KYN or the KYN-TRP

ratio have recently been suggested to predict cardiovascular prognosis, particularly in coronary artery disease (CAD). Data on chronic heart failure (CHF) are scarce. **Methods:** In 114 patients with diastolic or systolic CHF, we investigated a possible relation between KYN and CHF severity. KYN was determined using an ELISA (Neuroimmun GmbH and Immundiagnostik).

**Results:** Baseline KYN increased with NYHA class ($p < 0.001$) and was significantly higher in deceased versus survivors ($p = 0.01$). The discrimination between dead and alive by KYN (ROC AUC 0.70) was at least comparable to that afforded by NT-proBNP (AUC 0.66). In bivariate logistic regression, KYN was a significant predictor of death (OR 1.44 [1.03 - 2.00]) as opposed to NT-proBNP (OR 1.00). In univariate analyses, KYN associated negatively with left ventricular ejection fraction (Pearson's $r = -0.33$, $p < 0.001$), estimated GFR (Pearson's $r = -0.61$, $p < 0.001$), as well as peak oxygen uptake during exercise (subgroup of patients only) (Pearson's $r = -0.64$, $p = 0.004$); and positively with NT-proBNP (Pearson's $r = 0.52$, $p < 0.001$).**Conclusion:** This is one of the first reports to show that KYN reflects the severity of CHF and has predictive power therein. KYN furthermore associates with established markers of clinical severity, such as renal function, exercise capacity, NT-proBNP, and systolic function.

## A-078

### Development of immunoassay for the high sensitive measurement of cardiac Troponin I for LUMIPULSE® *G* systems

T. Tokunaga[1], C. Okamura[1], S. Kojima[1], V. Kostanjevecki[2], G. Jannes[2], K. Aoyagi[1]. *[1]Fujirebio Inc., Tokyo, Japan, [2]Fujirebio Europe N.V., Gent, Belgium*

**Background:** Troponin I is a subunit of the troponin (Tn) complex, which is a heteromeric protein bound to the skeletal and cardiac muscle thin filaments. The cardiac troponin I (cTnI) is expressed exclusively in heart. Normal cTnI levels in blood are very low and increase substantially with heart muscle damage. They start to rise within an hour after the onset of acute myocardial infarction (AMI), peak at approximately 24 hours and return to baseline over 7 to 10 days. Current high sensitive cTnI assays are able to detect elevated levels of cTnI within 1 to 3 hours after the onset of chest pain. Given the improved sensitivity, the longer diagnostic window and tissue-specificity, cTnI is the preferred biomarker for the detection of myocardial necrosis compared to other available biomarkers. We have developed a fully automated chemiluminescence enzyme immunoassay (CLEIA) for LUMIPULSE G systems (LUMIPULSE G1200 and LUMIPULSE G600II) for the quantitative and high sensitive measurement of cTnI. In this study, the analytical characteristics of the new Lumipulse G hs Troponin I assay were evaluated.

**Methods:** High-sensitivity cTnI assay for LUMIPULSE is a two-step sandwich CLEIA as a principal. The resulting reaction signals are derived within 30 minutes/test, and are proportional to the amount of cTnI in the serum or plasma sample allowing quantitative determination of cTnI. Analytical performances of the assay were evaluated on LUMIPULSE G1200 according CLSI guidelines.

**Results:** The detection limit (LoD) of the assay was 1.9 pg/mL, and the cTnI concentration corresponding to a total CV of 10% was 7.3 pg/mL (LoQ). Linearity was demonstrated over the range 1.0 to 43,098.1 pg/mL. The assay did not exhibit cross reactivity to cardiac TnT, cardiac TnC, skeletal TnI or skeletal TnT, and was not affected by various interferences in blood (bilirubin, hemoglobin, triglycerides, chyle, total protein, rheumatoid factor or HAMA). The assay's total imprecision was ≤ 7.2% CV. The correlation coefficient and the regression slope between serum and plasma (Li heparin, K2 EDTA or Na citrate) samples were 1.00 and ≥ 0.90 (0.96, 0.90 or 0.98), respectively. The estimated 99th percentile URL in serum for a healthy population were overall 26.9 pg/mL and, 29.4 pg/mL and 21.4 pg/mL in male and female, respectively. The imprecision estimated at the 99th percentile was ≤ 4.6%CV (calculated from the precision profile). The percentage of measurable cTnI values below the 99th percentile and above the LoD (= 2.1 pg/mL used) was 68.1%. The correlation coefficient and the regression slope of Lumipulse G hs Troponin I and ARCHITECT STAT High Sensitive Troponin I (Abbott) were 1.00 and 1.05, respectively (N=63).

**Conclusion:** The Lumipulse G hs Troponin I assay showed to be sensitive, specific and precise. The performance of the assay met current requirements for a high sensitive assay to be used as an aid in the diagnostics and risk management of acute coronary syndrome patients.

## A-079

### Hyperuricemia and clustering of cardiovascular risk factors in the Chinese adult population

J. Wu[1], L. Qiu[1], X. Cheng[1], T. Xu[2], W. Wu[1], X. Guo[1], G. Zhu[3]. *[1]Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing, China, [2]Department of Statistics, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, [3]Department of Pathophysiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China*

**Background:** Hyperuricemia is common in China and the relevance of hyperuricemia and cardiovascular disease (CVD) risk has been highlighted, but to date there has been no large-scale study for China adults covering different racial populations. The aim of this study was to estimate the current prevalence of hyperuricemia and evaluate the associations between hyperuricemia and cardiovascular risk factors (CRFs) clustering in a large sample of China adults including a plurality of ethnic minorities.

**Methods:** A cross-sectional survey in a nationally representative sample of 22983 adults aged 18 years and older was conducted from 2007 to 2011. Questionnaire data and information on anthropometric characteristics, and laboratory measurements were collected. Hyperuricemia was defined as SUA ≥ 416 mmol/L for men or SUA ≥ 357 mmol/L for women. Major CRFs including dyslipidemia, hypertension, diabetes, current smoking, and overweight were estimated and clustering of CRFs were analyzed.

**Results:** The prevalence of hyperuricemia was 13.0% (18.5% in men and 8.0% in women) among adults in China. Overall, hyperuricemic subjects had higher prevalence rates of ≥ 1, ≥ 2 and ≥ 3 CRFs clustering than non-hyperuricemic subjects (80.6%, 49.4% and 21.8% vs. 81.1%, 54.8% and 24.8% in men; 80.6%, 49.4% and 21.8% vs. 58.1%, 27.2% and 8.6% in women). Hyperuricemia is positively associated with dyslipidemia, hypertension and overweight in both men and women. Furthermore, the odd ratio and 95%CI of hyperuricemia with 1, 2 and ≥ 3 CRFs were 1.22(1.03-1.45), 2.03(1.72-2.40) and 3.04(2.57-3.59) for men, and 1.49(1.22-1.83), 2.49(2.01-3.07) and 4.06(3.23-5.11) for women, respectively.

**Conclusion:** A high prevalence of hyperuricemia and CVD risk factors clustering are common among Chinese adults. Coexistence of more CVD risk factors was associated with significantly increased risk of hyperuricemia and a stronger association of hyperuricemia with CVD risk factors clustering was found in females than in males. Guidance and effective lifestyle intervention are required to prevent hyperuricemia and reduce the cardiovascular disease risk in China.

## A-080

### Cardiac Troponin I Measured with the Singulex Sgx Clarity™ cTnI Assay: A Step Forward on High Analytical Sensitivity

Á. García-Osuna[1], A. Alquezar-Arbe[2], A. Duran-Cambra[3], M. Grau-Almirall[1], R. Perez-Calleja[1], U. Enkhbayar[4], J. Todd[4], A. Sionis[3], J. Ordonez-Llanos[1]. *[1]Department of Clinical Biochemistry. Hospital Santa Creu i Sant Pau, Barcelona, Spain, [2]Department of Emergency. Hospital Santa Creu i Sant Pau, Barcelona, Spain, [3]Department of Cardiology. Hospital Santa Creu i Sant Pau, Barcelona, Spain, [4]Singulex, Inc., Alameda, CA*

Background: High-sensitivity cardiac troponin (hs-cTn) assays measure the 99th reference percentile (p99) value with an appropriate low imprecision. The improved analytical sensitivity of hs-cTn assays permits to observe cTn kinetics in shorter periods than with previous assays. The Sgx cTnI Assay (Singulex Inc., Alameda, CA), currently for investigational use only, uses the Single Molecule Counting technology for cTnI detection at concentrations otherwise undetectable with previous assays. Aim: We calculated the p99 and evaluated the clinical performance of the Sgx cTnI Assay in chest pain (CP) patients. Methods: The hs-cTnI assay was run on the Sgx Clarity™ System. The limits of blank (LoB) and detection (LoD) were preliminary assessed by the manufacturer to be 0.02 and 0.08 pg/mL, respectively. The limit of quantitation (LoQ) was 0.53 pg/mL at 10% CV and 0.14 pg/mL at 20% CV.The percent hs-cTnI healthy-subject detection was analyzed in 359 self-declared healthy blood donors; those with antecedents of cardiovascular risk factors or diseases were excluded. The p99 was calculated with a non-parametric method. Hs-cTn was measured in 144 CP patients prospectively admitted to our emergency room; patients with ST-elevation myocardial infarction were excluded. Sgx cTnI and hs-cTnT (Roche Diagnostics) were measured at admission (0h) and 1h later. Sgx cTnI concentrations were evaluated using the p99 values obtained in the healthy population. Hs-cTnT concentrations were

evaluated using the cutoff values proposed in the 0-1h algorithm of the 2016 European Society of Cardiology (ESC) guidelines. Final diagnoses were adjudicated by two independent physicians. Results: Median age of healthy donors was 47 years (range 18-65 years), 49.9% were female and Sgx cTnI ranged between 0.06 and 10.9 pg/mL. Based on the observed preliminary LoD, Sgx cTnI was detectable in 99.5% of healthy subjects. The p99 of the overall population was 6.74 pg/mL (obtained at 1.8% CV), but sex differences existed: the p99 was 3.35 pg/mL in females (3.1% CV) and 9.24 pg/mL in males (1.34% CV). Median age of CP patients was 70 years (range 26-100) and 63.8% were males. Smoking, hypertension, diabetes, dyslipidemia, and renal dysfunction existed in 17%, 60%, 33%, 58%, and 12% of the patients, respectively. NSTEMI was diagnosed in 10.6% and unstable angina in 4.9% of the cases. Coronary angiography was performed in 14.9% of the patients. At 0-1h, 68% of patients showed Sgx cTnI values below the respective p99, whereas only 54% showed hs-cTnT below the limits proposed in the ESC guideline ($p$<0.001). Only one patient, ruled out by both assays, was diagnosed with NSTEMI, resulting in a negative predictive value (NPV) of 98.95% for both assays. Conclusions: The Singulex assay measured the cTnI p99 with an extremely low imprecision, was detectable in nearly 100% of healthy donors, although sex differences existed in the p99 values. In patients with CP NSTEMI, the Sgx cTnI Assay increases significantly the proportion of patients showing values below the limits of clinical decision at admission and 1h later, allowing the use of rapid rule-out strategies. Although more clinical studies are required, the Sgx cTnI Assay showed promising features for the evaluation of myocardial injury.

## A-081

### Carboxy-terminal Fragment of Insulin-like Growth Factor Binding Protein-4 (CT-IGFBP-4). A New Biomarker for Assessing Future Risk in ST-elevation Myocardial Infarction (STEMI)?

Á. García-Osuna[1], J. Sans-Roselló[2], L. C. Belarte-Tornero[2], A. Ferrero-Gregori[2], A. Sionis[2], J. Ordonez-Llanos[1]. *[1]Department of Clinical Biochemistry. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [2]Department of Cardiology. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

**BACKGROUND** One of the more life-threatening presentations of acute coronary syndromes (ACS) is the ST-segment elevation myocardial infarction (STEMI). Patients that survive are still at high risk of future cardiovascular events and could take advantage of more refined risk stratification. Pregnancy associated plasma protein-A (PAPP-A) is a matrix-metalloproteinase found in vulnerable atherosclerotic plaques where degrades extracellular matrix and cleaves IGFBP-4 into its amino- and carboxy-terminal fragments. PAPP-A was originally proposed as a prognostic biomarker of major adverse cardiac events (MACE) in STEMI, but its measurement is affected by several physiological and methodological factors and its usefulness as a biomarker of ACS has been questioned. Recently, it has been proposed that circulating IGFBP-4 fragments concentration could reflect the atherosclerotic plaque destabilization caused by PAPP-A. **AIM** To investigate whether CT-IGFBP-4 values measured at admission in STEMI patients could add value to or complement the prognostic role of the currently used variables. **METHODS** We evaluated 236 STEMI patients admitted in our hospital. CT-IGFBP-4 concentrations were measured with a research-use-only enzyme-linked immunosorbent assay (Mercodia, Uppsala, SW) in EDTA-plasma aliquots obtained at admission and stored at -80 ºC. Future risk of MACE was clinically calculated at admission with GRACE 2.0 (Global Registry of Acute Coronary Events) Risk Score. New non-fatal myocardial infarction (MI), need of percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) after discharge and cardiovascular death during hospital stay or follow-up were registered as MACE up to 6-months after discharge. **RESULTS** Mean age was 65 years and 25.3% of cases were female, 51.7% were Killip > 1 and 68 patients (25.7%) were Killip IV. In-hospital all-cause mortality was 12.5 % (33 patients), increasing to 15.8% (42 patients) at 6 months. 52 MACE [27 cardiovascular death (23 in-hospital), 10 MI, 14 PCI, 1 CABG] were registered after 6-months follow-up. Age, gender, antecedents of diabetes and renal disease, Killip class, GRACE risk score, high sensitivity troponin T, NT-proBNP and LDL cholesterol differed between patients with and without MACE (p<0.05) meanwhile antecedents of hypertension, dyslipidemia, smoking habit or IMC did not differed. The ability of CT-IGFBP-4 to predict MACE was investigated by area under ROC curves (AUC) analysis. CT-IGFBP-4 was a predictor of MACE [AUC 0.666 (95% CI 0.576-0.755; p=0.001)]. CT-IGFBP-4 cutoff that best discriminated patients with and without MACE was ≥62 µg/L. Kaplan-Meier survival curves for MACE showed that patients above the cutoff had higher event rate (log rank 16.683; p=0.001). CT-IGFBP-4 concentrations ≥62 µg/L were associated with an increased risk of future MACE [Hazard ratio (HR) = 3.03 (95% CI, 1.73-5.31), p=0.001]. After adjusting the model for the GRACE Risk Score 2.0, CT-IGFBP-4 concentrations were still associated with increased risk of MACE [HR= 1.96 (95% CI. 1.09-3.54); p=0.02]. **CONCLUSIONS** In STEMI patients, CT-IGFBP-4 concentrations ≥62 µg/L were associated with a 3 times higher risk of MACE during hospital stay or 6-months follow-up and added value to the risk stratification provided by the GRACE 2.0 score. If confirmed in other studies, CT-IGFBP-4 at admission could help to further stratify the future risk of STEMI patients.

## A-082

### Analytical performance characteristics of the Sgx Clarity™ cTnI Assay from Singulex for the detection of cardiac troponin I

J. Ordonez-Llanos[1], A. García Osuna[1], D. Gaze[2], Y. Cheung[3], U. Enkhbayar[3], P. Collinson[2]. *[1]Hospital de Sant Pau, Barcelona, Spain, [2]St George's University Hospitals NHS Foundation Trust, London, United Kingdom, [3]Singulex Inc., Alameda, CA*

**Background**: More precise tools for early detection and rule-out of coronary artery disease (CAD) and acute myocardial infarction (AMI) would avoid unnecessary diagnostics, reduce healthcare costs, and improve patient care. Cardiac troponins I (cTnI) and T (cTnT) are used in the management of suspected AMI and have been associated with risk of future CVD. The Sgx Clarity™ cTnI Assay (Singulex Inc., Alameda, CA) is a novel high-sensitivity Single Molecule Counting immunoassay currently for investigational use only. It is a fully-automated, quantitative, fluorescent, one-step sandwich immunoassay, currently in development for the Sgx Clarity system. Here, we report analytical performance characteristics of the assay for the detection of cTnI in plasma.

**Objective:** To evaluate the limit of blank (LoB), detection (LoD), and quantification (LoQ/ functional sensitivity), and precision of the Sgx Clarity cTnI Assay using EDTA plasma in a multisite study.

**Methods**: LoB and LoD was estimated by testing four blank samples and four samples with low cTnI values, respectively, in replicates of five on each day of testing over three days for each of two reagent lots. LoQ was estimated by testing ten samples with low cTnI values in replicates of four over three days on one instrument and two reagents lots, following CLSI guidelines. LoB, LoD, and LoQ were measured at Singulex.

Test panels in various cTnI concentrations were generated to perform and compare testing for functional sensitivity and precision across multiple external sites. Functional sensitivity was measured by testing ten samples near the LoQ in replicates of four in two runs. Precision was estimated by analysing ten samples across the dynamic range in triplicates in two runs by different operators over five days. Precision and functional sensitivity were evaluated on two external sites (St Pau Hospital Barcelona and St Georges Hospital London). All testing was performed in EDTA plasma.

**Results**: LoB and LoD for the Sgx Clarity cTnI Assay was preliminary assessed to be 0.02 and 0.08 pg/mL, respectively. LoQ was estimated to be 0.14 pg/mL at 20% CV and 0.53 pg/mL at 10% CV. Observed precision was 4.0% - 9.6% and 2.7% - 10.7%, respectively. Functional sensitivity was 0.36 and 0.31 pg/mL at 20% CV and 0.82 and 0.71 pg/mL at 10% CV, respectively.

**Conclusions**: The Sgx Clarity cTnI Assay has high and reproducible analytical sensitivity and precision for the detection of cTnI in plasma, and the assay allows for reliable measurements of very low analyte concentrations. The Sgx Clarity cTnI Assay also provides high-sensitivity cTnI detection that has the potential to provide a paradigm change in the diagnosis and risk stratification of AMI and CAD.

## A-083

### Sex Specific Versus Overall 99th Percentile Upper Reference Limits For The Diagnosis Of Acute Myocardial Infarction Using A High Sensitivity Cardiac Troponin I Assay

Y. Sandoval, S. Smith, S. Love, K. Schulz, J. Nicholson, A. Sexter, F. Apple. *Hennepin County Medical Center, Minneapolis, MN*

**Background:** Analytical differences in cardiac troponin (cTn) concentrations exist between sexes when measured by high sensitivity assays, with men having higher 99$^{th}$ percentiles than women. However, while analytical differences exist, whether the use of sex-specific vs. overall 99$^{th}$ percentile translates to different clinical outcomes remains uncertain. Our goal was to compare the diagnostic performance of a high-sensitivity (hs) cTnI assay using sex-specific vs. overall 99$^{th}$ percentiles for the diagnosis of acute myocardial infarction (MI). **Methods:** Consecutive patients presenting to the emergency department with serial cTnI measurements (0/3/6/9h) on clinical indication (UTROPIA, NCT02060760) using an investigational hs-cTnI assay (Abbott, 99$^{th}$ percentiles upper reference limits (URL): overall 26 ng/L; female

16 ng/L; male 34 ng/L) were enrolled. Diagnostic accuracy statistics were compared using sex-specific vs. overall 99th percentiles for diagnosing acute MI. **Results:** Acute MI occurred in 170 (10.4%) patients. Using the overall 26 ng/L URL for both genders (n=1631), diagnostic indictors at 0, 3, 6, and 9 h were as follows: sensitivities - 64.1%, 89.9%, 90.5%, and 89.8%, respectively; specificities - 88.0%, 87.7%, 84.8%, 84.6%, respectively. Conversely, using sex-specific URLs diagnostic indictors at 0, 3, 6, and 9 h were as follows: males (34 ng/L) only (n=911, sensitivities - 62.3%, 94.4%, 95.3%, and 95.6%, respectively; specificities - 89.2%,88.7%, 85.5%, 86.0%, respectively; females (16 ng/L) only (n=720), sensitivities - 70.5%, 92.7%, 92.0%, and 93.2%, respectively; specificities - 83.7%,82.0%, 81.3%, 81.4 %, respectively; **Conclusions:** The use of sex-specific 99th percentiles for high sensitivity cTnI improved the diagnostic sensitivity for acute MI compared to the overall 99th percentile.

## A-084

### High pre-operative inflammatory factors are associated with morbidity after cardiac valve replacement: a prospective cohort study

S. Chen[1], C. Liu[1], H. Chen[1], L. Ling[1], L. Du[2], J. Zhou[1]. *[1]Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, China, Chengdu, China, [2]Department of Anesthesiology and Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu 610041, China, Chengdu, China*

**Background.** Complications after cardiac surgery are common and could lead to patient death in some cases. Previous studies suggest that inflammation is one of major factors that contribute to adverse events (AEs) after valve replacement. This study is to examine whether pre-operative inflammatory factors are associated to morbidity after cardiac valve replacement.

**Methods.** A total of 244 patients received on-pump valve replacement at the West China Hospital, Sichuan University during a period from November 1, 2011 to September 30, 2012 were prospectively enrolled. Preoperative characteristics, operative parameters were collected. Inflammatory factors as tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) were determined before surgery. The primary outcome was major adverse cardiac and cerebrovascular events (MACCE), ischemic events and peri-operative adverse events (AEs).

**Results**

The total number of MACCE was 30 out of 244 (12.3%). Mortality of all causes was 13. Mortality that could be attributed to MACCE causes was 8. Ischemic events and peri-operative AEs occurred in 36 and 38 subjects respectively. Pre-operative median TNF-α and IL-8 were 1.4 pg/ml (interquartile range: 0.8 to 2.3 pg/ml) and 0.96 μg/L (interquartile range: 0.55 to 1.87 μg/L), respectively. In comparison to those who did not develop MACCE, patients who developed MACCE did not show higher TNF-α (1.57 vs. 1.42 pg/mL, p=0.930) or IL-8 (0.87μg/L vs. 0.97 μg/L, p=0.986). A multivariate Cox regression analysis revealed an association of MACCE with IL-8 (adjusted HR: 1.13; 95%CI: 1.04 to 1.23; p=0.003) but not TNF-α (adjusted HR: 0.94, 95%CI: 0.82 to 1.09; p=0.430). Also IL-8 have an association with ischemic events (adjusted HR 1.08, 95%CI: 1.00 to 1.17; p=0.048), but not TNF-α (adjusted HR: 0.99, 95%CI: 0.88 to 1.11;

p=0.853). Mortality of all causes was associated with elevated TNF-α (adjusted HR, 1.19 for 1 pg/mL increase; 95%CI: 1.01 to 1.41; p=0.037) but not IL-8 (adjusted HR for 1 μg/L increase; 0.94; 95%CI: 0.62 to 1.43; p=0.775). Neither TNF-α nor IL-8 was associated with perioperative AEs.

**Conclusions.** Elevated pre-operativeIL-8 is associated with the increased MACCE and ischemic events, TNF-α is associated with death from any cause after valve replacement.

## A-085

### Comparison of two POC cTnI Assays with High-Senstivity cTnI and cTnT Assays in a 0, 1 and 3 Hour Algorithm in the Diagnosis of Myocardial Infarction

E. Spanuth[1], Z. Tanja[2], D. Westermann[2], R. Thomae[3], E. Giannitsis[4]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]University Heart Center Hamburg, Clinic for General and Interventional Cardiology, Molecular Cardiology, Genomics and Systems Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Mitsubishi Chemical Europe, Düsseldorf, Germany, [4]Department of Medicine III, Division of Cardiology, University of Heidelberg, Heidelberg, Germany*

**Background**

There is a clinical need to rapid rule out or rule in AMI in patients admitted with chest pain to the ED or CPU. LSIM Corporation, Tokyo offers two POC cTnI assays which are identical except the standardization by using different concentrations of the NIST standard SRM 2921. Measurement ranges, reference intervals and 99th percentile upper reference limits (URLs) are different between both assays. First evaluation studies demonstrated that the assays might meet the criteria for high-sensitivity cardiac troponin assays (imprecision at the 99th percentile URL CV < 10%, quantifiable values below the URL > 50%, 99th percentile cutoffs in males higher than in females).

**Objective**

We thought to determine the analytical criteria of both POC assays and to examine the diagnostic validity in a 0, 1 and 3 hour algorithm in the diagnosis of myocardial infarction (MI) in comparison with established high-sensitivity cTn assays.

**Methods**

cTn values were measured by using the POC cTnI assys in plasma samples obtained from selected patients admitted with chest pain (n = 95, age 22-94 years, males 67%) at 0, 1 and 3 hours. Additionally, hs-cTnT (Roche Diagnostics) and hs-cTnI (Architect STAT, Abbott Laboratories) were measured for comparison. To examine the diagnostic validity of the POC cTnI methods the dichotomized cTn values were compared by cross table evaluation. The discriminative power between myocardial infarction rule-out and rule-in diagnosis was examined by ROC analysis at 0, 1 and 3 hours. The 99th percentile URLs of the POC cTnI methods were established using the cTnI values obtained in a normal healthy population of 474 individuals.

**Results**

According to CLSI C28-A3 the 99th percentiles URLs of 15.5 (males 16.9, females 11.5 ) and 27.5 (males 31.3, females 24.9 ) ng/L (females n=236, males n=238) were obtained for the POC cTnI assays A and B, respectively. Additionally, the limits of detection (LoD) of the POC cTnI assays A and B were estimated with 1.0 ng/L and 1.75 ng/L. The cross table comparison of the POC cTnI assays A and B with the hs-cTn assays revealed proportions of individuals getting the same classification for both POC cTnI assays of 95, 94% at 0 h, 92, 94% at 1 h and 93, 96 % at 3 h. ROC analysis for discrimination between patients with rule-out and rule-in diagnosis of MI revealed AUC values of 0.879, 0.908 for the POC cTnI asssays and 0.826, 0.842 for hs-cTnI and hs-cTnT at 0h compared to 0.914, 0.927 and 0.916, 0.885 at 1h and 0.951, 0.952 and 0.945, 0.913 at 3 h, respectively.

**Conclusion**

The results demonstrated comparable diagnostic validity of the POC cTnI assays in comparison with the high-sensitivity assays Abbott cTnI and Roche cTnT. Thus, both POC cTnI-II assays can be used in the chest pain unit in parallel with the hs-cTnI and hs-cTnT assays in the central laboratory without the risk of misinterpretation of the results. The different standardization of the POC cTnI assays resulted in different analytical criteria. Further investigation should identify the most advantageous standardization method.

## A-086

### Evaluation of POC Troponin I Assays on Alere Triage and Abbott iSTAT and Their Comparison to Abbott Architect High Sensitivity Troponin I Assay

A. Lou, M. Elnenaei, M. Fortin, I. Maclean, R. Gallant, E. Mayo, B. Nassar. *Nova Scotia Health Authority, Dalhousie University, Halifax, NS, Canada*

**Objectives:** To evaluate the precision of Point of Care (POC) Troponin I (TnI) assay on the Alere Triage and the Abbott iSTAT as well as the accuracy of each of these platforms compared to the Architect High Sensitivity Troponin I (hs-TnI) as the reference assay.

**Methods**: Venous whole blood (WB) was collected in Lithium Heparinized (LH) tubes. A patient sample pool was used to establish within run imprecision (20 replicates). Day-to- day precision was examined using the vendor's control materials (N=10). The Architect hs-TnI was used as the reference assay, against which the two POC devices were compared "head to head" using fresh LH patient samples (N=40). Both EDTA and LH WB samples were also run in parallel for comparison of TnI results on the Triage.

**Results:** A Coefficient of Variation (CV) of 20.3% at a mean value of 18 ng/L (one outlier <10 ng/L, the detection limit of the device) was obtained with the Triage and a CV of 19.7 % at a mean value of 35 ng/L with the iSTAT using the same patient pool. A mean value of 28.4ng/L was obtained for this patient pool using the Architect hs-TnI assay. As shown in the Table below, the iSTAT yielded a closer agreement with the reference method with a much lower positive bias at the proposed 99$^{th}$ percentile of the latter while the Triage gave a much higher negative bias. TnI results from the Triage with LH WB were more comparable to the reference assay than those obtained with EDTA samples.

**Conclusions:** The two POC devices overall have comparable precision. The iSTAT showed a better agreement with Architect hs-TnI assay at the clinical decision cutoff value. Additionally, LH WB is suitable, if not superior to EDTA, for Triage TnI analysis.

| Sample | Method | Passing & Bablok fit | % bias at 99th tile (26.2 ng/L) | N | Mean ± SD (ng/L) | Mean Bias | % Bias at the Mean | Data Range (ng/L) and N of samples <10 ng/L |
|---|---|---|---|---|---|---|---|---|
| Plasma (LH) | Reference method (Architect i1000) | na | na | 40 | 485 ± 1344 | na | na | 0.1-7020 (7) |
| WB (EDTA) | Triage | y= 0.67 x - 0.53 | -35.0% | 27 | 473 ± 945 | -12 | -2.5% | 10-3820 (13 ) |
| WB (LH) | Triage | y= 0.82 x - 1.87 | -25.1% | 31 | 499 ± 1026 | 14 | 2.9% | 10-4330 (9) |
| WB (LH) | iStat | y= 0.90 + 1.38 | 4.7% | 34 | 500 ± 1131 | 15 | 3.1% | 10- 3520 (6) |

## A-087

### Evaluation of Diagnostic Accuracy of Abbott Architect High Sensitivity Troponin I Assay

A. Lou, M. Elnenaei, I. Maclean, M. Fortin, T. Lee, W. Kim, S. Campbell, B. Nassar. *Nova Scotia Health Authority, Dalhousie University, Halifax, NS, Canada*

**Objectives:** To evaluate the diagnostic accuracy of the Abbott high sensitivity troponin I (hs-TnI) assay by comparing it against the Roche high sensitivity troponin T (hs-TnT) assay for acute myocardial infarction (AMI) in patients presenting to the emergency department (ED) with chest pain. **Methods:** Patients presenting to ED with symptoms of potential acute coronary syndrome were studied. Blood was drawn at baseline, 3h, and 6h or more, following presentation. The diagnosis of AMI was adjudicated by cardiologists using clinical information and investigations, including the baseline and follow-up hs-TnT results (e411, Roche). Comparison between the diagnostic accuracies of the hs-TnI (ARCHITECT i1000, Abbott) and the hs-TnT was done using the 99$^{th}$ percentile cutoffs of 26.2 ng/L and 14 ng/L, respectively. The 50 ng/L value for hs-TnT is considered as the 'rule-in' value, above which patients may be considered for triage. Hence a Passing-Bablok comparison was employed for hs-TnT values of between 25- 75 ng/L (N=75) against their corresponding hs-TnI values to establish the equivalent 'rule-in' value for hs-TnI. The diagnostic efficiency of each of the assays at their defined 'rule-in' cutoff values was then compared. **Results:** 200 pairs of hs-TnI vs. hs-TnT results were obtained; 68 (34%) from females and 132 (66%) from Males. Comparison between hs-TnT at the cutoff 14ng/L and hs- TnI at 26.2 ng/L showed a diagnostic agreement of 68.5%. None of the results that showed an hs-TnI above the relevant cut-off demonstrated an hs-TnT value below its cutoff. However, in the 63 pairs of discrepant results with an elevated hs-TnT but an hs-TnI below the cut-off, there was no evidence of new onset of AMI for these patients based on the assessment by cardiologists. This indicates a higher diagnostic specificity for hs-TnI at the cutoff of 26.2 ng/L to rule out AMI. The Passing-Bablok comparison revealed an AMI 'rule-in' cutoff of 140 ng/L for hs-TnI, as being equivalent to the cutoff of 50 ng/L for hs-TnT. Correlation between the two cutoffs revealed a diagnostic agreement of 83.5% between the two assays. Clinical assessment of the 7 patients who were positive for hs-TnI but negative for hs-TnT demonstrated no evidence of new onset of AMI. Analysis of the 26 pairs of discrepant results that were positive for hs-TnT but negative for hs-TnI revealed only one diagnosis of AMI. Hence using these 'rule-in' cutoffs, hs-TnI showed a12% higher specificity and 2% lower sensitivity for the diagnosis of AMI in the discrepant group compared to hs-TnT. **Conclusions:** hs-TnI assay using the 99$^{th}$ percentile cutoff of 26.2 ng/L showed a better diagnostic specificity than the hs-TnT at 14 ng/L, which improves the AMI rule-out efficiency. AMI 'rule-in' cutoff at 140 ng/L for the hs-TnI also showed a better specificity than that for hs-TnT at 50 ng/L, while it has a comparable diagnostic sensitivity for AMI.

## A-088

### Elevated CA-125 levels are associated with increased immune activation in coronary sinus blood samples of patients with chronic heart failure

A. E. STANCIU[1], R. G. Vatasescu[2], C. Iorgulescu[2], M. M. Stanciu[3], M. Dorobantu[2]. *[1]Institute of Oncology, Bucharest, Romania, [2]Clinic Emergency Hospital, Bucharest, Romania, [3]University Politehnica of Bucharest, Bucharest, Romania*

**Background:** Heart failure is considered to be a complex syndrome associated with the neurohormonal and cytokine activation, that contribute to its progression. Interestingly, there are evidences which showed that, carbohydrate antigen 125 (CA-125), a tumor marker widely used for ovarian cancer therapy monitoring, was significantly elevated in chronic heart failure (CHF) patients. Previous studies have found that CA-125 could be produced by mesothelial cells as a consequence of fluid overload/serosal effusions and/or inflammation, but the precise mechanisms leading to CA-125 elevation in CHF are still unknown. We hypothesized that increased chronic left ventricular filling pressure, inflammation and cytokine stimulation may be responsible for CA-125 production and release. The aim of the study was to investigate the serum levels of CA-125, N-terminal pro-brain natriuretic peptide (NT-proBNP), interleukins IL-1β, IL-6, IL-8, IL-4 and tumor necrosis factor-alpha (TNF-α) from peripheral venous blood (PVB) and coronary sinus blood (CSB) samples in patients with CHF during cardiac resynchronization therapy (CRT).

**Methods:** Twenty-seven patients (15M/12F) with CHF (III-IV NYHA functional class) implanted with a biventricular pacemaker/defibrillator and 40 healthy controls (23 M/17 F) were investigated. PVB samples were collected at baseline and 1 week, 3 months after CRT device implantation and CSB samples during CRT. Cardiac function was assessed echocardiographically.

**Results:** Median serum concentrations of NT-proBNP, CA-125, IL-1β and IL-6 have been found to be significantly higher in the CS than in periphery (1392 pmol/L vs. 1156 pmol/L; 82.4 IU/mL vs. 48.6 IU/mL; 21.4 pg/mL vs. 7 pg/mL and 112 pg/mL vs. 51 pg/mL, all P<0.001). In the CS, CA-125 inversely correlated with left ventricular ejection fraction (LVEF) (r= - 0.49, P<0.001) and positively with NT-proBNP (r=0.60, P=0.001), IL-6 (r=0.36, P=0.003), IL-1β (r=0.40, P=0.001). CRT induced significant improvement in the NYHA class (baseline 3.2±0.5 vs. 1.4±0.6 at 3 months, P=0.002), NT-proBNP (baseline PVB: 1160 pmol/L vs. 353 pmol/L at 3 months, P<0.001) and CA-125 (baseline PVB: 48.6 IU/mL vs. 14.2 IU/mL at 3 months, P=0.001). Throught the monitoring period, CA-125 was inversely correlated with LVEF increase (r= - 0.67, P<0.001) and positively, with left ventricular end-systolic volume (LVESV) and NT-proBNP reduction (r=0.40 and r=0.47, P<0.001).

**Conclusion:** More localized interactions within the heart may be better studied from sinus coronary blood samples. Our findings indicate that the heart secretes CA-125, which is also present in peripheral venous serum in patients with CHF. Higher CA-125 concentrations in the CS than in periphery demonstrate that inflammation and cytokine stimulation, especially increased IL-6 and IL-1β, are responsible for CA-125 production and release. More than that, because CA-125 serum levels are associated with severity of HF, it can be used as a biomarker for monitoring and guiding therapy in these subjects.

## A-089

### Sex-Specific 99th Percentile Cardiac Troponin Normal Limits with the Medience PATHFAST Point-of-Care Cardiac Biomarker Analyzer

R. H. Christenson, S. Duh, R. M. Ruiz, K. Mullins. *University of Maryland School of Medicine, Baltimore, MD*

**Objectives:** Primary: To determine sex-specific 99th percentile normal cutoffs for cardiac troponin (cTn) I and the proportion of normal samples detectable with the PATHFAST Point of Care (POC) analyzer using a sufficiently powered normal healthy population. Secondary: Evaluate the assay's classification as contemporary or high-sensitivity.

**Relevance:** cTn is the cornerstone for diagnosis of myocardial infarction (MI) and detection of cardiac injury. Use of POC cTnI measurements can result in earlier rule-out/rule-in decisions for acute MI. However the diagnostic sensitivity and analytic parameters among POC systems vary substantially so each must be characterized near the 99th percentile cutoff.

**Methods:** The PATHFAST cTnI-II system (LSI Medience) was used for all cTnI measurements. The system's limit of detection (LoD) was 2.3 ng/L and the concentrations above 19 ng/L have CVs< 10%. EDTA specimens from the AACC Sample Bank were used for determining sex-specific 99th percentile cutoffs. Exclusion criteria were subjects without sample, race, sex or birthdate information, and subjects having NTproBNP>300ng/L, eGFR<60 ml/min and HbA1c≥6.5%.

**Results:** Data are displayed in the table below.

| | Overall | Female | Male | No Sample/ Age/ Sex/ Race |
|---|---|---|---|---|
| Total AACC Sample Bank | 897 | 428 | 450 | 19 |
| Number excluded from Sample Bank | 73 | 19 | 35 | 19 |
| Normal Healthy Population | 824 | 409 (49.7%) | 415 (50.3%) | |
| # of normal healthy population > LoD | 418 | 155 | 263 | |
| 99th percentile Cutoff | 24.8 ng/L | 20.7 ng/L | 26.4 ng/L | |
| % of normal healthy population > LOD | 50.7% | 37.9% | 63.4% | |

The PATHFAST cTnI-II system yielded detectable results for 50.7% of a sex-balanced normal healthy population. Of the 897 total subjects from the AACC Sample Bank 19 had no sample/age/sex/race available; 35 males and 19 females did not meet NT-proBNP/creatinine/HbA1C criteria. The final normal healthy population consisted of 415 males and 409 females.

**Conclusion:** The sex-specific 99th percentile cutoffs for females was 20.7ng/L and for males was 26.4ng/L for the PATHFAST TnI-II POC system. Consistent with the current IFCC TF-CB definition of high-sensitivity cTn assays, the PATHFAST detected >50% of the normal healthy population above the assay's LoD, and the CV is <10% at the 99th percentile cutoff.

## A-090

### Circulating VCAM-1 as potential biomarker of atherosclerosis

J. N. G. de Araújo[1], J. C. dos Santos[1], M. S. Cruz[1], K. M. de Oliveira[1], V. H. R. Duarte[1], A. M. G. da Silva[1], I. C. C. dos Santos[1], J. M. de Oliveira[1], M. S. M. d. Paiva[1], A. D. Luchessi[1], A. A. Rezende[1], M. H. Hirata[2], R. D. C. Hirata[2], V. N. Silbiger[1]. *[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Universidade de São Paulo, São Paulo, Brazil*

**Background:** Adhesion molecules such a vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and selectins play an important role in the development of atherosclerosis, which is the primary origin of most common cardiovascular disorders. ICAM-1 facilitates monocyte/macrophage emigration and adherence to endothelial cells, whereas VCAM-1 facilitates macrophage uptake into the subintimal space. E-selectin is involved in the tethering, rolling, and activation of leukocytes. Together, these molecules form an integrated and overlapping system for the transport of leukocytes and recruitment of additional cytokines, growth factors and matrix metalloproteinases into the vascular wall. Matrix metalloproteinases provokes disruption of atherosclerotic plaques and participates in the degradation of the extracellular matrix, which may lead to acute cardiovascular events. In a search for molecules that complement invasive atherosclerotic disease diagnostic techniques, the present study aimed to investigate the relationships among VCAM-1, ICAM-1, E-selectin and matrix metalloproteinase 9 (MMP9) serum concentrations and the extent of coronary lesion. **Methods:** Seventy-four male and female individuals aged between 30 to 74 years who were undergoing coronary angiography for diagnostic purposes were enrolled in this study. Morning fasting blood samples were obtained for analysis of biochemical parameters (glucose, total cholesterol and fractions, urea, creatinine K, uric acid, alanine aminotransferase and aspartate aminotransferase) and for the determination of VCAM-1, ICAM-1, E-selectin and MMP9 serum concentrations. Biochemical tests were performed using colorimetric and colorimetric-enzymatic methods on a semi-automatic biochemical analyzer, and serum concentrations of the adhesion molecules and MMP9 were quantified using The Millipex® MAP Human CVD Panel 1 Immunoassay Kit, according to the manufacturer's instructions. The extent of the coronary lesion was assessed using the Friesinger Index and subjects were classified in four groups: no lesion, minor lesion, intermediate lesion and major lesion. **Results:** Significant differences in the clinical and biochemical data were not found among the groups. The VCAM-1 serum concentration was higher than 876 ng/mL in individuals with intermediate and major lesions ($p < 0.001$ and $p = 0.020$, respectively). Moreover, logistic regression analysis showed that these patients had an increased risk of having an intermediate lesion (odds ratio (OR): 9.818, 95% confidence interval (CI): 1.840-52.384, $p = 0.007$). Interestingly, all individuals with major lesions had VCAM-1 concentrations higher than 876 ng/mL. No association was found between the serum concentrations of the other proteins and the Friesinger Index. **Conclusion:** Therefore, circulating VCAM-1 may be strongly associated with the presence and extent of coronary lesions. Further investigation is needed to consider whether monitoring the VCAM-1 serum level would be valuable for the assessment of cardiovascular risk.

## A-091

### Genetic Diagnosis of Monogenic or Polygenic Familial Hypercholesterolemia in Northern Ireland: Evaluation of the Randox FH Arrays in Combination with the Randox 6SNP Polygenic Hypercholesterolemia Array

D. A. Bailie[1], M. Latten[2], H. A. McMillan[2], P. J. Hart[1], P. Hamilton[1], H. A. Murray[2], C. Patterson[3], J. V. Lamont[2], S. P. FitzGerald[2], I. S. Young[3], M. A. Crockard[2], C. A. Graham[2]. *[1]Regional Genetics Centre, Belfast Health and Social Care Trust, Belfast, United Kingdom, [2]Randox Laboratories Ltd., Crumlin, United Kingdom, [3]Centre for Public Health, Queen's University, Belfast, United Kingdom*

**Background** Familial Hypercholesterolemia (FH) is a common genetic disorder characterised by elevated LDL-C and early onset coronary heart disease. The condition is primarily monogenic caused by a single mutation in 1 of 3 genes (LDLR, APOB or PCSK9) with a prevalence of 0.2-0.5%. Despite its commonality, FH is highly underdiagnosed in most countries (<10%). With a clinical diagnosis of 'possible FH', 60% of patients are mutation-negative and may have polygenic hypercholesterolemia, most likely due to an accumulation of common small-effect LDL-C raising alleles. The aim of this study was to determine the effectiveness of the Randox FH Arrays for monogenic mutation detection and the 6SNP Polygenic Hypercholesterolemia Array (Randox Laboratories Ltd. Crumlin, UK) to identify possible polygenic hypercholesterolemia. **Methods** A total of 414 cases of 'possible FH' based on Simon Broome criteria, were selected for analysis. Mutation analysis for monogenic FH was carried out by Sanger sequencing or with the FH Arrays which permit detection of the 40 most common monogenic mutations associated with FH in the UK and Ireland. 6SNP genotyping was conducted by Sanger sequencing and the results used to validate the 6SNP Polygenic Hypercholesterolemia Array. This panel permits genotyping of six SNPS associated with raised LDL-C values that occur across five genes (APOE: rs7412, rs429358, APOB: rs1367117, ABCG8: rs6544713, CELSR2: rs629301 and LDLR: rs6511720). A weighted LDL-C Genetic Risk Score (GRS) was calculated for all 414 possible FH cases. **Results** A definite monogenic FH diagnosis (mutation positive) was confirmed in 196 cases, the FH Array panel detects 72% of the point mutations identified, and the remaining 218 clinically 'possible FH' cases were mutation negative. The mean LDL-C GRS for the mutation positive group was 0.705 but this value was significantly higher in the mutation negative group with a mean LDL-C GRS of 0.777 [p<0.001]. Mutation negative patients had 2.3 times the odds of having a LDL-C GRS exceeding 0.81 than mutation positive patients, thus increasing the chance of polygenic hypercholesterolemia. **Conclusion** The FH Arrays and 6SNP Polygenic Hypercholesterolemia Array successfully genotyped a subset of the Northern Irish population deemed clinically to have 'possible FH'. Recent literature concludes that the LDL-C GRS consistently distinguishes mutation negative patients from healthy individuals and that a high proportion of mutation negative patients are likely to have a polygenic basis to their condition (Futema *et al*, 2015). This combined approach of using the Randox FH Arrays and the 6SNP Polygenic Hypercholesterolemia Array can be used to define patients affected with monogenic FH and likely polygenic hypercholesterolemia and assist with clinical

management decisions. The approach may reduce the need for further comprehensive DNA sequencing in many patients and negate the use of family cascade screening. This combined method has the potential to improve the management and treatment of patients with hypercholesterolemia.

## A-092

### Prognostic value of copeptin on adverse clinical outcomes after successful percutaneous coronary intervention in patients with acute myocardial infarction

H. Choi[1], M. Kim[2], Y. Ahn[2], M. Shin[1], S. Suh[1]. *[1]Department of Laboratory Medicine, Chonnam National University Hwasun Hospital, Hwasun, Korea, Republic of, [2]Department of Cardiology, Cardiovascular Center, Chonnam National University Hospital, Gwangju, Korea, Republic of*

**Background**: Copeptin has been demonstrated to be of utility in early risk stratification and prognostication of acute myocardial infarction (AMI) patients. However, it is uncertain about the prognostic role of copeptin measured right after successful percutaneous coronary intervention (PCI) in patients with AMI. We aimed to evaluate the association between blood copeptin levels immediately after successful PCI and major adverse cardiac events (MACE) in patients with AMI.

**Methods**: We enrolled 149 patients with AMI who successfully received PCI with or without coronary stenting in the Chonnam National University Hospital between February 2013 and December 2014. Serum copeptin levels were analyzed using a time-resolved amplified cryptate emission immunoassay (Thermo Fisher Scientific Clinical Diagnositics BRAHMS GmbH, Germany) from blood samples taken from enrolled patients immediately after successful PCI. We examined the associations of serum copeptin levels with incidence of major adverse cardiac events (MACE; composite of death, repeat PCI, recurrent MI or coronary artery bypass grafting) during follow-up period.

**Results**: The serum copeptin was analyzed in total 149 patients (ST-segment elevation myocardial infarction [STEMI] 40 and NSTEMI 109 patients). Of 149 patients, MACE occurred in 34 patients (22.8%) during a median follow-up of 30.1 months (IQR, 22.9-36.8 months). Although the area under the receiver operating curve for copeptin (0.631) was not large for the prediction of MACE, the copeptin levels (mean ± SD) were higher in patients with MACE than those without MACE (40.7 ± 95.4 pmol/L versus 180.6 ± 396.6 pmol/L, *P*=0.049). Because copeptin level exhibited a right skewed distribution, the data were subjected to a natural log transformation. In a multiple logistic regression model, a e-fold (approximately 2.71828) increase in copeptin level was associated with an odds ratio for MACE of 1.6 (95% CI, 1.15-2.20, *P*=0.005) after adjusting confounding variables (age,

diabetes mellitus, previous PCI history, low density lipoprotein-cholesterol and high sensitivity CRP). The MACE-free survival of patients with high copeptin levels was significantly shorter than that of patients with low copeptin level (*P*=0.045).

**Conclusions**: High concentration of copeptin measured immediately after successful PCI was associated with MACE in patients with AMI during long-term clinical follow-up. It suggests that copeptin can be served as a prognostic marker for risk stratification improvement in patients with AMI after successful PCI.

Key Words: Copeptin, Major adverse cardiac events, Percutaneous coronary intervention

## A-093

### miRNAs as potential biomarkers for new-onset fibrillation: *in silico* and *in vivo* analysis.

A. M. G. Silva, A. D. Luchessi, A. M. Novaes, M. B. Lopes, J. C. V. Sousa, A. A. A. Filho, A. A. Rezende, M. H. Hirata, V. N. Silbiger. *Universidade Federal do Rio Grande do Norte, PARNAMIRIM, Brazil*

**Background:** Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. The AF diagnostic methods are given by electrocardiogram (ECG) and Holter for 24hrs, and considering this limitation, some non-coding RNAs (miRNAs) have been involved in regulatory activity in arrhythmogenesis, targeting genes that contribute to the development of AF. However, the role of these non-coding RNAs (miRNAs) as a circulating biomarker for diagnosis of atrial fibrillation is not well reported. Therefore, the objective of this study was to evaluate the expression of miRNAs of patients with AF, new-onset AF and its application as biomarkers, as well as to search for interactions of target miRNAs with mRNAs and cardiovascular processes that may involve AF.

**Methods:** In the *In Vivo* study, Six miRNAs miR-21, miR-133a, miR-133b, miR-150, miR-328 and miR-499 were selected as targets in this study. They were isolated from plasma of individuals aged from 20 to 85 years old with AF (n = 17), new-onset AF (n = 5) and without AF (n = 15). The results were analyzed by Real-Time PCR (RT-PCR) with *miScript SYBR Green PCR*. An *In Silico* study was carried out to search for potential miRNA targets differentially expressed in the *In Vivo* study, correlating with the top 30 mRNAs targets of these miRNAs by the *Target Scan 7.1* tool, and then integrative analysis through the *Ingenuity Pathway Analyses* 6 (IPA) software, seeking interactions miRNA-mRNA-cardiovascular process.

**Results:** *In vivo* data, we observed that miR-21, miR-133b, miR-328 and miR-499 had a different level of expression between the three groups (p <0.05). There was increase expression of miR-21 (0.6-fold), miR-133b (1.4-fold), miR-328 (2.0-fold) and miR-499 (2.3-fold) in patients with new-onset AF, when compared to AF and control subjects. The miR-133a and miR-150 expression did not differ among the groups. In silico data, the miRNA-mRNA interactions showed 14 mRNAs regulated by the miRNAs miR-21, miR-133 and miR-499 and observed the association of these miRNAs in different pathophysiological processes that may trigger or be a consequence of AF. We also observed that of the 14 mRNAs, 10 were related to cardiac pathophysiological processes and of these, 3 (SMAD7, FASLG and TIMP3) are directly related to the pathophysiology of AF, acting in the processes of atrial apoptosis and atrial fibrosis.

**Conclusion:** Our data suggest that miR-21, miR-133b, miR-328 and miR-499 may be potential biomarkers for AF as well as for new-onset AF, for monitoring and for the diagnosis. These miRNAs have also demonstrated important association with several cardiovascular pathophysiological processes, regulating different mRNAs that are expressed in these conditions and these findings may contribute to the understanding of the process that triggers AF outcome, encouraging the development of new studies to evaluate the application of these molecules as future clinical markers for this disease.

## A-094

### *TREML4* polymorphisms and mRNA expression in blood leukocytes are associated with the extension of the atherosclerotic lesions in patients with coronary artery disease

V. H. R. Duarte[1], M. K. R. N. Duarte[1], J. N. G. Araújo[1], A. M. Q. S. Santos[1], I. C. C. Santos[1], C. T. O. Fernandes[1], J. C. Santos[1], A. M. G. Silva[1], M. S. Cruz[1], J. M. Oliveira[1], M. S. M. O. Paiva[1], A. A. Rezende[1], M. H. Hirata[2], R. D. C. Hirata[2], A. D. Luchessi[1], V. N. Silbiger[1]. *[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Universidade de São Paulo, Natal, Brazil*

Cardiovascular disease (CVD) is the leading cause of death and morbidity in developed and developing countries. The discovery of novel circulating biomarkers involved in the pathophysiology of the atherosclerosis may contribute to evaluate the cardiovascular risk. In a microarray based gene expression study, we observed that the expression of the receptor *Trem-like transcript 4* (*TREML4*) in blood leukocytes mRNA was increased in patients with acute coronary syndrome (ACS). It is likely that TREML4 is involved in the atherosclerosis process, and it may have an innovative potential as a biomarker of cardiovascular risk. We investigated the association of *TREML4* polymorphisms and mRNA expression in blood leukocytes with the extension of the atherosclerotic lesions in CAD patients without ACS.

One hundred and fifty one CAD patients aged 30 to 74 years old, submitted to the first coronary angiography were enrolled in this study. The extension of coronary artery lesion was assessed by the Friesinger index. Subjects were stratified in fuor group. Peripheral blood samples were obtained before coronary angiography. Blood samples were used for biochemical analysis, DNA extraction and isolation of leukocyte. Total RNA was extracted from leukocytes using RiboPureTM Blood kit. DNA synthesis was performed using High Capacity cDNA Reverse Transcription Kit. *TREML4* mRNA expression was analyzed by real time PCR (qPCR). The reference gene selected was *ACTB.* Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit. *TREML4* polymorphisms rs2803495 (A>G) and rs2803496 (C>T) were genotyped by real time PCR using the TaqMan SNP Genotyping Assays and the 7500 Fast Real-Time PCR System.

Patients with intermediate and major artery lesions had greater age when compared to those without lesion (p=0.002 and p=0.001, respectively). Subjects with intermediate lesions had higher systolic pressure than those without lesion (p=0.01) and low lesion patients presented higher body mass index than those without lesion (p=0.02). Other clinical variables and the biochemical parameters did not differ among the groups (p>0.05). Subjects carrying rs2803495 G allele (AG+GG genotypes) are more likely to be *TREML4* expressors than AA genotype carriers (OR= 2.4, 95%CI=

1.0-5.7, p<0.05). However rs2803495 variant was not associated with the degree of *TREML4* mRNA expression (p>0.05). Analysis of the *TREML4* rs2803496 variant showed that carriers of C allele (CT+CC genotypes) have high likelihood of being in Expressor group than subjects carrying TT genotype (OR=7.8, 95%CI= 2.9-20.9, p<0.01). Moreover C allele was associated with high mRNA expression levels (O.R.=5.1, 95%CI= 1.5-15.6, p<0.01). CAD patients with major artery lesions had higher *TREML4* mRNA levels than those with intermediate (1.4-fold) or low (1.2-fold) artery lesions (p<0.05). Patients with major artery lesions also expressed 1.4-fold higher the *TREML4* than those without lesion ($p$<0.01). The results of this study suggests that mRNA expression of *TREML4* in leukocytes is influenced by the extension of coronary artery lesion and gene polymorphisms in CAD patients, and therefore the measurement of mRNA levels may be a potential biomarker to evaluate the progression of CAD. However, further investigations are necessary to confirm these results in larger population samples of CAD patients.

## A-095

### Evaluation of free and complex cardiac troponin I in serial samples of MI patients

X. M. R. van Wijk, S. Claassen, K. L. Lynch, A. H. B. Wu. *University of California, San Francisco, San Francisco, CA*

**Background:** Cardiac troponin (cTn), part of the myofiber complex, is the preferred biomarker for detection of myocardial infarction (MI). It is predominantly present as a complex of three subunits (troponin I, T and C), while ~6-8% is unbound ('free') and presumably located in the cytosol. It has been hypothesized that free troponin can be released by ischemia without cell death. Furthermore, it is thought that during an MI free troponin is released first, after which the structurally bound complex troponin leaks out slowly. If proven, an assay that is able to distinguish between free and complex troponin may become a valuable clinical assay to evaluate timing and severity of cardiac damage, and to distinguish between type 2 MI (ischemia due to a supply/demand imbalance) and type 1 MI (spontaneous event related to the rupture of an atherosclerotic plaque).

**Methods:** To distinguish free from complex cTnI, we coupled antibody 20C6 (Hytest, Finland), which recognizes complex troponin (IC/TIC) and not the individual subunits, to Dynabeads® magnetic beads. Patients samples or controls, diluted 1:2 with PBS/4% BSA/0.5% Tween-20, were incubated with or without the 20C6-coupled beads. After removal of the beads using a magnet, the samples were measured on a Siemens ADVIA Centaur® analyzer using the TnI-Ultra™ assay. The percentage reduction after bead incubation was then calculated. Up to now we have selected serial troponin samples, which displayed a rise and fall, from three type 1 and twenty-six type 2 MI patients and we are selecting more type 1 MI samples.

**Results:** Using standards of free and complex (TIC) cTnI, we determined the percentage reduction after incubation with 20C6-coupled beads at multiple cTnI concentrations. When complex or free cTnI was spiked in TnI-free serum at 30, 7.5, 1.88, and 0.47 ng/mL, we observed a reduction in complex cTnI of 95, 94, 95, and 100%, respectively. At low concentrations, however, a significant amount of free cTnI was removed as well (13, 24, 33, and 39%, respectively). So far, we have analyzed serial samples of 5 patients with a type 2 MI, and one patient with a type 1 MI. With the exception of one sample set, all samples showed a reduction >75%. In one type 2 MI sample, values were reduced between 6.7 and 12.1%. We compared the reduction percentage of the first rising cTnI with the last falling cTnI. The reduction was 75.4±29.8% (average±SD) for the first rising cTnI and 73.5±29.7% for the last falling cTnI (paired *t*-test; $p$=0.34, $n$=6). Corresponding cTnI values were 1.25±0.4 and 1.71±1.0 (paired *t*-test; $p$=0.41, $n$=6).

**Conclusions:** In 5 out of 6 samples, the majority of released cTnI was present as complex cTnI. When comparing early vs. late serial samples, there was no significant difference in the percentage of cTnI reduction using 20C6-coupled beads, suggesting the proportion of complex cTnI was similar at both time points. We are currently testing more patient samples and we are working on new methods to directly measure free and complex cTnI.

## A-096

### Performance Evaluation of the ADVIA Centaur® High Sensitivity Troponin I Assay

R. C. Payne[1], L. Halik[1], J. Ma[1], H. Zhang[1], J. Zhang[1], J. Conklin[1], K. Yokoyama[1], Y. Bahrainy[1], M. Gaylord[1], N. Ozgen[1], J. Balderson[1], M. E. Grosett[1], A. Chase[1], J. Lei[1], R. Gorman[1], B. Plouffe[1], F. Apple[2], R. Christenson[3]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Hennepin County Medical Center, Minneapolis, MN, [3]University of Maryland School of Medicine, Baltimore, MD*

**Background**: The 2015 European Society of Cardiology guidelines propose algorithms for faster rule-in or rule-out of AMI in patients admitted in the acute care setting and for the management of NSTEMI. High-sensitivity cardiac troponin I assays will more accurately and precisely measure changes in cTnI concentrations in serial draws providing useful data to assist in identifying acute versus chronic cTnI elevations, and acceptable rule-in and rule-out performance within 1 to 3-hours of presentation. This study evaluated the performance of the ADVIA Centaur High Sensitivity Troponin I (TNIH) assay developed for use on the ADVIA Centaur family of immunoassay analyzers.[1] The ADVIA Centaur TNIH assay is a dual-capture sandwich immunoassay using preformed magnetic latex particles, a proprietary acridinium ester for chemiluminescence detection, and three monoclonal antibodies. **Method:** The limit of blank (LoB) and limit of detection (LoD) assessments used three reagent lots on two ADVIA Centaur XP and two ADVIA Centaur XPT Immunoassay Systems for both Lithium Heparin and Serum matrixes following CLSI EP-17A. The 99$^{th}$ percentile cutoff values were established non-parametrically using a well-characterized population of healthy subjects. Clinical correlation of cTnI levels above the 99$^{th}$ percentile to AMI diagnosis as adjudicated by three cardiologists was assessed in all-comer emergency department (ED) subjects in both sample matrixes. **Results:** LoB results ranged from 0.11 to 0.90 ng/L with a typical value of 0.5 ng/L, and LoD results ranged from 1.10 ng/L to 2.21 ng/L (95% confidence interval 1.05 to 2.54 ng/L). The cTnI concentration at 20% $CV_{Total}$ (LoQ) had a pooled value of 2.50 ng/L. The 99$^{th}$ percentile estimated for 2026 apparently healthy volunteers with an equal number of males and females was a gender-combined 48 ng/L. The 99$^{th}$ percentile observed on the Centaur XP and Centaur XPT for females is 37ng/L to 39 ng/L, respectively, for males 57 to 62 ng/L. The percentage of the normal population above 1.10 ng/L ranged from 66% to 80% on the Centaur XP and XPT, respectively. Identical 99$^{th}$ percentiles were obtained with Lithium Heparin plasma and Serum samples. The with-in Lab CV at the 99$^{th}$ percentile at 37 ng/mL was less than 3%. Clinical sensitivity and clinical specificity in pooled-genders at 1, 2, and 3-hours post-ED presentation ranged from 87.6 to 93.2% and 90.0 to 91.5% respectively. **Conclusion:** The ADVIA Centaur TNIH assay under development has a 10% total CV at a cTnI concentration 10-fold lower than the 99$^{th}$ percentile. This new assay allows the establishment of gender-specific 99$^{th}$ percentile cutoffs and shows acceptable clinical utility in an all-comer ED population. [1] Under development. Not available for sale. The performance characteristics of this device have not been established. Product availability will vary from country to country and will be subject to varying regulatory requirements.

## A-097

### DIAGNOSTIC PERFORMANCE OF A HIGH SENSITIVITY TROPONIN I ASSAY IN PATIENTS WITH CHEST PAIN

A. A. FERREIRA[1], L. F. B. PORTO[2], M. F. DUTRA[3], M. G. Iorio[1], M. D. FREIRE[2]. *[1]HOSPITAL PROCARDIACO, RIO DE JANEIRO, Brazil, [2]DASA, RIO DE JANEIRO, Brazil, [3]HOSPITAL RIOS D'Or, RIO DE JANEIRO, Brazil*

**Background:** There are many methodologies available in the market, which use different targets and formats to assembly an assay, and depending on the type of assay and type of antigen used, different results and interpretations could be made. Due to the higher sensitivity and diagnostic accuracy for the detection of Acute Coronary Syndrome (ACS) at presentation and after 1 hour, the time interval to the second cardiac troponin assessment can be shortened with the use of high-sensitivity assays. Hospital Procardiaco is a reference in cardiology in Rio de Janeiro and has well-structured protocols for evaluation of chest pain.

**Methods:** 137 consecutive samples collected from patients of different ages admitted to the Emergency Department (ED) with chest pain and suspected ACS. The determination of Troponin I in serum samples were initially analyzed with method of high-sensitivity LOCI Cardiac Troponin I using a homogeneous sandwich chemiluminescent immunoassay in the *Dimension EXL 200* (*Siemens*) ® (HS).

**Results:** Excellent diagnostic accuracy was obtained in patients with a higher probability of pre-testing for ACS and with a shorter time onset of pain with a sensitivity of 71.4%, specificity of 96.8%, positive Likelihood ratio of 22 , 3%, AUROC 0.81 and CI of (0.571-1). For the Rule out of these patients at 1 hour post admission, an excellent result was obtained with sensitivity of 93.8% specificity of 100%, AUROC 0.98 and CI of (0.968-1).

**Conclusion:** In the present study the population evaluated with the Troponin I HS methodology presented accurate results for the diagnosis of AMI (Rule in) and for the release of the patients from ED (Rapid 1-hour Rule out).

## A-098

### High-sensitivity cardiac troponin I (hs-cTnI) detects postoperative myocardial damage after elective knee or hip replacement

M. T. Tesfazghi[1], A. R. Bass[2], B. F. Gage[1], C. F. Eby[1], J. L. Anderson[3], S. M. Stevens[4], S. C. Woller[4], M. G. Scott[1]. *[1]Washington University School of Medicine, St. Louis, MO, [2]Weill Cornell Medical College, New York City, NY, [3]Intermountain Healthcare, Salt Lake City, UT, [4]University of Utah School of Medicine, Murray, UT*

**Background:** More than 200 million patients worldwide undergo non-cardiac surgery every year. Despite the benefits of treating disease and improving quality of life, non-cardiac surgery is associated with a risk of vascular complications including myocardial injury (JAMA. 2012; 307:2295). The VINO trial showed that preoperative high sensitivity troponin T (hs-cTnT) levels predicted both postoperative myocardial infarction (MI) and long-term mortality (Am Heart J. 2013;166:325).

**Objective:** The aim of this study was to examine the change from pre- to post-operative day 2 (POD 2) plasma concentrations of cardiac troponin I (hs-cTnI) among elderly patients undergoing elective knee or hip replacement.

**Method:** This was an ancillary study to a randomized controlled trial, the Genetics-InFormatics Trial (GIFT) (Pharmacogenomics J. 2012;12:417.). We measured hs-cTnI using the Abbott Architect assay on pre-operative and POD 2 plasma samples from 791 subjects that underwent elective hip or knee replacement. At 26 pg/mL (the overall 99$^{th}$ percentile for this method) the coefficient of variation was 4%. We quantified how many patients had a delta hs-cTnI $\geq$ 10 pg/mL, a common (but conservative) delta for ruling in/out MI at 3 hours in an emergency setting. We also examined whether in patients with a delta hs-cTnI $\geq$ 10 pg/mL, the delta exceeded the short-term biological variability of hs-cTnI (Reference change value = 50 – 60%).

**Result:** Before surgery, 10/791 patients had hs-cTnI values $\geq$ 26 pg/ml (range 27-217) but only one of these patients exhibited $\geq$ 10 pg/mL delta at POD 2. Ninety-nine patients (12.5%) had a delta $\geq$ 10 pg/ml (range 10 –8901 pg/mL, median 29 pg/mL) rise in hs-cTnI levels at POD 2. Of these, only one had a pre-operative hs-cTnI > 26 pg/mL (99$^{th}$%). Of the 99 patients with a > 10 pg/dL delta, 60 also had a POD 2 above 26 pg/mL (range 27- 8906 pg/mL, median 62 pg/mL). All but two of the patients with a > 10 pg/mL rise had a percent delta change > 100% (median 992%). Six patients had a clinical diagnosis of MI during this trial.

**Conclusion:** In this study of elderly patients undergoing elective arthroplasty, 12.5% of patients showed a biologically significant (> 10 pg/mL) rise in hs-cTnI at POD 2. In contrast, <1% of patients had a clinical diagnosis of MI. The clinical significance of the rise in hs-cTnI after elective non-cardiac surgery requires further study.

## A-099

### Sex-Specific 99$^{th}$ Percentiles Derived from the AACC Universal Sample Bank for the Roche Gen 5 cTnT Assay

F. S. Apple, A. Sexter, K. Schulz, A. K. Saenger, A. S. Jaffe. *Hennepin County Medical Center, Minneapolis, MN*

**Background**: The recent FDA clearance of the Roche Elecsys Troponin T Gen 5 Stat Immunoassay provides 99$^{th}$ percentile upper reference limits (URL) for 1301 apparently healthy adults. The concentrations were: overall 19 ng/L, women 14 ng/L, men 22 ng/L. These concentrations represent a substantial shift from their overall 14 ng/L (no sex-specific URLs) used internationally. The purpose of our study was to determine the overall and sex-specific 99$^{th}$ percentile URLs based on the AACC Universal Sample Bank. **Methods**: Lithium heparin plasma from 838 normal subjects, enrolled following informed consent and completion of a health questionnaire, were purchased from the AACC. Blood was collected from 423 men and 415 women recruited from both the 2015 annual AACC meeting in Atlanta and at the University of Maryland. The e602 instrument was used to measure cTnT by the Gen 5 assay, with a limit of detection (LoD) of 3 ng/L. Hemoglobin A1c (URL 6.5%), NT-proBNP (URL 250 ng/L) and eGFR (60 ml/min) were measured, along with identification of statin use, to help define a normal status. 99$^{th}$ percentile URLs were determined by the non-parametric (NP) and Harrell-Davis Bootstrap (HDB) methods. **Results**: 83 of 838 subjects were excluded based on abnormal surrogate biomarkers or based on statin use. Ages ranged from 19 to 91y; race: Caucasian 58%, African American 27%, Pacific Islander/Asian 11%, other 4%; ethnicity: Hispanic 8%, non-Hispanic 92%. Overall <50% of subjects had measureable concentrations >LoD (46% no exclusion, 44% after exclusion); males: 68% no exclusion, 66% post exclusion); females: 22% (both). Excluding subjects decreased the 99$^{th}$ percentiles by both statistical methods as follows: overall, 17 to 16 ng/L for both NP and HDB methods; males 18 to 16 ng/L for NP method, and 21 to 18 ng/L for HDB method. For females, there were no differences before and after exclusion, but there were differences by statistical method: 13 ng/L NP and 14 ng/L HDB. **Conclusions**: Our findings are consistent with the majority of the literature in that a) the Gen 5 cTnT assay does not meet the IFCC guideline for high-sensitivity assays which requires >50% of measureable concentrations above the LoD, b) using surrogate biomarker criteria lowers 99$^{th}$ percentiles, and c) the statistical method used impacts 99$^{th}$ percentiles. Our data suggest lower sex-specific cTnT 99$^{th}$ percentile concentrations than FDA approved should be used in clinical practice for the Gen 5 assay. Our study highlights the importance of detailing the criteria used to include and exclude subjects for defining a healthy population to determine cTn 99$^{th}$ percentiles and the statistical method used to calculate 99$^{th}$ percentiles.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Clinical Studies/Outcomes

## A-100

### Selected pro- and anti-inflammatory cytokine serum concentrations in different clinical forms of multiple sclerosis

A. Kost, L. Lapovets, U. Pidvalna. *Danylo Halytsky National Medical University, Lviv, Ukraine*

**Background:** Multiple sclerosis (MS) is an immune-mediated central nervous system disease characterized by inflammation, demyelination and axonal degeneration. The pathology of MS suggests an autoimmune cause involving cellular and humoral components of the immune system. Active MS lesions are characterized by T-cell and macrophage infiltration and the presence of immune mediators, including adhesion molecules, chemokines, and cytokines. Cytokines are proven mediators of immunological process in MS. The aim of this study was to delineate the serum cytokine profile in patients with MS and the controls and to determine in different clinical forms of MS.

**Methods:** This study involved 62 consecutive MS patients--28 patients with progressive MS and 34 patients with relapsing-remitting MS (RRMS). The control group consisted of 18, age and sex matched, non-immunological, neurological patients. The patients were evaluated using the Expanded Disability Status Scale (EDSS) and magnetic resonance imaging (MRI) with gadolinium. Serum samples for cytokine measurements were collected on admission. Plasma levels of proinflammatory T-helper (TH)1 (interferon (IFN)-γ) cytokines, peripheral monokines (IL-1beta, IL-6, TNF-alpha) and anti-inflammatory or down-regulatory TH2 cytokines (IL-4, IL-10) were determined by an enzyme-linked immunosorbent assay (ELISA) method.

**Results:** All patients with MS had significantly higher cytokine (IFN-γ, IL-1β, IL-6, TNF-α, IL-4, IL-10) concentrations compared with controls ($p < 0.001$). Increased IL-1beta, IFN-γ ($p=0.032$ and 0.041, respectively) and decreased IL-4, IL-10 ($p=0.038$ and 0.02, respectively) levels were found in progressive MS compared with RRMS. Patients with progressive MS with disease progression presented higher IL-1beta, TNF-alpha, IFN-γ and IL-10 levels than those without disease progression ($p < 0.05$). There was a significant inverse correlation between IL-10 levels and EDSS score in patients with progressive MS ($R = - 0.43$, $p < 0.05$).

**Conclusion:**

Profiling cytokines in multiple sclerosis may help to identify mechanisms involved in the pathogenesis of the disease, and, potentially, lead to new therapies directed at cytokines or their receptors. The level of IL-10 can serve as an additional diagnostic criterion for assessing the disability in patients with progressive MS.

## A-101

### Comparison of Neutrophil / Lymphocyte Ratio & Red Blood Cell Distribution Width with Cardiac Markers in Acute Coronary Syndrome

S. CELIK, E. NEZAKET, E. ARZU, F. TURGAY, S. CIGERLI, K. KILICKESMEZ, E. SERIN. *Sisli Hamidye Etfal Research and Training Hospital, ISTANBUL, Turkey*

**Background:** Acute coronary syndrome (ACS) is a group of symptoms for acute myocardial ischemia. The RDW (red cell distribution width), recently described as a novel risk marker, has been associated with an increased risk of cardiovascular events and routinely reported as part of the complete blood count. We assessed the relationship of hematologic parameters (RDW and NLR) with cardiac markers (Troponın I, CK-MB) in patients admitted with ACS.

**Methods:** In this study 228 patients were included. For all patients, a baseline blood sample was collected for routine hematological testing. The patients were diagnosed as Non ST elevation myocardial infarction (NSTEMI), ST elevation myocardial infarction (STEMI) or unstable angina (USAP) based on the elevation of cardiac troponin I levels in the first and sixth hours of admission. SPSS version 15.0 program was used for statistical analysis.

**Results:** There was no difference in the levels of RDW in NSTEMI and USAP groups (p=0,154). In the ACS subtypes groups, NLR showed a negative correlation with EF ($p\leq 0{,}001$)and significant positive correlation with the first hour levels of CK-MB and TnI ($p\leq 0{,}001$). It was also found that NLR showed positive correlation with STEMI groups ($p\leq 0{,}001$).

**Conclusion:** It was determined that there was no statistically significant difference between RDW and ACS subtypes. Finding NLR levels being significantly high in STEMI group of ACS patients revealed out that NLR can be a useful marker in ACS. We suggest that these findings should be evaluated and supported with prospective studies.

## A-102

### High Heme Oxygenase -1 Levels and HMOX1 Gene Promoter Alleles Are Associated with Malaria in Ghanaian Children

C. E. Lekpor[1], F. A. Botchway[2]. *[1]Korle-Bu Teaching Hospital/ University of Ghana/Department of Pathology, Accra, Ghana, [2]KorleBu Teaching Hospital/ University of Ghana, Accra, Ghana*

Background: Malaria is a tropical disease that kills in its own right and weakens immune defenses. Malaria infects red blood cells, causing them to burst, releasing parasites and parasite by-products. Heme is a breakdown product of red blood cell haemoglobin. Heme is degraded by an enzyme (heme oxygenase-1; HO-1). Heme Oxygenase is an essential enzyme induced by Heme and multiple stimuli associated with critical illness. In humans, polymorphisms in the HMOX1 gene promoter may influence the magnitude of HO-1 expression. In many diseases including murine malaria, HO-1 induction produces protective anti-inflammatory effects, but observations from patients suggest these may be limited to a narrow range of HO-1 induction, prompting us to investigate the role of HO-1 in malaria infection. Method: In 382 Ghanaian children with either severe or uncomplicated P. falciparum malaria, we characterized the associations of HMOX1 promoter polymorphisms, HMOX1 mRNA inducibility, HO-1 protein levels in leucocytes (flow cytometry), and plasma (ELISA) with disease severity. Results: The (GT)n repeat polymorphism in the HMOX1 promoter was associated with HMOX1 mRNA expression in white blood cells in vitro, and with severe disease and death, while high HO-1 levels were associated with severe disease. Neutrophils were the main HO-1-expressing cells in peripheral blood, and HMOX1 mRNA expression was upregulated by heme-moieties of lysed erythrocytes. We provide mechanistic evidence that induction of HMOX1 expression in neutrophils potentiates the respiratory burst, and propose this may be part of the causal pathway explaining the association between short (GT)n repeats and increased disease severity in malaria and other critical illnesses. Conclusion: Our findings suggest a genetic predisposition to higher levels of HO-1 is associated with severe illness, and enhances the neutrophil burst leading to oxidative damage of endothelial cells. These add important information to the discussion about possible therapeutic manipulation of HO-1 in critically ill patients

## A-103

### Validation of an adjusted calcium formula using the roche calcium (nm-bapta) & albumin (bcg) methods at groote schuur hospital

M. T. Ndlovu. *National Health Laboratory Service & University of Cape Town, Cape Town, South Africa*

**Background**: Most laboratories continue to adjust serum total calcium (tCa) concentration for serum albumin as a surrogate marker of calcium status, despite the availability of ionised calcium (iCa) measurement. Current recommendations by the Association for Clinical Biochemistry and Laboratory Medicine (ACB) advocate that laboratories should use formulae specific for their tCa and albumin methods and analytical platforms. The National Health Laboratory Service at Groote Schuur Hospital (GSH) undertook to investigate this recommendation. An adjusted calcium (aCa) formula specific for the Roche serum tCa and albumin methods was derived from 3131 patients. We investigated the validity and clinical utility of this locally derived aCa formula. **Methods**: The tCa, albumin and iCa were analysed in blood from 162 inpatients and outpatients at GSH. Corrected calcium (cCa) was calculated using the Payne cCa formula, and aCa was calculated with the new aCa formula. Patients were classified as hypo-, normo- or hypercalcaemic using iCa, tCa, cCa and aCa measurements. Cohen's kappa statistic, log-linear and logistic regression models and interclass and concordance correlation coefficients were used to assess agreement between tCa, Payne cCa and aCa against iCa (gold standard). Agreement was further assessed according to renal status and albumin concentrations. **Results**: The aCa demonstrated good correlation with iCa, but its performance was not significantly better than tCa or Payne cCa in correctly classifying calcium status. Furthermore, albumin concentration was demonstrated to predict the performance of the calcium

status classification by the aCa and cCa formulae, irrespective of renal status. **Conclusion**: The laboratory-specific aCa formula did not perform significantly better than tCa and the Payne cCa formula. This implies that aCa does not add value over tCa where iCa measurements are not readily available.

## A-104

### Liver Fibrosis Biomarkers and Acoustic Radiation Force Impulse Imaging for non-invasive assessment of Non-Alcoholic Steatohepatitis in patients with morbid obesity before bariatric surgery

I. Cebreiros López, J. A. Noguera Velasco, F. Guzmán Aroca, M. D. Frutos Bernal, J. A. Luján Mompeán, Á. Bas. *Clinical University Hospital Virgen de la Arrixaca, Murcia, Spain*

**Background:** There is a wide spectrum of liver histology in Non-Alcoholic fatty liver disease (NAFLD).Simple steatosis is generally considered benign and reversible, but steatohepatitis (NASH) can evolve to progressive fibrosis and liver cirrhosis in the long term. The early detection of NASH would make it possible to anticipate the natural course of the disease, allowing therapeutic action to be proposed before the appearance of fibrosis, therefore, identification of NASH patients is crucial. Obesity is a recognized risk factor for NAFLD. The prevalence of NAFLD in the general population ranges from 15 to 30% and increases with obesity. In overweight subjects, the prevalence of steatosis is at least two times greater than in lean subjects and in morbid obesity the prevalence of NAFLD and NASH is nearly 90% and 37% respectively. Liver biopsy is the standard for the diagnosis of NAFLD but has risks and limitations, so that non-invasive diagnostic tools such as serum biomarkers, imaging methods have been developed. The ELF test measures three markers of liver matrix metabolism in serum (hyaluronic acid, procollagen-III amino terminal peptide and tissue inhibitor of metalloproteinase-1) and the result becomes a score that indicates the level of fibrosis. Acoustic Radiation Force Impulse (ARFI) is a imaging technique that provides a quantitative measure of the tissue elasticity, shear wave velocity (SWV), that correlates with the degree of fibrosis. We aimed to develop a model that combines ELF and ARFI and to assess its feasibility for detecting NASH in morbid obese patients with suspected NAFLD.

**Methods:** We selected 57 morbidly obesity patients who were to undergo bariatric surgery and were classed according to their biopsy findings. Group A (n=28): normal liver or simple steatosis; Group B (n=29): NASH and/or fibrosis. All patients were evaluated with ARFI before surgery and ELF test was calculated.

**Results:** Significant differences in ELF and SWV results were found between the two groups ($p$=0.002, $p$=0.003). AUROC for differentiating patients of both groups using ELF and SWV were 0.780 ($p$=0.002) and 0.729 ($p$=0.003). We developed a logistic regression model that combined clinical variables, biomarkers and the SWV and defined the probability of presenting NASH and/or fibrosis (group B). The variables included were age, body mass index, metabolic syndrome, mean corpuscular volume, AST, ALT, SWV and ELF. The AUROC of the model was 0.890 ($p$=0.044) and was significantly higher than any of the individual variables.

**Conclusion:** The combination of the ELF and the Arfi represents a efficient option for the identification of obese patients with NAFLD and signs of NASH and fibrosis. Using complementary methods together with different biological bases is an advantage over the use of individual techniques and increases confidence in diagnosis. In the case of patients who are going to be subjected to bariatric surgery, the use of this model would make it possible to presurgically identify those patients with more severe hepatopathy, thus establishing a population at risk in which surgery could be prioritized, and to propose prior therapeutic actions to reduce postsurgical morbidity and mortality related to hepatopathy.

## A-105

### Accuracy of various Estimated Creatinine Clearance equations in estimating Glomerular Filtration Rate in Indians

R. K. Nigam[1], M. Mathur[2]. *[1]Rajiv Gandhi College, Barkatullah University, Bhopal, India, [2]Paramedical Institute, JLN Cancer Hospital, Bhopal, India*

**Background:** The aim of this study was to compare the efficacy of GFR derived from various estimated creatinine clearance methods like Jelliffe, Cockcroft and Gault, and 4MDRD equations as compared to measured glomerular filtration rate (GFR) with in Indians

**Methods:**We enrolled 80 patients in the study. GFR was determined by technetium-99m diethyl triamine penta-acetic acid (Tc99mDTPA) clearance . Height, body weight and serum creatinine were measured, and GFR and creatinine clearance (CrCl) estimates calculated by various equations. Spearemans correlation was used to assess relationships between measured GFR (Tc99mDTPA clearance) and estimated clearances using the three formulae. Difference between the measured GFR and estimated clearances compared with measured GFR were examined to determine whether prediction error was independent from measurement magnitude. Analyses of differences were used to determine bias and precision. Bias was assessed by mean percentage error (MPE), calculated as the percentage difference between the estimated clearances for each formula and measured GFR. A positive bias indicates overestimation of GFR, and a negative bias indicates underestimation. Relationships were also assessed by gender and varying levels of renal function: GFR <60 ml / min, and GFR >60 ml/ min.

**Results:** The mean measured GFR was 77.2 ml / min (range 17 to 152 ml / min). The mean bias (mean percentage error) was -4.9, -10.3 and -1.57% respectively for the, Jelliffe , Cockcroft and Gault, and 4MDRD formulas, respectively. The 4 MDRD formula slightly overestimates the GFR in patients having GFR less than 60ml/ min, where as,it underestimates for GFR more than 60ml / min..

**Conclusion:** In Indians 4 MDRD equation of estimated creatinine clearance seems to be most accurate in estimating GFR .

## A-106

### Derivation of nepalese reference intervals for clinical chemistry parameters of liver disease by use of up to date statistical methods

R. VINOD MAHATO[1], B. YADAV[1], M. LAMSAL[2], K. ICHIHARA[3]. *[1]Tribhuvan University Institute of Medicine, Kathmandu, Nepal, [2]BP Koirala Institute of Health Science, Department of Biochemistry, Dharan, Nepal, [3]Yamaguchi University Graduate School of Medicine, Department of Laboratory Medicine, Ube, Japan*

**Background:** The reference interval (RI) is important for the screening, diagnosis, treatment, and monitoring of common disorders like liver diseases. During the last decades, the number of routine clinical chemistry tests has increased dramatically in Nepal. However, there are no valid RIs for any biochemical parameter in Nepal. RIs currently in use are those supplied by reagent manufacturers which are not derived from local population. Therefore, we conducted this study of establishing RIs of 25 major biochemistry parameters for Nepalese population, as a part of the IFCC global multicenter study on RVs. In this report, results of 7 parameters belonging to liver function tests are described.

**Method:** Total 617 apparently healthy individuals were recruited nationwide from five different regions of the country. Blood samples were collected and sera were separated and stored at $-80^0$ C. All the samples were measured collectively by auto-analyzer AU480 (Beckman-Coulter).Test results were standardized by measuring the value-assigned panel of sera which was provided by IFCC committee on Reference Intervals and Decision Limits (C-RIDL). With application of the latent abnormal values exclusion (LAVE) method, reference intervals (RIs) were derived by both parametric and non-parametric method by use of Reference Master software provided by C-RIDL.

**Results:** The reference intervals (lower limit−upper limit) derived for males[M], females [F], and both [MF] were total protein MF:6.8−8.3,M:7.0−8.5, F:7.0−8.3 g/dL albumin MF:3.9−5.0, M:4.1−5.0, F:3.8−4.6 g/dL; total bilirubin (TBil) MF :0.26−1.23,M:0.28−1.37,F:0.24−1.02mg/dL;ALTMF:5−27U/L, M:6−32, F:4−19 U/L;ASTMF:13−33,M:14−36,F:12−28U/L;ALPMF:140−397,M:138−423,F:143−379 U/L and γGT MF:10−76,M:12−115, F:9−40 U/L. Furthermore, gender-wise evaluation showed prominent increase in ALP and γGT in females after 45 years of age. While albumin in males showed linear reduction with age. Other than the 7 parameters, we also noted lower-sided shift of RIs for urea, HDL-C and LDL-C in Nepalese.

**Conclusion:** This is the first study to establish clinical reference intervals for healthy adult Nepalese population. RI of Total Protein is shifted to the higher side and TBil and ALT are shifted to the lower side in Nepalese compared to those of other countries. Some of our RIs which were standardized based on the serum panel differ from those of other countries, indicating the importance of deriving country specific RIs.

**Key words:** LFT, Reference Intervals, Liver enzymes, Total Proteins, Albumin

## A-107

### Assessment of Vitamin B12 Levels in Type 2 Diabetics on Metformin Therapy

P. Nsiah[1], E. F. Laing[2], M. Yakubu[2]. *[1]University of Cape Coast., Cape Coast, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

**Background:** Metformin, which belongs to the biguanide class, is one of the most widely used oral hypoglycemic agents for type 2 diabetes mellitus patients. Despite existing evidence of metformin-associated vitamin $B_{12}$ deficiency, large numbers of diabetics on metformin are not assessed routinely for vitamin $B_{12}$ levels in the Ghanaian setting. Methylmalonic acid (MMA) is a substance produced in very small amounts and is necessary for human metabolism and energy production. Vitamin B12 promotes the conversion of methylmalonyl CoA (a form of MMA) to succinyl Coenzyme A. Vitamin B12 deficiency results in increase in MMA concentration. This study primarily aims to determine the prevalence of vitamin B12 among type 2 diabetics on metformin and also whether there is a need for supplementation.

**Methods:** This cross-sectional study was conducted at Effia-Nkwanta Regional Hospital in the Western Region of Ghana. A total of 196 type 2 diabetic patients were recruited, consisting of 173 who were on metformin and 23, not on metformin. Vitamin B12 and methylmalonic acid (MMA) investigations were carried out on Selectra Pro S System (Elitech Clinical Systems-Elitech Group). Descriptive statistics described frequency and means.

**Results:** Vitamin B12 deficiency was defined as vitamin B12 <150ng/L and MMA concentration > 0.4µmol/L was considered high. In the type 2 diabetics taking metformin (n=173) the prevalence of B12 deficiency was 32.9% compared to 26.0% (p=0.637) in non-metformin users (n=23). Moreover, the prevalence of increased MMA was 16.2% in metformin users compared to 4.2% of non-metformin users (p=0.008). Serum vitamin B12 levels were significantly correlated with level MMA levels negatively ($R^2$=0.25, p<0.0001). Patients on metformin therapy for more than 10years increased (OR=2.68, p=0.026) the odds of having a vitamin B12 deficiency in logistic regression.

**Conclusion:** This study contributes to the existing body of literature by suggesting that long term use of metformin increases the risk of vitamin B12 deficiency. The study also demonstrated that vitamin B12 deficiency was significantly correlated with the duration of metformin use. Type 2 diabetes patients treated with metformin may require clinical attention for vitamin B12 deficiency and regular monitoring of their vitamin B12 levels.

## A-108

### THE ROLE OF CXCL10 AND HEME IN MALARIA PATHOGENESIS

F. A. BOTCHWAY[1], N. Wilson[2], J. Stiles[2]. *[1]Korlebu Teaching Hospital, Accra, Ghana, [2]Morehouse School of Medicine, Atlanta, GA*

**Background:** Plasmodium falciparum malaria remains one of the most frequently lethal diseases affecting children in sub-Saharan Africa, yet the immune mediators that regulate pathogenesis and the wide variation in clinical manifestations of malaria are poorly understood. Malaria mortality is associated with exaggerated host responses to inflammatory factors such as free heme and C-X-C motif chemokine 10 (CXCL10). The aim of this study was to determine whether the overproduction of CXCL10 and Heme play roles in malaria pathogenesis **Methods:** This was a case control study involving a total of 499 children (382 malaria subjects and 117 non malaria subjects) aged 1 -16 years. Full blood count was estimated using Haematology Analyzer BC-5300. Plasma heme was measured using chromogenic method. Plasma levels of CXCL10 were measured among the study participants using Quantikine ELISA kit using optimal concentrations of standards and antibodies according to the manufacturer's instructions. The test was analyzed at 450 nm wavelength using a Spectra Max 190 fluorescence micro plate reader. Data was presented as mean ± standard error or median and interquartile range (IQR). A p-value < 0.05 was considered statistically significant. Pearson's rank test was used to determine if there was any association between CXCL10 and Heme levels and malaria infection **Results**: There was significantly lower hemoglobin levels (12.1g/dL) in the malaria patients compared with 12.5g/dL in non-malaria subjects (p<0.001). There were significant difference in hematocrit values between malaria and non-malaria subjects (36.4 and 38.0 respectively, p <0.001) and in White cell counts in malaria compared to non-malaria white cell counts (6.9x109/L and 5.8x109/L respectively, p<0.0001). There was also significance difference in platelets counts between malaria and non-malaria platelets levels (140x109/L and 271x109/L p<0.0001). There were significant increases in plasma concentrations of CXCL10 in malaria subjects compared to non-malaria controls. (Non-malaria 180.4 pg/mL (IQR 101.1-328.6), malaria 705.7 pg/mL (IQR 459.0-1154), p < 0.0001). There was significant increase in plasma concentration of Heme in malaria compared to non -malaria controls 60.33 µM (IQR 47.67-74.34), malaria 119.57 µM (IQR 72.34-192.41), p < 0.0001) There was strong linear relationship between CXCL10 and Heme levels in malaria subjects (r =0.492, p<0.0001 **Conclusion**: Plasma levels of heme and CXCL10 were significantly increased in malaria subjects compared with non-malaria subjects. The present study has shown that the CXCL10 and Heme are markers of susceptibility to malaria in individuals living in malaria endemic areas such as Ghana.

## A-109

### Evaluation of Glycated Albumin as a Useful Indicator for Renal Dysfunction in Diabetic and Nondiabetic Population

N. Duan, H. Li, L. Pang. *Peking University First Hospital, Beijing, China*

**Background:** Glycated albumin (GA) reflects serum glucose of preceding 2-3 weeks and plays an important role in diabetes mellitus (DM). This study aimed at investigating whether GA can assess renal dysfunction in population.

**Methods:** 3818 individuals attending physical examination were enrolled in this cross-sectional study and divided into 5 groups: healthy subjects (n = 3238), impaired fasting glucose (n = 83), DM without renal complications (n = 317), DM with albuminuria (n = 64) and nondiabetic chronic kidney disease patients (n = 116). All analyses were conducted using the subjects with fasting venous blood and morning urine samples. Statistical analysis was done by SPSS 16.0.

**Results:** Among all groups, mean GA, hemoglobin A1c, fasting plasma glucose, serum creatinine were the highest and estimated glomerular filtration rate (eGFR) was the lowest in DM with albuminuria group. When eGFR was 90-105 ml/min/1.73m$^2$ or mildly decreased to 60-90 ml/min/1.73m$^2$, GA increased significantly with elevating albumin-to-creatinine ratio (ACR) from 0-10 mg/g to 10-30 mg/g to > 30 mg/g ($P$ < 0.01 and $P$ < 0.001). GA increased further when eGFR decreased moderately to severely as renal function continuing to deteriorate (eGFR ≤ 60 ml/min/1.73 m$^2$). When ACR ≤ 30 mg/g and eGFR ≤ 60 mL/min/1.73m$^2$, more than 50% subjects were DM patients and had significant higher GA levels than other subjects with eGFR > 105 mL/min/1.73m$^2$. After adjusting demographics (age at enrollment, gender and body mass index [BMI]), every 5% rise of GA levels showed a 1.778-fold increased risk in all subjects (adjusted odds ratio [OR], 1.778; 95% confidence interval [CI], 1.373-2.302; $P$ < 0.001) and 1.737-fold risk in DM subjects (adjusted OR, 1.737; 95% CI, 1.221-2.471; $P$ = 0.002) for occurrence of ACR > 30 mg/g in contrast to ACR ≤ 30 mg/g. Compared to eGFR > 90 mL/min/1.73m$^2$, 5% rise of GA levels showed a 1.482-fold risk for eGFR 60-90 mL/min/1.73m$^2$ (adjusted OR, 1.482; 95% CI, 1.112-1.975; $P$ = 0.007) and 1.996-fold risk for eGFR ≤ 60 mL/min/1.73m$^2$ (adjusted OR, 1.996; 95% CI, 1.366-2.916; $P$ < 0.001).

**Conclusions:** Increased GA serves as a risk marker for renal dysfunction and an effective complement to ACR for DM patients. GA combined with eGFR and ACR can reflect renal function changes in population, especially those DM and early diabetic kidney disease subjects whose eGFR might be in normal values.

## A-110

### Distinct expression profile of HCMV encoded miRNAs in plasma from oral lichen planus patients

C. Zhang[1], M. Ding[2], X. Wang[3], C. Wang[1], Z. Ke[2], W. Wang[3], C. Zhang[2]. *[1]Jinling Hospital, Nanjing, China, [2]Nanjing University, Nanjing, China, [3]Nanjing Stomatological Hospital, Nanjing, China*

**Background:** Human cytomegalovirus (HCMV) infection is a causal factor in the development of various diseases and some HCMV-encoded miRNAs in human circulation have been demonstrated to be novel potential biomarkers for some autoimmune diseases. Oral lichen planus (OLP) is a T-cell-mediated autoimmune disease and its aetiology and molecular mechanisms remain unclear. In this study, we examined HCMV-encoded microRNA (miRNA) expression profiles in plasma from OLP patients.**Methods:** The expression profile of HCMV-encoded miRNAs were initially measured using RT-qPCR in a cohort of individual plasma samples from 21 OLP patients and 18 healthy controls. Markedly upregulated miRNAs in patients were further verified in an additional cohort of 41 patients and 33 controls. HCMV DNA in peripheral blood leukocytes was examined by quantitative PCR and anti-HCMV IgG and IgM antibodies in plasma samples were detected by ELISA assays using a HCMV IgG/IgM kit.

**Results:**All of the examined 23 HCMV-encoded miRNAs were detectable in plasma from both OLP patients and normal controls. Seven of the 23 HCMV-encoded miRNAs were differentially expressed in OLP samples compared with controls ($P$<0.05). Six miRNAs, hcmv-miR-UL112-3p, hcmv-miR-UL112-5p, hcmv-miR-UL22a-5p, hcmv-miR-UL148D, hcmv-miR-UL36 -5p and hcmv-miR-UL59, were upregulated (1.8-7.2-fold increase, at least $P$<0.05), and 1 miRNA (hcmv-miR-US5-2-3p) was downregulated ($P$=0.04) in plasma from patients compared with controls. Of the 7 miRNAs, five miRNAs including hcmv-miR-UL112, hcmv-miR-UL22a-5p, hcmv-miR-UL148D, hcmv-miR-UL36-5p and hcmv-miR-UL59, were confirmed in the validation set (at least $P$<0.05). HCMV DNA in peripheral blood leukocytes was also significantly higher in patients than in control subjects, while the positive rates of anti-HCMV IgG and anti-HCMV IgM were not different between patients and controls(100.00% versus 93.65%, $P$ = 1.0; 0% versus 0%, $P$ = 1.0, respectively).

**Conclusion:**Our results demonstrate a distinct expression pattern HCMV-encoded miRNAs in OLP patients, which warrants additional study in the diagnosis and aetiology of OLP.

## A-111

### Effects of laboratory results and lifestyle parameters on the development of non-alcoholic fatty liver disease: The Korean National Health Insurance Service-National Sample Cohort 2009-2013

J. Rim[1], T. Youk[2], J. Cho[1], H. Gee[3], J. Yoo[2]. *[1]Yonsei University College of Medicine, Severance Hospital, Seoul, Korea, Republic of, [2]National Health Insurance Service Ilsan Hospital, Goyang, Korea, Republic of, [3]Yonsei University College of Medicine, Seoul, Korea, Republic of*

**Background:** The purpose of this study was to investigate the effects of anthropometric, laboratory, and lifestyle factors on the development of nonalcoholic fatty liver disease (NAFLD) in a nationwide, population-based, 4-year retrospective cohort.

**Methods:** The propensity score-matched study and control groups contained 1,474 subjects (940 men and 534 women) who had data in the Korean National Health Insurance Service-National Sample Cohort in 2009, 2011, and 2013. NAFLD was defined using medical records of a diagnosis confirmed by primary clinicians and meeting two previously validated fatty liver prediction models: the hepatic steatosis index and fatty liver index. Chronological changes in anthropometric variables, laboratory results, and lifestyle factors were compared to baseline values and those of the control group.

**Results:** Among the 5 anthropometric, 10 laboratory, and 3 lifestyle factors, chronological change in no single variable appeared to be statistically associated with NAFLD development in either men or women. Interestingly, baseline characteristics before the diagnosis of NAFLD seemed to be important regardless of time-dependent change throughout the 4-year period. Nevertheless, triglycerides showed prominent decrease in men during the period of NAFLD development, while weight and exercise changes were noticeable in women.

**Conclusion:** Although baseline characteristics might be important in NAFLD development, chronological changes in anthropometric, laboratory, and lifestyle factors are insufficient to predict development of NAFLD. However, we propose that early screening strategies for NAFLD should be strongly recommended for people with abrupt chronological changes in specific parameters, especially waist circumference and exercise degree for women and serum triglycerides level for men.

## A-112

### PREVALENCE OF METABOLIC SYNDROME IN GRANITE WORKERS

S. Panakala[1], R. Kondreddy[1], K. Kumar[2], A. Rawal[3], J. R. Peela[3], J. R. Kooriyil[4], M. Prasad[5], M. G. I. El Fituri[6]. *[1]Department of Biochemistry,Mamatha Medical College, Khammam, India, [2]Department of Biochemistry, Varun Arjun Medical College and Rothilkhand Hospital,, Banthra, Shahjahanpur, UP, India, [3]Department of Biochemistry and Medical Genetics,School of Medicine,St Matthews University,Grand Cayman, Grand Cayman, Cayman Islands, [4]Department of Physiology,School of Medicine,St Matthews University,Grand Cayman, Grand Cayman, Cayman Islands, [5]Narayana Medical College Hospital, Nellore,Andhra Pradesh, India, [6]Department of Global Health,College of Global Public Health,New York University, New York, NY*

**BACKGROUND:** The prevalence of the metabolic syndrome (MS) has significantly increased over the last few decades and has become a main health challenge worldwide. Prevalence of MS is quickly rising in developing countries due to changing lifestyle. It was considered worthwhile to study MS and its components in granite workers since granite factories are situated in and around Khammam area. Moreover, no studies of MS in granite workers have been reported in literature. **OBJECTIVES:** Aim of our study is to assess the prevalence of metabolic syndrome and its components in granite workers. **MATERIALS AND METHODS:** 210 male workers in the age group of 20-50 working in granite industries located in and around the Khammam town of Telangana State are selected for the present study. Blood pressures (BP), waist circumference (WC) were measured. Fasting blood samples were collected for the estimation of glucose and lipids. **RESULTS:** 69 subjects out of 210 were identified as having MS based on updated National cholesterol education program- Adult Treatment Panel III (NCEP-ATP III) guidelines. **CONCLUSION:** MS should be identified and remedial measures may be suggested, so that the risk of hypertension, cardiovascular risk, diabetes and the resultant morbidity is minimized and can be delayed.

| Prevalence of MS and its components | | |
|---|---|---|
| Paramaters | No Of Granite Workers | Percentage |
| Elevate BP | 85 | 49 |
| Elevate waist Circimference | 61 | 29 |
| Elevated TG | 52 | 25 |
| Reduced HDL | 72 | 34 |
| Elevated Glucose | 42 | 20 |
| Metabolic Syndrome | 69 | 33 |

## A-113

### Evaluating neonatal minimum volumes with Abbott Architect c8000 and Sysmex XN

P. Dolph[1], J. Baker[1], R. Wonderling[2]. *[1]MultiCare Health System, Tacoma, WA, [2]Abbott Diagnostics, Abbott Park, IL*

**Background:**Our neonatologists approached the laboratory to find a solution to reduce neonatal blood collection volumes. Nurses draw specimens in wide bore BD Microtainers (Cat # BD 365967) which can potentially reduce the amount of blood drawn from neonates, but short collections lead to high rates of QNS and redraws. Nurses frequently collected whole blood in spun hematocrit tubes and phase platelet devices in situations of previous QNS or other collection issues. We wanted a solution that was efficient and productive for both nursing and the laboratory that would maximize quality and safety for the neonates.**Methods:**We started by reviewing actual patient hematocrit values to assess the number (%) of high/low hematocrit specimens. 155 specimens in one month showed 87% of neonates with a hematocrit ≤50 and 60% of neonates ≤40. Based on this population, we conservatively based the study on a hematocrit of approximately 60% to establish extreme minimum volumes. We selected 20 most frequently ordered chemistry tests and included the volume necessary for HIL (Hemolysis, Icterus, and Lipemic specimen integrity testing.) Hematology focus was on replacing the manual parameters spun hematocrit (0.225 mL) and phase platelets (0.020 mL) with automated testing.

**Results:**The Abbott Architect c8000 Sampling Dead Volume + Over Aspiration Volume (DV/OAV) is 0.058 mL. This DV/OAV plus the accumulative aspiration volumes (calculated from the Abbott Architect Sample volumes) ranges from 0.083 mL (minimum 4 assay panel + HIL) to 0.1337 mL (maximum 20 assay panel plus

HIL). Whole blood was adjusted to resemble elevated neonate hematocrits at >60%, centrifuged and transferred to Evergreen Scientific sample cups placed in standard aliquot tubes by plastic transfer pipette. We determined the following recovery volumes of plasma after centrifugation: 0.200 mL whole blood yielded 0.076 mL plasma; 0.300 mL whole blood yielded 0.114 mL plasma; 0.400 mL whole blood yielded 0.152 mL plasma. The Abbott Architect instrument dead volume is 0.058 mL. Using similarly adjusted specimens (hematocrits at >60%), we performed volume trials on Sysmex XN for determination of a repeatable minimum volume that produces 22 parameters. We demonstrated that 0.150 mL is required for an automated panel including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, PLT, MPV, NRBC, IPF, and automated differential plus adequate volume to make a slide. This reduced the specimen volume from of 0.245 mL (2 manual tests) to 0.150mL while optimizing the availability of test options and improved quality. A visual chart of testing volumes and specimen requirements was created for NICU nurses with color photos as guides.

**Conclusion:**Abbott Architect c8000 instrument can perform 10 tests with initial volume of 0.3 mL whole blood and 20 tests on 0.4 mL whole blood (prior to centrifugation). Sysmex XN can perform CBC with platelets and a slide can be made with 0.150 mL whole blood. This project resulted in significant reduction of specimen volume collected, reduced manual processes, and promoted reliable chemistry and hematology testing to clinicians in caring for our most tiny and vulnerable patients. Satisfaction among our NICU partners has been enormous.

## A-114

### The current status of mucopolysaccharidosis I and II newborn screening, and the confirmation in Taiwan

C. Chuang[1], H. Lin[2], C. Chiang[3], M. Chan[3], L. Wang[4], R. Tu[1], T. Chen[1], S. Lin[2]. *[1]MacKay Memorial Hospital, New Taipei City, Taiwan, [2]MacKay Memorial Hospital, Taipei City, Taiwan, [3]The Chinese Foundation of Health, Taipei City, Taiwan, [4]Taipei Institute of Pathology, Taipei City, Taiwan*

**Background:** Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases. Mutations of genes encoding for lysosomal enzymes leading to defects in stepwise degradation of glycosaminoglycans (GAGs). GAGs accumulating in lysosomes result in cellular dysfunction and clinical abnormalities. Early initiation of enzyme replacement therapy (ERT) can slow or prevent the development of severe clinical manifestations. ERT for MPS I and II have become available with optimal outcomes associated with early diagnosis and treatment. MPS I and II newborn screening has been executed in all Newborn Screening Centers of Taiwan since October, 2015. The infants who failed on the recheck at the recall were referred to the Biochemical Genetics laboratory of MacKay Memorial Hospital for detailed confirmatory diagnosis.

**Methods:** From August 1st, 2015 to December 31, 2016, a total of 170,755 and 93,025 infants had been screened by mass spectrometry for MPS I and MPS II, respectively. In those, sixty-six suspicious cases including 5 for MPS I and 61 for MPS II were referred for confirmation. Urine and EDTA blood samples were collected. The urine first-line biochemistry examination was first performed, including urinary GAG quantification, two-dimensional electrophoresis, and tandem mass spectrometry assay for predominant disaccharide units of urinary GAGs. If the results showed positive, the confirmative diagnoses, including leukocyte enzymatic assay and molecular DNA analysis were then performed. Leukocyte pellet was isolated from EDTA blood and used for fluorescent α-Iduronidase (IDUA) or iduronate-2-sulfatase (IDS) enzymatic assay. In addition, DNA sequencing analysis was also performed.

**Results: N**ormality of IDS or IDUA enzyme activities was found in most referred cases except four highly suspected MPS I and one MPS II infants, which were closely correlated with individual gene variations. In those, one infant with a novel mutation of IDS gene, c.817C>T; p.273W, and four infants with four missense mutations of IDUA gene (p.D115N, p.M1T; p.Y625S, C.300-3C>G; p.D119Y, p.S206L; and p.L346R, p.T364M) showed significant deficiencies of IDS and IDUA enzyme activities (less than 5% of mean normal activity), respectively. DS and HS quantitative analysis by tandem mass spectrometry also demonstrated significant elevation.

**Conclusion:** The performance of MPS I and MPS II newborn screening, and the confirmation in Taiwan are remarkable and well-done. Early initiation of ERT for MPS results in better clinical outcome. Optimal benefits from ERT would require commencement of treatment before the onset of irreversible clinical disease, and this can be achieved by MPS newborn screening. Early confirmatory diagnosis increases the probability of receiving appropriate medical care, like ERT, soon enough to avoid irreversible manifestations.

## A-115

### Risk indices for predicting mortality and morbidity in acute pancreatitis patients: a retrospective study

C. Liu, S. Chen, H. Chen, L. Ling, J. Zhou. *Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China*

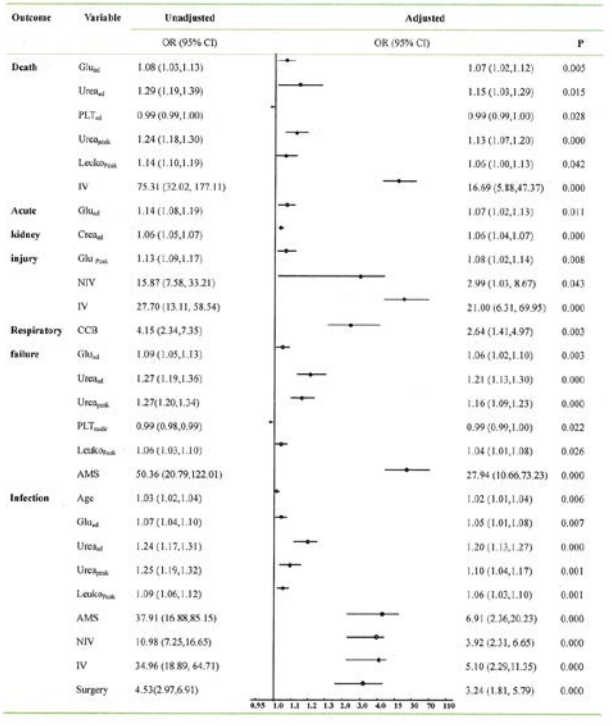
**Background:** Acute pancreatitis is a common acute abdominal disease complicated by high mortality and morbidity. This study explored simple clinical indices to predict mortality and morbidity associated with acute pancreatitis.

**Methods:** We retrospectively reviewed medical records of 1,297 patients with AP between January 2011 and March 2015. Forward univariate and multivariate logistic regression analyses were performed to assess the independent risks at admission and during hospitalization.

**Results:** In the entire cohort, 39 patients died, 71 suffered acute kidney injury, 145 suffered respiratory failure, and 159 experienced infection. After excluding patients with morbidity at admission (n = 31, 50 and 20, respectively), we found simple clinical indices for predicting mortality and morbidity. In the logistic regression analysis, glucose (OR (95%CI) : 1.07 (1.02,1.12) for death, 1.07 (1.02,1.13) for acute kidney injury, 1.06 (1.02,1.10) for respiratory failure and 1.05 (1.01,1.08) for infection) and urea levels (OR (95%CI) : 1.13 (1.07,1. 20) for death, 1.16 (1.09,1.23) for respiratory failure and 1.10 (1.04,1.17) for infection) as well as the use of invasive ventilation (OR (95%CI) : 16.69 (5.88,47.37) for death, 21.00 (6.31,69.95) for acute kidney injury and 5.10 (2.29,11.35) for infection) were identified as independent risks for mortality and morbidity of acute pancreatitis. Furthermore, platelet nadir (OR,0.99) and peak leukocyte (OR,1.06) were independent risks for death, so creatine (OR,1.06) and noninvisve ventilation (OR,2.99) for acute kidney injury. Calcium antagonists (OR,2.64), platelet nadir (OR,0.99), peak leukocyte (OR,1.04) and altered mental status (OR,27.94) were independent risks for respiratory failure. Age (OR,1.02), peak leukocyte (OR,1.06), altered mental status (OR,6.91), noninvisve ventilation (OR,3.92) and surgery (OR,3.24) were independent risks for infection.

**Conclusions:** We have found routine clinical indices to predict morbidity and mortality of acute pancreatitis patients. Early and dynamic prediction of prognosis may improve patient management.

**Fig legend** Logistic regression to identify risk factors of mortality and morbidity.

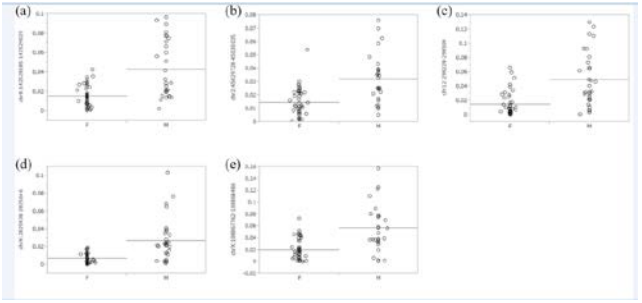


## A-116

### Within-pair Differences of DNA Methylation Levels between Identical Twins are Different between Male and Female pairs

M. Watanabe[1], C. Honda[2], T. Twin Research Group[2], Y. Iwatani[1]. *[1]Department of Biomedical Informatics, Osaka University Graduate School of Medicine, Suita, Japan, [2]Center for Twin Research, Osaka University Graduate School of Medicine, Suita, Japan*

**BACKGROUND:** DNA methylation levels will be important for detection of epigenetic effects. However, there are few reports showing sex-related differences in the sensitivity to DNA methylation. To evaluate their sex-related individual differences in the sensitivity to methylation rigorously, we performed a systematic analysis of DNA methylation in monozygotic twins, an optimal model to evaluate them because the genetic backgrounds are the same. **SUBJECTS and METHODS:** We examined 30 male and 43 female older monozygotic twin pairs recruited from the registry established by the Center for Twin Research, Osaka University. Their methylation levels were determined using the Infinium HumanMethylation450 BeadChip Kit (Illumina), which interrogated 485577 highly informative CpG sites at the single-nucleotide resolution, and the median methylation level was calculated for each of the 25657 CpG islands. **RESULTS:** Within-pair differences of methylation levels (WPDMs) were greater in male pairs than female pairs for 86.0% of autosomal CpG islands, but were higher in female pairs than male pairs for 76.7% of X chromosomal CpG islands. Mean WPDMs of CpG islands in each autosomal chromosome were significantly higher in male pairs than in female whereas that in X chromosome was significantly higher in female pairs than in male. Multiple comparison indicated that WPDMs in three autosomal and two X-chromosomal CpG islands were significantly greater in male pairs (Figure), whereas those in 22 X-chromosomal CpG islands were significantly greater in female pairs.**CONCLUSION:** Sex-related differences were present in the WPDMs of CpG islands in individuals with the same genetic background. These differences may be associated with the sexual influences in susceptibility of some diseases.




## A-117

### Growth Differentiation Factor-15 is a New Biomarker with Independent Prognostic Significance for Survival and Renal Outcomes in Different Cohorts of Patients with Light Chain (AL) Amyloidosis

I. Papassotiriou[1], G. Merlini[2], E. Terpos[3], A. Akalestos[1], F. Apostolakou[1], P. Milani[2], M. Basset[2], F. Russo[2], E. Psimenou[3], M. Roussou[3], M. Gavriatopoulou[3], D. Fotiou[3], D. Ziogas[3], C. Pamboucas[3], E. Papadopoulou[3], M. A. Dimopoulos[3], G. Palladini[2], E. Kastritis[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Amyloidosis Center, University Hospital Policlinico San Matteo, Pavia, Italy, [3]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece*

**Background**: Growth differentiation factor-15 (GDF-15), a member of TGF-beta family, is involved in several pathological conditions, which include inflammation, cancer, cardiovascular, pulmonary and renal diseases. Serum GDF-15 levels add prognostic information to conventional prognostic factors, such as NT-proBNP and troponins, in cardiovascular disorders and also have shown to be associated with renal damage and risk of end stage renal disease in patients with diabetes. Based on the above data, we evaluated the prognostic value of GDF-15 levels in patients with AL amyloidosis and showed that in a cohort of 77 patients GDF-15 was associated with early death, shorter survival and progression to dialysis, independently of the cardiac biomarkers and renal stage. In order to validate the prognostic value of serum levels of GDF-15, we evaluated GDF-15 in two independent cohorts of patients treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens). **Methods**: Circulating levels of GDF-15 were measured by a novel pre-commercial immunoassay (Roche Diagnostics), along with measurements hs-Troponin-T and NT-proBNP in more than 800 serial stored samples using electrochemiluminescence techniques. The Pavia cohort included 202 and the Athens cohort included 107 patients with AL amyloidosis. Standard criteria were used for the diagnosis, evaluation of organ involvement and cardiobiomarker-based risk stratification. Renal staging was based on baseline proteinuria >5g/day and eGFR<50ml/min. Median age and involved FLC levels were similar between the two cohorts but there were differences in other baseline characteristics including heart involvement and Mayo stage Median follow up for the Pavia cohort was 18-months and for the Athens cohort was 45-months (p<0.001), while survival at 2 years was identical. **Results**: Median GDF-15 levels was 3,027pg/ml in Pavia (range 624->100,000pg/ml) and 3,854pg/ml (range 626-71,475pg/ml) in Athens cohort (p=0.09); the upper quartile of GDF-15 levels however was ≥5,658pg/ml for the Pavia and ≥7,553pg/ml for Athens cohort, while 90% and 94% of patients in the two cohorts had GDF-15 levels >1,200pg/ml (the upper limit of normal for individuals without cardiovascular disease). We then evaluated the prognostic significance of GDF-15 levels in the two cohorts by applying the previously identified cutoff of 7,575pg/ml. GDF-15 above cutoff was associated with significantly shorter survival both in Pavia (17 months vs not reached, p=0.003) and in Athens cohort (13 vs 47 months, p=0.03). We then evaluated the prognostic significance regarding renal outcomes (dialysis): GDF-15 >4,000pg/ml was associated with a HR of 6 (95% CI 2015.6, p=0.001) in Athens cohort (progression to dialysis within 2 years in 7% vs 47%); however, very few events have occurred in Pavia cohort and analysis was inconclusive. Although renal stage discriminated 3-groups in univariate analysis (p=0.03), in multivariate analysis, GDF-15 >4,000pg/ml outperformed renal stage and was the only independent prognostic factor for dialysis risk. **Conclusion**: This study validated and confirmed in two independent cohorts, with differences in their characteristics, the prognostic value of GDF-15, which emerges as a novel biomarker with prognostic implications for different outcomes in patients with AL amyloidosis. Importantly, GDF-15 emerges also as new biomarker for renal outcomes in patients with AL-amyloidosis.

## A-118

### Prostate-Specific Antigen (PSA) Screening Rates in the United States, 2010-2015: Implications for Practice Interventions

S. Shahangian, L. Fan, T. H. Taylor. *Centers for Disease Control and Prevention, Atlanta, GA*

Background: Prostate-specific antigen (PSA) is the most commonly used cancer screening test in men. U.S. Preventive Services Task Force (USPSTF) recommended against PSA screening of all men regardless of age in 2012. This recommendation is endorsed by both American Academy of Family Physicians and American College of Preventive Medicine. By 2013, other medical organizations (e.g., American Cancer Society, American Urological Association and American College of Physicians), had recommended offering PSA screening to men between 50 and 69 years of age and with at least 10 years of remaining life expectancy, but only after a process of informed and shared decision making. The objective of this study was to evaluate the rate of PSA screening and its trend in different age groups from 2010 through 2015 using large databases covering approximately one-third of all privately insured men in the U.S. plus all men insured by Medicare. Analyzed data are expected to provide information on gaps in clinical practice, thus informing interventions needed to promote good laboratory screening practices.

Methods: Claims data for men under 65 years of age were collected using Truven Health Analytics' MarketScan database for commercial claims and encounters in 2010-2015 (9.9-16.6 million men). For men aged 65 years or older, claims data for the entire Medicare-insured population were evaluated using CMS' virtual research data center encompassing 17.7-21.8 million men for these years. A two-sided Poisson regression was used to determine $P$ for trend, calling $P < 0.05/n$ as significant, where n is the number of groups analyzed (Bonferroni correction). To consider PSA testing for screening only, all claims with any one or more of 62 prostate or urinary conditions implying use of testing for purposes other than screening were deleted.

Results: PSA-based screening rates in 2010-2015 were: 2% in men age 30-39, 5%-6% in men age 40-49, 29%-31% in men age 50-59, 33%-36% in men age 60-64, 9%-12% in men age 65-69, 11%-14% in men age 70-74, and 6%-9% in men older than 74. There were no significant temporal trends in men aged 30-39 years ($P = 0.18$), 50-59 years ($P = 0.08$), 65-69 years ($P = 0.16$), 70-74 years ($P = 0.05$) and older than 74 years ($P = 0.16$). There were significant downward trends in men aged 40-49 years ($P = 0.005$) and 60-64 years ($P = 0.003$). For all age groups, PSA screening rate decreased from 2010 to 2015 by 1%, 6%, 2%, 6%, 9%, 13% and 10%, respectively, for men aged 30-39, 40-49, 50-59, 60-64, 65-69, 70-74 and

greater than 74 years (1%-13% decrease in PSA screening rate for the 7 age cohorts).

Conclusion: All medical organizations recommended against PSA screening of men older than 69 and younger than 50 soon after the earlier recommendation by the USPSTF in 2008, but there are still a substantial proportion of younger (2%-6%) and older (6%-14%) men who are screened with PSA, contrary to all existing practice guidelines. Further research is needed to understand the reasons for overuse of PSA screening in order to identify more effective means to prevent overuse.

## A-119

### The dibucaine number is required to accurately predict pseudocholinesterase phenotypes

K. Volzing, T. L. Kautiainen, J. Ulloor, O. K. Ndimbie, M. A. Beischer, K. B. Sikder. *Abbott Laboratories, Irving, TX*

**OBJECTIVE:** The relationship between pseudocholinesterase (PChE) phenotypes and dibucaine numbers (DNs) is used to identify individuals who may experience prolonged effects of neuromuscular-blocking general anesthetics administered during surgery (Table 1). **The objective of this study is to determine if uninhibited or dibucaine-inhibited PChE activity sufficiently predicts enzyme phenotype.**

**METHODS:** Pairs of uninhibited and dibucaine-inhibited PChE activity data were analyzed for 39,719 patient samples across 43 laboratories. DNs were calculated for each sample from this paired data (Equation 1). The uninhibited and dibucaine-inhibited PChE activity of each sample was then evaluated relative to its corresponding DN to determine if a correlation exists between enzyme activity and DN.

**Equation 1** DN=100*(1-PChEDi activity/PChE activity)

**RESULTS:** Wild-type, heterozygous, and homozygous atypical PChE phenotypes were present in the data set as indicated by the DN calculation (Table 1).

**Table 1**

| DN | PChE Phenotype | Recovery from general anesthesia | Frequency - this study |
|---|---|---|---|
| DN>72 | Wild-type ($E^u_1$ $E^u_1$) | Normal | 94.2% (34,565) |
| 35≤DN≤72 | Heterozygous atypical ($E^u_1$ $E^a_1$) | Prolonged | 5.6% (2083) |
| DN<35 | Homozygous atypical ($E^a_1$ $E^a_1$) | | 0.2% (71) |

Consistent with literature, over 90% of PChE samples in this study were wild-type (DN>72) and less than 0.5% were homozygous atypical (DN<35). 100% of uninhibited homozygous atypical PChE samples displayed activity <18,000 U/L, as did 97.1% of uninhibited heterozygous, and 98.5% of uninhibited wild-type samples. Despite the small fraction of heterozygous and wild-type samples with uninhibited PChE activity >18,000 U/L, there was no overall correlation between uninhibited activity and phenotype or DN.

Dibucaine-inhibited PChE activity for all phenotypes did not exceed 16,000 U/L and did not correlate with phenotype or DN.

**CONCLUSIONS:** The DN calculation is essential for accurate PChE phenotyping. Taken in isolation, neither uninhibited nor dibucaine-inhibited PChE activity correlates with enzyme phenotype.

These data may be used to determine the DN for a patient receiving general anesthesia if they have previously suffered a prolonged recovery from anesthesia or have a family history of atypical phenotypes. Application of DN to management of these patients may prevent unexpected postoperative respiratory muscle paralysis that lasts hours to days and necessitates mechanical ventilation.

## A-120

### An observational prospective single center study to assess utility of serum cystatin C and its estimated GFR among various subgroups of patients- an experience in semi urban locality

M. S. Patwardhan[1], H. Shah[2], A. Ahuja[3], S. Babu[4]. *[1]Dr Patwardhan,s Pathology Laboratory, Ulhasnagar, India, [2]Bombay Hospital & Research Centre, Mumbai, India, [3]Shivneri Hospital, Ulhasnagar, India, [4]Agape Diagnostics, Kochi, India*

**Background:** Serum creatinine and cystatin C are complementary markers of GFR. Whereas serum creatinine is affected by age, race, sex and muscle mass, cystatin C remains unaffected. Cystatin C has been used extensively as a research tool but it is not commonly done in routine laboratories in India because of its cost. Study aims to assess the subgroups of patients with nephropathy who get additional benefit from cystatin c test over creatinine. **Methods:** Inclusion criteria- Consecutive 131 subjects seen and referred by nephrologist and physician.Exclusion criteria- Patients with age greater than 75 years, those suffering from hypo or hyperthyroidism, patients taking steroids.1) Normal male: 20 normal female: 20 (forcomparison). 2)Hypertensive patients (s. creatinine: >1.5mg/dl in males, >1.3 mg/dl in females): 21 3)Chronic glomerulonephritis (urine protein +++): 15. 4)Diabetic nephropathy (s. creatinine >1.5mg/dl in males, >1.3 mg/dl in females) : 55.

We estimated s. creatinine and urine creatinine by Jaffe kinetic. Cystatin c estimated by by turbidimetry method. Microalbumin done by turbidimetry method (Agappe India) Urine protein done by suphosalicylic turbidimetry method. Tests done on semiautomated instrument. EGFR estimation from creatinine calculated by Cockcroft-Gault equation, and from cystatin C by Hoek formula GFR = -4.32+ (80.35 × 1/cystatin C (mg/l).

**Results:**We report good correlation in healthy male & female subjects between values of creatinine, cystatin C, GFR estimated from the above and albumin/ creatinine Ratio. Reference range of cystatin c: (male) 0.78 to 1.2 mg/l, (female) 0.4 mg to 0.8 mg/l . Out of 21 Hypertensives patients (mean age 50 years), mean for creatinine eGFR was 52±18 ml with 4 patients in mild/stage 2 and rest 17 in moderate/stage 3 whereas cystatin C eGFR was reported mean of 83ml ±40ml with 2 patients in mild reduction, 8 in moderate reduction and 11 patients within normal range. Therefore only 50% of hypertensive patients showed eGFR values in abnormal range with cystatin C as compared to creatinine as verified by normal urine microalbumin. This variation in creatinine results might be due to above mentioned factors. Out of 15 patients of glomerulonephritis mean for age was 41±15 years. About 40% of these patients had borderline creatinine values (mean 1.64 mg/dl) while all cystatin C values are in pathological range (mean 2.5 mg/dl). In 55 patients suffering from diabetic nephropathy, all values show good correlation between creatinine and cystatin C. Mean for creatinine was 2.21±0.9 mg/dl with eGFR with 37±24 ml; mean for Cystatin c was 2.19±1.29 mg/L with eGFR 37.2±25 ml. All the above patients had albuminuria.**Conclusion:**Above results show the importance of cystatin C and its eGFR in hypertensives and patients suffering from glomerulonephritis. It can be used as a complementary test in patients suffering from diabetic nephropathy.

## A-121

### Graft-derived cell-free DNA - a promising rejection marker in cardiac transplantation - Results from a prospective observational trial

E. Schütz[1], L. Reinhard[2], F. Knüttgen[3], J. Beck[1], U. Schulz[4], M. Harden[5], U. Fuchs[4], N. Mettenmeyer[2], C. Knabbe[3], A. Zittermann[4], J. Gummert[4], I. Birschmann[3], M. Oellerich[2]. *[1]Chronix Biomedical, Göttingen, Germany, [2]Dept. Clinical Pharmacology University Medical Center, Göttingen, Germany, [3]Inst. for Laboratory and Transfusion Medicine, Heart and Diabetes Center NRW, Bad Oeynhausen, Germany, [4]Clinic for Thoracic and Cardiovascular Surgery, Heart and Diabetes Center NRW, Bad Oeynhausen, Germany, [5]Dept. of Medical Statistics, University Medical Center Göttingen, Göttingen, Germany*

**Background:** Reliable noninvasive markers for early detection of rejection after heart transplantation are lacking. Immunosuppressant therapeutic drug monitoring is more useful to prevent toxicity rather than to predict efficacy. Graft-derived cell-free DNA (GcfDNA) has shown promise as a biomarker for the early detection of graft injury.

**Methods:** In a prospective observational trial, GcfDNA was monitored in 84 adult cardiac transplant recipients followed over at least one year post transplantation. cfDNA was extracted from >1 ml EDTA plasma, obtained in cell-free DNA BCT tubes. GcfDNA was determined as described elsewhere (Clin Chem 2013; 59: 1732-1741). The turnaround time for an initial sample is about 2 days and one working day for any consecutive sample. Biopsies were done upon clinical suspicion of acute rejection and compared to GcfDNA test results.

**Results:** GcfDNA percentage was highly elevated (>5% of total cfDNA) on the first day after transplantation, evaluated in a subset of 15 patients, presumably due to ischemia/reperfusion damage. The median GcfDNA percentage decreased in stable patients with no signs of graft injury within the first week to a baseline value <0.25%, where it remained throughout the one year observation period. In 19 patients with samples drawn during biopsy-proven acute rejection periods (n=25), values were about 5-fold higher (median: 0.51%, 95%-CI 0.42-0.87%) than median values observed in samples (n=208) from 67 stable patients without rejection (median: 0.10%, 95%-CI 0.08-0.12%). In comparison to 23 patients with negative biopsy (n=23 samples; median: 0.19%, 95%-CI 0.16-0.26%), the median observed in patients with biopsy-proven rejection was 2.7-fold. In 14 otherwise clinically stable patients with samples available 9 to 30 days prior to diagnosis of biopsy-proven acute rejection (n=22), 5-fold elevated GcfDNA values (median: 0.47%, 95%-CI 0.26-

0.61%) compared to stable patient samples were observed. hsTroponinI showed only a moderate correlation with GcfDNA (Spearman correlation coefficient (R): 0.51, 95%-CI 0.40-0.60, n=222).

**Conclusion:** Plasma GcfDNA determinations allowed for early detection of cardiac transplant patients with acute rejection and may be helpful to personalize post-HTx immunosuppression.

## A-122

### The Application of EFIRM technology in ALDH2 rs671 Genotyping

X. Lin, X. Wang, X. He, H. Liang, X. Sun, C. JI, Y. Mo, Z. Huang, W. Liao. *Guangzhou Ezlife Biotechnology Co., Ltd., Guangzhou, China*

**Background:** Mitochondrial aldehyde dehydrogenase-2 (ALDH2) is an enzyme that oxidizes acetaldehyde into acetic acid in the alcohol metabolism. The enzyme also catalyzes vascular bioactivation of the antianginal drug nitroglycerin. As a dominant negative inhibitor, Glu504Lys (also named rs671) variant significantly reducesthe enzyme activity in heterozygotes and abolishesthe activity in homozygotes. The variant, which commonly occurs in East Asian populations, has been reported to relatewith many different kinds of disorderssuch as alcohol liver disease, colorectal cancer, and esophageal cancer. Based on our novel core technology-electric field induced release and measurement (EFIRM), EZlife Bio. has developed an effective, accurate and inexpensive genotyping method to detect the mutants of ALDH2.

**Methods:** EFIRM is an electrochemical technique that can rapidly and efficiently capture DNA targets in the fluid environment. EFIRM method has a number of key characteristics: 1. A large amount of the specific DNA probes (for ALDH2 mutants) can be efficiently immobilized to the surface of an electrode through the unique conducing polymer. The immobilized DNA probes are so sensitive to detect a single base pair mutation in the gene. 2. After this immobilization occurs, the PCR amplified ALDH2 fragments are added to the surface of the electrode and pulsed electric fields are used to drive the hybridization process between the probes and the target DNA (ALDH2 DNA fragment).And the signals are quantified using an enzymatic readout process. 3. A high-throughput array of electrodes allows 96 different samples to be simultaneously assayed using the EFIRM method.

**Results:** We collected 19 humans' buccal epithelial cells, and extracted genomic DNAs from each samples. Those DNA samples were further amplified by polymerase chain reaction (PCR). The PCR products amplified with different primers were used for genotyping by EFIRM *versus* Sanger sequencing method as a control.The EFIRM testing results of all the 119 samples including 4 mutant homozygote and 35 heterozygotes were 100% concordant to the data obtained from Sanger sequencing. The used amount of genomic DNA for PCR assay was in the range of 0.2-20 ng.

**Conclusion:** Our data indicate that EFIRM is effective, accurate, rapid, user-friendly, and cost effective method for the genotyping of ALDH2 rs671.

## A-123

### Multicenter Evaluation of Imipenem MIC Results for Gram Negative Bacilli Using MicroScan Dried Gram Negative MIC Panels

R. M. Humphries[1], J. A. Hindler[1], P. C. Schreckenberger†[2], J. Tjhio[2], M. P. Weinstein[3], S. Wood[4], A. Chipman[4], J. Y. Chau[4], D. E. Roe-Carpenter[4]. *[1]University of California Los Angeles David Geffen School of Medicine, Los Angeles, CA, [2]Loyola University Medical Center, Maywood, IL, [3]Rutgers Robert Wood Johnson Medical Center, New Brunswick, NJ, [4]Beckman Coulter, West Sacramento, CA*

**Background:** A multicenter study was performed to evaluate the accuracy of reformulated imipenem on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels.

**Materials/Methods:** For efficacy, MSDGN panels were evaluated at four sites by comparing MICs obtained using the MicroScan panel to MICs using a CLSI broth microdilution reference panel. A total of 419 gram-negative bacilli clinical isolates (39 *Acinetobacter* species, 295 *Enterobacteriaceae*, and 85 *Pseudomonas aeruginosa*) were tested using the turbidity and Prompt™* methods of inoculation. For reproducibility, a subset of 10 organisms was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ±2ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI/ISO methodology. FDA breakpoints (µg/mL) used for interpretation of MIC results were: *Acinetobacter* spp. ≤ 2 S, 4 I and ≥ 8 R; *Enterobacteriaceae* ≤ 1 S, 2 I, ≥ 4 R; *Pseudomonas aeruginosa* ≤ 2 S, 4 I, ≥ 8 R.

**Results:** When compared to frozen reference panel results, essential and categorical agreements for isolates tested in Efficacy are as follows:

| Read Method | Essential Agreement % | | Categorical Agreement % | | Very Major Errors % | | Major Errors % | | Minor Errors % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T | P | T | P | T | P | T | P | T | P |
| Visual | 98.3 (412/419) | 97.4 (408/419) | 92.4 (387/419) | 93.6 (392/419) | 0.0 (0/61) | 0.0 (0/61) | 0.9 (3/332) | 0.3 (1/332) | 6.9 (29/419) | 6.2 (26/419) |
| WalkAway | 97.9 (410/419) | 96.4 (404/419) | 91.6 (384/419) | 92.4 (387/419) | 0.0 (0/61) | 0.0 (0/61) | 0.9 (3/332) | 0.6 (2/332) | 7.6 (32/419) | 7.2 (30/419) |
| autoSCAN-4 | 98.3 (412/419) | 96.7 (405/419) | 93.1 (390/419) | 93.6 (392/419) | 0.0 (0/61) | 1.6 (1/61) | 0.9 (3/332) | 0.6 (2/332) | 6.2 (26/419) | 5.7 (24/419) |
| T = Turbidity inoculation method, P = Prompt* inoculation method | | | | | | | | | | |

Reproducibility among the four sites was greater than 95% for all read methods for both the turbidity and Prompt* inoculation methods.

**Conclusion:** This multicenter study showed that reformulated imipenem MIC results for gram-negative bacilli obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels.

†Deceased 29 November 2016.

* PROMPT is a registered trademark of 3M.



Pending clearance by US FDA, CE compliance, and other regulatory registrations.

## A-124

### Evaluation of the Bio-Rad D-100™ HemoglobinTesting System

B. Etchells, W. J. Candelaria. *Maricopa Integrated Health System, Phoenix, AZ*

**Background:** The D-100 is a high throughput automated test system used to quantify hemoglobin A1c (A1c) in whole blood samples. The D-100 uses High Performance Liquid Chromatography (HPLC) to separate hemoglobin fractions and obtain an HbA1c result that is expressed in NGSP or IFCC units. The D-100 also utilizes a bi-directional LIS interface and offers the capability of automatically reviewing (flagging or releasing) the A1c results on the system.

**Objective:** The purpose of this study is to verify and validate the manufacturer claims of 10,000 tests (injections) per analytical cartridge with prefilter change every 2,000 tests on a single calibration. This testing included precision and accuracy checks, sample throughput, hemoglobin variant detection with no interference from heterozygous hemoglobins S, C, D and E, and workflow studies.

**Methods:** Beginning January 4, 2017 a new analytical cartridge and prefilter were installed on a D-100. Calibration and QC were run per guidelines. During the 10,000 test sample run study, 4 patient EDTA whole blood samples at ~ 5, 6.5, 8 and 12 % A1c were selected and run as precision samples in duplicates, twice a day for 10 days. Additionally, 40 samples whose NGSP values were pre-assigned by SRL (Secondary Research Laboratory) were run in duplicates along with a linearity kit and 2 levels of diabetes controls. The A1c values from D-100 were compared to the NGSP values. Workflow timing was evaluated on 2 separate daily runs from start to finish. Hemoglobin variants were compared to gel electrophoresis.

**Results:** The analytical cartridge performed flawlessly over 30 days for 10,000 tests without recalibration, loss of precision or abnormal chromatography. Total precision calculations for the 4 precision samples were 0.86, 0.73, 0.87, and 0.86%. The sample throughput was confirmed to be 45 seconds per sample. The "Time to first result" was confirmed to be 2 minutes 15 seconds, where the 45 second assay of the first sample in the run follows a 1 minute 30 second system flush. Each successive sample of the run is 45 seconds.

**Conclusion:** The performance of the Bio-Rad D-100 met all of the manufacturers stated claims. The D-100 saves >80% in tech time over our previous system, the Bio-Rad VARIANT™ II TURBO. With the built-in result review criteria and the bi-directional LIS interface, >90% of our patient samples are run and verified with no tech intervention.

## A-125

### Liver injury and endotoxemia in male and female alcohol-dependent individuals admitted to an alcohol treatment program

I. Kirpich[1], V. Vatsalya[1], K. Falkner[1], C. McClain[1], J. Umhau[2]. *[1]University of Louisville, Louisville, KY, [2]Food and Drug Administration, Silver Spring, MD*

**Background:** Interactions between the liver, the gut, and the immune system are critical components of alcoholic liver disease (ALD). The aim of this study was to explore the associations between alcohol-induced liver injury, endotoxemia, and inflammation at admission and over time during abstinence; and to examine the sex-related differences in these parameters in alcohol-dependent individuals admitted to an alcohol treatment program. **Methods**: A cohort of 48 otherwise healthy participants with alcohol use disorder, but no clinical signs of alcoholic liver injury (34 males (M)/14 females (F)) admitted to an alcohol detoxification program, was stratified into two groups based on baseline plasma ALT levels (as a marker of liver injury). Group 1 (ALT < 40 U/L, 7M/8F) and Group 2 (ALT ≥ 40 U/L, 27M/6F) were identified. Plasma biomarkers of liver damage, endotoxemia and inflammation were examined at baseline (T1), day 8 (T2) and day 15 (T3) of the admission. The drinking history was also evaluated. **Results**: Sixty-nine percent of patients had elevated markers of liver damage, including ALT (98.55±9.82 *vs* 26.47±2.28 U/L), AST (130.7±17.49 *vs* 34.53 ±5.18 U/L), and cytokeratin 18 (CK18) M65 and M30 (890.3±164.6 *vs* 308.1±105.9 U/L and 378.2±59.6 *vs* 241.6±36.1 U/L, respectively; Group 2 *vs* Group 1) at baseline, indicating the presence of mild ALD. Elevated CK18 M65:M30 ratio suggested a greater contribution of necrotic rather than apoptotic hepatocyte cell death in the liver injury observed in these individuals. Positive correlations were observed in Group 2 at T1 between both CK18 M65 and ALT ($r = 0.473$, $p = 0.005$) and CK18 M30 and ALT ($r = 0.357$, $p = 0.041$). CK18 M30 was positively correlated with average drinks per drinking day at T1 in males ($r = 0.503$, $p = 0.007$). Females showed greater elevations of liver injury markers compared to males (e.g., ALT 136.2±43.8 *vs* 90.19±6.9 U/L), although they had fewer drinks per day (10.5±1.9 *vs* 15.1±1.0) and shorter lifetime duration of heavy drinking (11.5±3.6 *vs* 18.9±1.9). Liver injury was associated with systemic inflammation, specifically, elevated plasma TNF-α levels. Compared to patients without liver injury, patients with mild ALD had greater endotoxemia (increased serum LPS levels), which decreased with abstinence and this decrease preceded the drop in CK18 M65 levels. A positive correlation between LPS and ALT levels was observed at T1 only in Group 2 males ($r = 0.404$, $p = 0.037$). **Conclusions**: The study documented the association of mild alcohol-induced liver injury and endotoxemia, which improved with two weeks of abstinence, in a subset of alcohol-dependent individuals. Females demonstrated higher levels of biomarkers of liver injury compared to males, although they had lower daily alcohol intake and shorter lifetime duration of heavy drinking

## A-126

### Investigated Genotype and Phylogenetic Relationship of Carbapenem-Resistant *Enterobacteriaceae* in Regional Hospital in Taiwan from 2011 to 2016

Y. Tsai, H. Chiu, L. Wen. *En Chu Kong Hospital, New Taipei City, Taiwan*

**Background:** *Enterobacteriaceae* are inhabitants of the intestinal flora and are among the most common human pathogens, causing infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, and meningitis. Carbapenems are important last-line β-lactams antibiotics for treatment of multi-drug resistant *Enterobacteriaceae*. Carbapenem-resistant *Enterobacteriaceae* (CRE) are difficult to treat because they confer on the bacteria which resistant to most of β-lactams antibiotics which may result in global health problems. Therefore, the monitoring of CRE becomes an important issue in the clinical workplace. CRE have been reported worldwide as a consequence largely of acquisition of carbapenemase genes. In this study, we investigated the presence of carbapenemase genes expression and then follow the data to analyzed homology of *Enterobacteriaceae* with carbapenem resistance.

**Methods:** According to Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S21), the clinical specimens collected from August 2011 to July 2016 were tested for drug susceptibility to imipenem, meropenem and ertapenem using disk diffusion method. Polymerase chain reaction (PCR) was using specific primers to detect beta-lactamase (bla) genes (KPC-2, NDM-1, IMP and VIM). Pulse-filed gel electrophoresis (PFGE) was analyzed genetic relationship among isolates using restriction enzyme *Xba*I.

**Results:** The 255 CREs clinical specimens were isolated and analyzed from En Chu Kong hospital of Taiwan (with 498 beds of teaching hospital). Among those bacterial strains, the most common species were *Klebsiella pneumoniae* (n=149), followed by *Escherichia coli* (n=32), *Morganella morganii* (n=24), and other species (n=50). Of which, fourteen isolates contained KPC-2 gene (13 *K. pneumoniae* and 1 *E. coli*), nine strains detected to contain IMP gene (4 *K. pneumoniae*, 2 *E. hermannii*, 2 *Enterobacter cloacae*, and 1 *Citrobacter freundii*), one strain expressed VIM (*E. aerogenes*) and found two NDM-1 positive strain (*E. aerogenes* and *E. coli*). For this data in Taiwan, we first reported presence of NDM-1 and VIM in *E. aerogenes*. PFGE analysis found >90% belonging to same pulsotype for $bla_{KPC-2}$-carrying *K. pneumoniae* isolates that displayed high level correlation of antibiotic resistant strains. However, the 4 IMP-positive *K. pneumoniae* no major pulsotype was found.

**Conclusion:** Our results showed that the prevalence of carbapenemase genes in CREs was 10.2%. Among 255 CREs, 229 were carbapenem-resistant did not presence carbapenemase gene, and we speculated that those strains might harbor other carbapenemase genes or resistance mechanisms (ex: decreased expression of outer membrane protein). In the future work, we could use multiplex PCR or reverse transcription PCR continuous surveillance and investigation of carbapenem-resistant isolates to understand carbapenem-resistant mechanisms. In conclusion, this study indicated CRE with $bla_{KPC-2}$ was mainly in *K. pneumoniae* with high level phylogenetic relationship in our hospital. Hence, we should establish the appropriate infection control policies to prevent spread of potential resistant strains for patients' healthcare to avoid the nosocomial transmission and cluster infection.

## A-127

### Analysis of Vaginal Microecosystem In 255 Cases of Vaginal Secretions

S. Li, L. Li, W. Miao, Y. Wang, X. Pang, Z. Wang, Q. Lv, W. Xu. *The First Hospital of Jilin University, Changchun,China, China*

**BACKGROUND:** There are a variety of normal micro-organisms in the vagina to maintain the ecological balance between the human body and the environment under normal conditions. Once the balance is destroyed, the body will get sick. The vaginal microecological evaluation system refers to the comprehensive analysis of the vaginal ecological environment by describing the five parameter (including bacterial density, bacterial diversity, dominant bacteria, organism inflammatory response and pathogenic microorganisms),combining with the detection of vaginal pH value and related biochemical indexes.As a new method to detect vaginal secretions, the vaginal microecological evaluation systemis based on the theory of microbiology and the research results of infectious disease in clinical obstetrics and gynecology. Studies have shown that different degrees of micro-ecological disorders are found in current clinical vaginal inflammation. Therefore,it is essential to evaluate the vaginal microecosystem for the diagnosis and treatment of vaginitis and other vaginal-related disease.

**METHODS:** Collecting vaginal secretions of the healthy women visited in our hospital from July 2016 to October 2016 and detected the specimens respectively by the traditional cleanliness grading system and the vaginal microecological evaluation system (including bacterial density, polymorphism, dominant bacteria, pathogenic microorganisms and Nugent score, act.). Comparing the two methods and analyzing the results statistically.

**RESULTS:** Among the 255 specimens, 129 cases (50.59%) were normal in results of the cleanliness grading system test , while 93.80% (121/129) among which existed varying degrees of microecological imbalance. Among the 255 cases of specimens, the proportion of specimens with imbalanced vaginal microecosystem was as high as 96.86%, in which people who were not diagnosed clearly accounted for the highest proportion of 63.14%.On the other hand, people with bacterial vaginosis (BV) occupied the highest percentage of 25.10% among those with clear diagnosis, followed by people who were diagnosed of vulvovaginal candidiasis (VVC). And there were a certain proportion of mixed infections as well. The dominant bacteria of BV group was mainly $G^-$ short bacilli/$G^-$ Vibrio, while other groups with clear or not clear diagnosis were mainly $G^+$ coli (principally lactobacillus), which were significantly different from each other ($P<0.05$).

**CONCLUSIONS:** To compare with the traditional method of cleanliness grading system , the new method of vaginal microecological evaluation system has a higher detection rate of vaginal related diseases. Even these investigators in this study did not show clinical symptoms, there were a certain percentage of vaginal diseases among them. So it is necessary for those women to detect their vaginal secretions routinely. In addition, there are a high proportion of women with vaginal micro-ecological disorders who can not be diagnosed clearly through previous diagnostic criteria, as they did not find clinical symptoms and signs, it is inconclusive whether they should

be treated furtherly or not. So in my opinion, it is necessary to improve the diagnostic criteria of vaginal secretions for vaginal-related diseases furtherly, especially for the vaginal microecological evaluation system, so as to provide a more effective basis for the diagnosis and treatment of vaginal-related diseases.

## A-128

### Decrease of Urotensin-II Activity in Women with Restless Legs Syndrome

E. Kilic[1], G. Halac[1], S. Kesgin[1], K. Celik[1], M. Cikrikcioglu[1], A. Erek Toprak[2], M. Nasifov[1], B. Gulen[1], G. Kocaman[1], N. Ozaras[1]. *[1]Bezmi Alem Vakif University, Istanbul, Turkey, [2]Istanbul Medeniyet University, Istanbul, Turkey*

**Background**

Restless legs syndrome (RLS) is a chronic sensorimotor disorder characterized by an irresistible urge to move the legs. The pathophysiological mechanism of RLS has not been completely elucidated. This study was undertaken to compare the levels of urotensin II, a vasoactive peptide, in patients with restless legs syndrome and healthy controls and to examine possible roles of urotensin in the development of restless legs syndrome.

**Patients and Methods**

A total of 64 female patients and 53 age- and body mass index-matched healthy female controls were included. Patients with conditions known to influence the vascular tonus such as hypertension, diabetes, or vascular diseases were excluded. The diagnosis of RLS was based on the International RLS Working Group criteria and all patients met the four essential diagnostic criteria required to establish a diagnosis of RLS.

International Restless Legs Syndrome Study Group (IRLSSG) which is an assessment tool directing questions on the typical symptoms of RLS using a 5-point scale (i.e. between 0 and 4). A total score of 0 to 10, 11 to 20, 21 to 30, and 31 to 40 is indicative of mild, moderate, severe, and very severe RLS, respectively .Also the severity of RLS was assessed using John Hopkins Restless Legs Severity Scale (severity score 1=mild ,2= middle ,3= severe). The two groups were compared with respect to demographic data, routine blood tests, complete blood count parameters, and Urotensin II levels.

**Results**

Average serum Urotensin II levels were significantly lower among restless legs syndrome patients than among controls (1.43±0.46 and 1.94±0.67, respectively). A significant negative correlation with increasing disease severity scores and Urotensin II levels was found (p=0.001, r = -301) . Diastolic and systolic blood pressure values were normal in both groups with no significant differences (Table 1) . On the other hand restless legs syndrome patients had significantly lower Hb, MCV, and MCH (p=0.014, p=0.015, p=0.04, respectively). No correlations between Urotensin II and CRP, ESR, glucose, HbA1c, vitamin B12, folic acid, albumin, total protein, and calcium were found.

**Conclusion**

RLS is a disease of unknown etiology. Since a proposed mechanism for the development of RLS involves the alterations of the peripheral vascular tone, we decided to examine the association between RLS and urotensin, which is a neuropeptide with known effects on the vascular tone. Our results suggest that urotensin may have a role in the pathophysiology of RLS both through vascular mechanisms and also through its effects on the central nervous system. Further clinical multi-center studies may shed more light on potential therapeutic modalities that take this hypothesis into consideration.

## A-129

### Evaluation of the Stratus CS Acute Care Diagnostic System and ADVIA Centaur XP Immunoassay System for Cardiac Troponin I

C. Bethoney. *Siemens Healthineers, Norwood, MA*

**Objective:** The objective of this study was to demonstrate diagnostic equivalence between the cardiac troponin I (cTnI) method on the point-of-care Stratus® CS Acute Care™ Diagnostic System and the laboratory-based ADVIA Centaur® TnI-Ultra™ assay on the ADVIA Centaur XP Immunoassay System (both from Siemens Healthcare Diagnostics Inc.) in the determination of myocardial infarction (MI).

**Relevance:** Troponin is recognized as the preferred biomarker in detection of MI given its high clinical sensitivity and myocardial tissue specificity. The Third Universal Definition of MI, endorsed by national societies of cardiology and heart foundations, requires at least one cTnI value above the 99th percentile upper reference limit (URL) for detection of MI. The Stratus CS and ADVIA Centaur XP systems display optimal precision at their 99th percentile URL with a CV≤10%, allowing reliable detection of changing cTnI values.

**Methodology:** A comparison study was performed on the Stratus CS and ADVIA Centaur XP systems using frozen plasma samples from 110 patients suspected of having MI at two time points per patient. Patients were categorized as positive or negative for MI based on the presence or absence of at least one elevated cTnI value for each platform relative to the URL. Final diagnosis according to the Third Universal Definition of MI was provided for each patient as the reference standard for comparison.

**Validation:** Contingency tables were generated comparing patient outcome as determined by each system to the reference standard. The 99th percentile URL for MI is 0.07ng/mL for the Stratus CS System and 0.04ng/mL for the ADVIA Centaur XP System. Comparative receiver operating characteristic (ROC) curves were generated for each time point.

**Conclusion:** Clinical concordance was demonstrated between the Stratus CS System and ADVIA Centaur XP Immunoassay System for troponin I.

**Table 1.** Diagnostic performance measures for cTnI on Stratus CS and ADVIA Centaur XP systems.

| Estimate | ADVIA Centaur XP System | Stratus CS System |
|---|---|---|
| Sensitivity,<br>95% confidence interval (%) | 100.0<br>(92.9–100.0) | 95.9<br>(86.3–98.9) |
| Specificity,<br>95% confidence interval (%) | 100.0<br>(94.0–100.0) | 100.0<br>(93.8–100.0) |
| Positive predictive value (%) | 100.0 | 100.0 |
| Negative predictive value (%) | 100.0 | 96.7 |
| Draw 1 area under the curve,<br>95% confidence interval | 0.947<br>(0.902–0.991) | 0.865<br>(0.801–0.929) |
| Draw 2 area under the curve,<br>95% confidence interval | 1.00<br>(1.00–1.00) | 0.99<br>(0.97–1.00) |

## A-130

### The use of procalcitonin as a sepsis marker in a community hospital

N. De Oro, M. E. Gauthreaux, J. Scott, J. Lamoureux. *West Kendall Baptist Hospital, Miami, FL*

**Background:** Procalcitonin is a biomarker that shows good sensitivity and specificity in identifying septic patients. This study investigated the diagnostic accuracy of procalcitonin in a community hospital setting and how it compared to lactic acid. It also explored the impact on patient care pre and post implementation of procalcitonin in regards to direct costs and length of stay.**Methods:** There were two methods used for this study. First, two comparative groups were analyzed using an exploratory descriptive case-control study with secondary analysis of retrospective data from a 19 month period when procalcitonin was implemented. Next, a retrospective quasi-experimental study was done using a control group of emergency department patients with a sepsis diagnosis. Data included 19 months prior to the implementation of procalcitonin.**Results:** In objective 1, the sample consisted of 165 sepsis cases and there was a positive correlation between lactic acid and procalcitonin values ($r=0.377$, $p < 0.001$). From the 165 sepsis cases who had positive blood cultures, procalcitonin had a sensitivity of 89.7%. In comparison, lactic acid was not as good a predictor as procalcitonin, its sensitivity at the current cutoff of 2.0 mmol/L was 64.9%. In objective 2, there post-procalcitonin samples were 165 cases and the pre-procalcitonin sample was composed of 69 cases. There was a significant decrease after the implementation of procalcitonin in cost of hospitalization compared to costs pre-implementation (Wilcoxon $Z = 2.034$, $p = 0.042$). Although this cost is highly correlated with length of stay, neither the hospital (Wilcoxon $Z = 0.006$, $p = 0.995$) or ICU length of stay (Wilcoxon $Z = 0.037$, $p = 0.970$), showed a difference with pre and post implementation.**Conclusion:** Our findings indicate that procalcitonin values had a higher predictive usefulness than the lactic acid. A consideration for lactic acid was to lower the cutoff to 1.4 mmol/L so its sensitivity is similar to that of procalcitonin but the specificity (45.7%) and positive predictive value (35.5%) were lowered. Based on information from objective 2, the decrease in costs could be associated with a variable outside of the length of stay, or the parametric test was not powerful enough to a show a decrease in length of stay, or the decrease in costs is a spurious association. Although procalcitonin is a relatively new test, it demonstrates to be a better marker for early diagnosis of sepsis and positively impact the health care practices in a community hospital setting as indicated in objective 1. Although this test is not routinely utilized to its full potential, it offers an opportunity to reevaluate the sepsis protocols as an alternative use of procalcitonin versus lactic acid for early diagnosis.

## A-131

### Is There a Relationship Between Serum Fetuin A Levels and Hematological Parameters with Behcet Disease Activity

A. Erek Toprak[1], H. Bilen[2], A. Karadag[1], N. Akdeniz[1], R. Karadag[1]. *[1]Istanbul Medeniyet University, Istanbul, Turkey, [2]Ataturk University, Erzurum, Turkey*

**Background:** Behcet's disease (BD) is a multisystemic inflammatory condition which has unclear etiology. Serum fetuin A concentrations were shown to be inversely related with serum C reactive protein (CRP) concentrations and cytokines which are participating in inflammation, thereby fetuin A categorized as a negative acute phase protein (APP). Also Neutrophil / lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) values are suggested as simple and inexpensive inflammation markers. Fetuin-A, a glycoprotein is composed by two cystatin like domains and a smaller unrelated domain; also known as the alpha-2- Heremans-Schmid (HS)-glycoprotein, is produced principally by the liver and has an important role in normal and pathological situations, including insulin resistance, obesity, vascular and valvular calcification, bone metabolism regulation, tumor cell proliferative signaling and protease activity control.

**Objective:** We aimed to evaluate the association between serum concentrations of fetuin A, NLR, PLR and disease activity and clinical characteristics of BD.

**Methods:** The study population includes 53 BD patients (39 active, 14 inactive) and 31 healthy controls. Fetuin A, CRP, erythrocyte sedimentation rate (ESR), neutrophil, lymphocyte and platelet test were analysed and NLR and PLR values were calculated in all subjects.

**Results:** BD patients in active phase had significantly lower serum Fetuin A concentrations than both of passive BD(p=0.009) and control (p=0.006) group . Mean serum levels of Fetuin A were 294.21±85.69 µg/mL in active BD patients and 374.84±118.02 µg/mL in passive BD patients and 359.64±63.46 µg/mL in control subjects.There was no significant difference between passive BD and control group fetuin A levels(p=1.000). P/L ratio was significantly lower in active (p<0.001) and passive (p<0.001) BD patients than control group

**Conclusion:** Our findings suggest that serum Fetuin A levels are lower in patients with active BD. Fetuin A might be used in following the disease activity.

## A-132

### Serum fetuin A levels and Hematological Parameters in Chronic Kidney Disease and Hemodialysis Patients for Assessing Inflammation

A. Erek Toprak[1], F. Gerin[1], H. Erman[1], I. Duran[2], E. Atalay[3], F. Korlaelci[3], U. Ozturk[4]. *[1]Istanbul Medeniyet University, Istanbul, Turkey, [2]Bingol Government Hospital, Bingol, Turkey, [3]Kafkas University, Kars, Turkey, [4]Derince Training and Research Hospital, Kocaeli, Turkey*

**Background:** Chronic kidney disease (CKD), includes conditions that destroy the kidney and causes loss of its functions. Fetuin-A, also known as α-2 herman schemid glycoprotein belongs to the cystatin super family, a cluster of cysteine protease inhibitors. It is mainly synthesized by liver and secreted into the bloodstream. Its production is closely regulated by the inflammatory status of the body. The objective of the current study is to examine the association between serum Fetuin A concentrations and some other basic inflammation markers (neutrophyl to lympcyte ratio(NLR), mean plathelet volüme(MPV) and C reactive protein(CRP)) in chronic kidney disease and hemodialysis patients.

**Methods:** This study subjects is composed of healthy volunteers (n=47) and two patient groups; chronic kidney disease patients (n=26) and hemodialysis patients (n=33). We measured serum glucose, urea, creatinin, total protein, albumin, sodium, potassium, calcium, phosphorus, iron, alkaline phosphatase (ALP), parathyroid hormone (PTH), ferritin and CRP levels by otoanalyzer and fetuin A levels by ELISA method. Also Complete blood count parameters analysed and NLR was calculated.

**Results:** There were significant difference in serum Fetuin A concentrations, NLR and MPV values among three groups (p<0.001,p<0.001,p<0.001). The correlation analyses revealed that fetuin-A negatively correlated with urea, creatinine, PTH, ferritin, and CRP concentrations (r: -0.349, -0.367, -0.295, -0.399, -0.550, respectively, p<0.05)

**Conclusion:** Fetuin A is lower than control group in CKD and hemodialysis patients, remarking as a negative acute phase reactant. NLR and MPV values are higher in CKD group comparing with control subjects. Determination of serum fetuin-A, NLR and MPV might present useful information to assess inflammation in chronic kidney disease and hemodialysis patients.

## A-133

### External validation of lab data-based mortality risk scores in patients undergoing coronary catheterization

Y. Dong[1], M. Gerling[1], M. James[2], S. Wilton[2], M. Knudtson[2], C. Naugler[1], L. de Koning[1]. *[1]Calgary Laboratory Services, Calgary, AB, Canada, [2]University of Calgary, Calgary, AB, Canada*

**Background:** Risk scores based on results from simple laboratory tests such as the basic metabolic panel are being used more often in the acute care setting as they provide fast, reliable and accurate predictions of serious outcomes such as death and readmission. However few studies have evaluated the performance of these risk scores in different geographic locations. Our objective was to test the prognostic power of a laboratory data-based mortality risk score developed in Calgary, Alberta, Canada (population 1.4 million) in Edmonton - the second largest city in Alberta (population 1.3 million). **Methods:** Age, sex, $CO_2$, chloride, hemoglobin, MCHC, MCV, platelet count, potassium, RBC, RDW, sodium, eGFR, WBC, INR, provincial healthcare number and test verification date were extracted from the laboratory information systems of Calgary Laboratory Services and Alberta Health Services (November 2009-December 2015) and merged to patient demographic (age, sex, smoking status) and outcome data from the Alberta Provincial Project for Outcome Assessment (APPROACH) , a province-wide cardiac catheterization registry. We merged only the last lab panel up to 30 days prior to catheterization. Lab variables were divided into quintiles and programmed as four dummy variables to allow non-linear relationships. Using Calgary as a 'development' sample, logistic regression with a group LASSO penalty was used to select a panel of tests that yielded the highest c-index (measure of discrimination between deaths and survivors) and best calibration (difference from predicted vs actual death probabilities) slope p-value (non-significant = better calibration of model) for <60-day post-catheterization mortality and >= 60 day post catheterization mortality. Validation in Edmonton was done by multiplying scoring coefficients developed in Calgary by Edmonton patient values, and re-calculating c-indices, calibration slopes and slope p values. Model re-calibration was done by multiplying a scaling factor by Calgary coefficients if c-indices and calibration slopes did not match. Risk scores for each population were created by multiplying patient lab values by regression coefficients and adding the products together, after which discrimination and calibration were recalculated. **Results:** In the Calgary development sample, there were 14135 patients among which there were 216 deaths < 60 days, and 941 deaths >=60 days available for analysis. Final models contained age, sex, $CO_2$, Sodium, Chloride, hemoglobin, RBC, WBC, RDW, eGFR, and INR. The c-indices, calibration slopes and slope p-values for each score were: < 60 days: c=0.85, slope=1.01, slope p=0.75; >= 60 days: c=0.80, slope=0.99, slope p=0.73 - indicating good discrimination and model calibration for deaths < or >= 60 days after catheterization. In the Edmonton validation sample, there were 12394 patients among which there were 347 deaths < 60 days and 1073 deaths >= 60 days available for analysis. The c-indices, calibration slopes and calibration slope p-value for scores based on re-calibrated coefficients were: < 60 days: c=0.82, slope=1.05, slope p=0.07; >= 60 days: c=0.80, slope=1.02, slope p=0.05 - indicating similar discrimination but slightly worse calibration. **Conclusion:** Lab-based risk scores developed to predict mortality performed similarly in two large urban centres in Alberta, Canada. Our long-term plan is to implement these scores in coronary catheterization labs across Alberta.

## A-134

### Frequency of Mutations in the Galactose-1-Phosphate Uridyl Transferase Gene in California: Findings from an eight year study

M. R. Sartippour, R. Doroudian, P. Roworth, C. Aznar, R. Alikhani Koupaei. *Genetic Disease Laboratory Branch, Richmond, CA*

Background: The deficiency of galactose-1-phosphate uridyl transferase (GALT) which converts galactose-1-phosphate to uridine diphosphate galactose results in an inherited metabolic disorder: classic galactosemia. Common mutations/variants of this enzyme are well-identified: IVS2-2A>G, S135L, T138M, Q188R, L195P, Y209C, L218L, K285N, and N314D. The California newborn screening program uses newborns' dried blood spots to screen for GALT deficiency. Follow-up testing for presumptive positive cases uses a whole-blood specimen to identify common mutations in the GALT gene. Prior studies have reported a high frequency of some mutations, such as Q188R, in select populations. Other mutations have been described as more common in certain race/ethnic groups, including the IVS2-2 mutation, which has been reported as being more prevalent in Hispanic populations. Using data from eight years of newborn screening in California, our aim was to describe the frequency of the most common GALT mutations in the racially and ethnically diverse California Population.

Design: The Newborn Screening Program, administered by the California Department of Public Health, screens for GALT deficient newborns as part of routine newborn screening. Each year, approximately half a million newborns are tested for GALT deficiency using the API 300 Analyzer using dried blood spot specimens. Presumptive positive cases are followed up by DNA testing using a well-defined single nucleotide extension method to identify common GALT gene mutations. We studied the frequencies of 7 mutations and 2 variants in newborns tested between July 2008 and June 2016.

Results: Out of nearly 4 million babies screened in California during the 8-year period, 247 GALT-deficient newborns had follow-up mutation analysis. The Q188R (66.40%) mutation and the variant N314D (66.40%) had the highest frequency in one or 2 alleles. This was followed by IVS2-2G (7.69%), S135L (6.88%), K285N (2.43%), L195P (2.02%), Y209C (0.81%), and L218L (0.4%). The T138M mutation was not detected in any newborns. The frequency of homozygosity was 6.07% for the Q188R mutation, 4.45% for the N314D variant, and 1.21% for the S135L. We also found a higher prevalence of the IVS2-2 mutation, known to be more common in Hispanic populations.

Conclusion: To the best of our knowledge this is the largest study to describe the frequency of GALT mutations in a racially and ethnicity diverse population. This is also the first study to use single nucleotide extension to study the IVS2-2 mutation. In concordance with other studies, the Q188R was the mutation with a very high prevalence; and similar to other studies with extremely low frequency for T138M mutation, none of the specimens were detected with this mutation. The low percentage of L218L is due to the GALT deficient population being tested. L218L variant increases GALT and we didn't expect high frequency of this variant in our GALT deficient population. Some of our findings were similar to studies from Texas, another state with a high Hispanic population. This includes a higher prevalence of the N314D and IVS2-2 mutations. The frequency of the IVS2-2 mutation was even higher than the Texas study and may reflect more births to newborns with Hispanic origin in California.

## A-135

### Prevalence and laboratory characterization of monoclonal gammopathy of undetermined significance by physical examination in Beijing

J. Han, Y. Liang, X. Liang, J. Li, X. Cao, T. Xu, J. Wu, C. Jin, W. Ji, Q. Di, J. Zhang, W. Su. *Peking Union Medical College Hospital, Beijing, China*

**Background**

Monoclonal gammopathy of undetermined significance (MGUS) is a benign condition present to monoclonal plasma cell proliferation in the people aged more than 50 years old. No studies have far evaluated a large population of apparently healthy adults in ethnically Chinese population. To gain further understanding about the prevalence and laboratory characters of MGUS in China mainland, the current study was designed as a general analysis that know the related lab features in people who underwent physical examinations in Beijing with MGUS.

**Methods**

A total of 44443 adult patients from the physical examination center were retrospectively enrolled in the study. The age range of these subjects was from 18 to 96 years old and the mean age was 42.44±12.62 years. Capillary serum protein electrophoresis (SPE) was carried out as main screening test on the subjects. Serum samples which were found monoclonal bands or suspicious monoclonal protein (M protein) were subjected to agarose gel immunofixation electrophoresis (IFE) to identify. Serum free light chain (sFLC) and β2 micro globulin were quantified to the samples which IFE results were positive.

**Results**

A total of 295 patients were diagnosed as MGUS according to the definition and suggestion of clinicians. The overall prevalence of MGUS was 0.66%. The prevalence was significantly higher in males than that in females (0.78% vs. 0.52% respectively, $P$=0.001, $P$<0.01). MGUS prevalence also significantly increased with age and it was 1.05%, 1.79%, 4.17%, 4.53% respectively in subjects older than 50, 60, and 70 and 80 years old. Immunofixation electrophoresis analysis (IFE) showed that IgG kappa (39.32%), IgG lamda (32.88%) and IgA lamda (12.54%) were the predominant monoclonal immunoglobulin pattern in these MGUS subjects. Double-type and lamda light chain pattern were simultaneously found in MGUS patients. The monoclonal immunoglobulin concentration was from 0.02 g/dL to 1.92 g/dL in the MGUS patients. The median concentration was 2.80 g/dL and 95% confidence interval was 3.49 g/dL to 4.50 g/dL. The serum free light chain (sFLC) κ: λ ratio range was from 0.00075mg/L to 34.077mg/L. There were respectively 32 (11.03%) and 33 (11.38%) serum samples which showed sFLC κ: λ ratio lower or higher than the reference range. We found 12 (4.17%) samples in MGUS were higher than 3.5 mg/L and 4 (1.39%) samples were higher than 5.5 mg/L with the concentration of serum $\beta_2$-microglobulin.

**Conclusions**

The overall prevalence of MGUS in China mainland was 0.66%. The prevalence was gender and age dependent. Some main lab characters of MGUS patients were evaluated in Chinese population.

## A-136

### Diagnostic value of different detecting technologies in antinuclear antibody (ANA) in autoimmune diseases

Y. Huang, W. Wang, K. Chen. *Sun Yat-sen University affiliated Zhongshan hospital, Zhongshan, China*

**Background:** Autoimmune diseases refer to immune reaction between the body antibody and its own antigen, which results in tissue damage. Antinuclear antibodies (ANA) play an important role in the diagnosis, treatment monitoring and prognosis judgement of autoimmune disease. Antinuclear antibodies (ANA) is an important screening index of autoimmune disease . But detection methods of ANA are varied.So our study is to investigate the positive rate and the coincidence rate between different methods,and our further research is to investigate the inconsistent phenomenon and the solutions, in order to provide more valuable reference information for clinical diagnosis, treatment monitoring and prognosis judgement in autoimmune diseases.
**Methods:** We collected 61 cases of patients with systemic lupus erythematosus (SLE), 181 cases of patients with autoimmune diseases but without SLE, 476 cases of patients without immune diseases, 710 cases of volunteers for control group. ANA was detected by ELISA and indirect immunofluorescence (IIF) method. 12 kinds of specific autoantibodies in ANA were detected by western blot method (LIA).

**Results:** Average coincidence rate between ELISA and IIF method to detect the ANA was 77.7% in the four groups of participants. The results of ANA detected by ELISA method were positively correlated with that by IIF method (correlation coefficient of 0.598, $P < 0.01$). Then we study the inconsistent results of ANA respectively detected by IIF method and LIA method,we found that patients with AID and no AID groups both existed the phenomenon of IIF ANA (+) LIA ANA (-), their fluorescence titers were given priority to with 1:100.Their fluorescence modes were given priority to with granularity (S) in AID group, significantly higher than that of no AID group; no AID group fluorescence mode was given priority to with type cytoplasm particles (Cyto). At the same time, the study found that patients with IIFANA (-) LIA ANA (+) were mostly AID patients (73.3%), the ratio was higher than no AID group, among these patients ,AID patients were with SSA/Ro60, SSA/Ro52, SSB/La as the main positive autoantibodies, rather than no AID patients with anti - Sm, SSA/Ro52, SSB/La, Cenp B as the main positive antibodies. SLE group, the other AID group compared with control group, in addition to Jo - 1 (LIA), the positive rate of other specific autoantibodies were Statistic different ($P < 0.05$). Among these specific autoantibodies,we collect six indexes well related with SLE: ANA, anti dsDNA antibodies, nRNP/Sm, Sm, AnuA, AHA to multi-index joint detection . In SLE group, the positive rate of ANA ,dsDNA, AnuA joint detection model was higher than other detection models(ANA, dsDNA, Sm model;ANA, dsDNA, AHA model,;dsDNA, Sm model).

**Conclusion:** Different detecting technologies in ANA have differences between sensitivity and specificity.If you use only one kind of methods ,it is likely to cause the positive ANA result omission and AID missed diagnosis. Therefore, clinical application, especially in patients with clinically suspected AID, we should carry out ELISA-ANA or IIF - ANA screening and LIA-ANA for various specific autoantibodies detection at the same time, and pay attention to multi-index joint detection when we diagnose autoimmune diseases.

## A-137

### An Evaluation of the Automated Analysis of Common Urinary Sediments on the Sysmex UX-2000

M. A. Hey, C. Yeoh, B. Lee, A. Omar, M. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

**Background:** Urinalysis is a routinely ordered test to screen for urinary tract infections and renal abnormalities. During a recent open tender process, we evaluated the UX-2000, an automated test strip and particle analysis urinalysis instrument, to supersede our existing UF-1000i analyser. A comparison of the common urine sediments between the two instruments was used to evaluate the UX-2000.

**Methods:** Fresh mid-stream urine was collected from 113 patients in a multi-disciplinary acute care hospital and promptly sent to the laboratory for examination.

These samples were analysed on both analysers concurrently, using an established workflow. The principle of analysis for both instruments is based on fluorescence flow cytometry. The parameters for the evaluation included: Red blood cells(RBCs), White blood cells(WBCs), Epithelial cells(ECs) and Bacteria. Trueness, imprecision, clinical sensitivity and clinical specificity were determined.

**Results:** Trueness studies against the UF-1000i yielded a relationship of y=0.85x+4.36, y=1.01x+0.95, y=0.97x+0.43, y=1.17x-20.14 for RBCs, WBCs, ECs and Bacteria respectively. The correlation coefficients ranged between 0.978 and to 0.999.

Analytical specificities were 98.1%, 95.6%, 94.6% and 97.9% while analytical sensitivities were 97.9%, 96.4%, 88.9% and 90.6% at their cutoffs of >13 cells/ul, >10 cells/ul, >7 cells/ul and >20 cells/ul for RBCs, WBCs, ECs and bacteria, respectively.

The imprecision study performed using low level quality control material yielded a Coefficient of Variation of 5.4%, 7.9%, 9.8% and 9.4% for RBCs, WBCs, ECs and Bacteria respectively. Additionally, the imprecision study using high level quality control material yielded a Coefficient of Variation of 2.6%, 1.3%, 4.0% and 4.8% for RBCs, WBCs, ECs and Bacteria respectively. These values fall within the manufacturer's recommendations of 10% for RBCs and WBCs, 30% for ECs and 20% for Bacteria.

**Conclusion:** Our study showed that the UX-2000 had an acceptable performance and is a suitable replacement for our current analyser, the UF-1000i. We found good correlation between the two analysers for the automated quantification of RBCs, WBCs, ECs and Bacteria. In addition, the UX-2000 analyser's built-in automated Dipstick analyser allows for lesser hands-on time which, which translates to increased productivity.

## A-138

### Development and evaluation of a serum biomarker panel in diagnosis of lung cancer

Q. Du[1], H. Wang[2], W. Zhang[3], A. Beshiri[4], A. Soh[5], Y. Zheng[6]. *[1]Department of Pulmonary Medicine , Da'lian Medical University 2nd Hospital, Da'lian, China, [2]Department of Pulmonary Medicine , Da'lian Medical University 2nd Hospital, Da'lian, China, [3]Department of Mathematics & Statistics, University of Arkansas at Little Rock, AR, AK, [4]Medical Scientific Affairs, Diagnostics Division,Abbott Laboratories, Chicago, IL, [5]Medical Scientific Affairs, Diagnostics Division,Abbott Laboratories, Singapore, Singapore, [6]Medical Scientific Affairs, Diagnostics Division,Abbott Laboratories, Shanghai, China*

**Background**

Lung cancer is the most common cancer and leading cause of cancer-related deaths worldwide. With the progress of tumor stage, the prognosis was significantly worse. Thus, early diagnosis of lung cancer is important to improve patient survivals. Blood-based biomarkers are easily accessible measurements that define high-risk patients and enhance diagnostic capabilities. Although published data on individual serum biomarkers for lung cancer have shown limited sensitivity and specificity, the combination of several biomarkers could improve the detection of early-stage lung cancer. Thus, the present study is to investigate the clinical diagnosis model for lung cancer diagnosis.

**Methods**

A total of 230 lung cancer patients (cancer group) and 80 subjects with benign lung tumors (control group), were recruited from Da'lian Medical University 2nd Hospital in China. The study received ethical approval from the site.

Serum CEA, SCC, CYFRA21-1, HE4 and ProGRP levels were examined by the ARCHITECT i2000( Abbott,USA).

**Results**

We examined the diagnostic value of CEA, SCC, CYFR211, ProGRP and HE4 individually or in combination for lung cancer using ROC curve analysis. The optimal cut-off value of each biomarker was obtained to yield the maximum Youden index.

Compared with control, AUC of individual biomarker CEA, SCC, CY211, ProGRP and HE4 exhibited from 0.648 to 0.857. To increase the diagnostic value, these five biomarkers were combined and equation model was set up. The equation was P = 0.121*CEA + 0.568*SCC + 0.513*CY211 + 0.011*ProGRP + 0.036*HE - 3.972, with cut-off of 0.729. AUC in biomarker combination increased to 0.907 with sesitivity of 70.6% and specificity of 94.90%. According to sub-type analysis, biomarker panel combination showed better performance in SCC(Squamous carcinoma) and SCLC(Small cell lung cancer) compared with AD(Adenocarcinoma).

**Conclusions**

This study established a biomarker combination model for lung cancer diagnosis with significant clinical performance.

**Table 1 Evaluation of a serum biomarker panel for lung cancer**

| | AUC | 95%CI | sensitivity | specificity | Youden's index |
|---|---|---|---|---|---|
| **LC VS Control** | | | | | |
| CEA | 0.717 | 0.672-0.763 | 36.20% | 94.90% | 0.311 |
| SCC | 0.702 | 0.655-0.748 | 32.40% | 95.50% | 0.279 |
| CY211 | 0.758 | 0.715-0.8 | 44.70% | 94.90% | 0.396 |
| pro-GRP | 0.648 | 0.599-0.697 | 29.00% | 94.90% | 0.239 |
| HE4 | 0.857 | 0.825-0.889 | 56.70% | 94.90% | 0.516 |
| panel | 0.907 | 0.881-0.932 | 70.6% | 94.90% | 0.655 |
| **AD vs Control** | | | | | |
| CEA | 0.74 | 0.69-0.79 | 43.20% | 94.90% | 0.381 |
| SCC | 0.674 | 0.619-0.728 | 28.10% | 95.50% | 0.236 |
| CY211 | 0.696 | 0.643-0.750 | 36.50% | 94.90% | 0.314 |
| proGRP | 0.593 | 0.536-0.651 | 18.20% | 94.90% | 0.131 |
| HE4 | 0.829 | 0.789-0.870 | 50.50% | 94.90% | 0.454 |
| panel | 0.875 | 0.840-0.909 | 60.40% | 94.90% | 0.553 |
| **SCC vs Control** | | | | | |
| CEA | 0.685 | 0.61-0.76 | 19.70% | 94.90% | 0.146 |
| SCC | 0.906 | 0.859-0.954 | 60.70% | 95.50% | 0.562 |
| CY211 | 0.91 | 0.858-0.961 | 75.40% | 94.90% | 0.703 |
| proGRP | 0.634 | 0.547-0.721 | 27.90% | 94.90% | 0.228 |
| HE4 | 0.93 | 0.895-0.965 | 70.50% | 94.90% | 0.654 |
| panel | 0.987 | 0.975-0.999 | 93.4% | 94.90% | 0.883 |
| **SCLC vs Control** | | | | | |
| CEA | 0.658 | 0.558-0.759 | 28.9% | 94.90% | 0.238 |
| SCC | 0.524 | 0.419-0.63 | 11.1% | 95.50% | 0.066 |
| CY211 | 0.82 | 0.747-0.894 | 42.20% | 94.90% | 0.371 |
| proGRP | 0.928 | 0.87-0.986 | 91.10% | 94.90% | 0.86 |
| HE4 | 0.88 | 0.812-0.948 | 73.30% | 94.90% | 0.682 |
| panel | 0.989 | 0.000-1.0000 | 95.00% | 94.90% | 0.899 |

## A-139

### Clinical evaluation of PIVKA-II in Hepatocellular carcinoma diagnosis in Chinese population-a single center analysis

T. Yang[1], H. Xing[1], S. Dai[1], C. Yan[2], N. Wang[3], W. Zhang[4], A. Tran[5], A. Beshiri[6], A. Soh[7], Y. Zheng[8], F. Shen[1]. *[1]Department of Hepatic Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China, [2]Peking University First Hospital, Beijing, China, [3]The Second Hospital of Nanjing, Affiliated to Medical School of Southeast University, Nanjing, China, [4]Department of Mathematics & Statistics, University of Arkansas at Little Rock, RA, AR, [5]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Ho Chi Minh City, Viet Nam, [6]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Chicago, IL, [7]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Singapore, Singapore, [8]Medical Scientific Affairs, Diagnostics Division, Abbott Laboratories, Shanghai, China*

**Background and Aims**

Liver cancer is a leading cause of cancer-related deaths in Asia. There are serval subtypes of liver cancer, of which hepatocellular carcinoma (HCC) is the major one. Persistent viral infections with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) contribute to the pathogenesis of HCC. Unfortunately, there are around 250,000,000 HBV carriers in Asia, presenting the unmet medical needs for liver health management and early HCC diagnosis.

Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is an abnormal des-gamma-carboxyprothrombin (DCP) in patients with vitamin K deficiency and recently showed the clinical value in HCC diagnosis. Most of the clinical data is from Japan. However, its clinical application still needs more evidence supports in Asian population. In addition, most of HCC patients in Japan are HCV related while other Asian countries are HBV related. Thus, we initiated an Asian study on the application of PIVKA-II in HCC diagnosis and differential diagnosis including China, Singapore, Vietnam and Thailand. Here we report the preliminary results from a site in Eastern China.

**Methods**

A total of 161 cases of HCC and 136 cases of cirrhosis were recruited from Eastern Hepatobiliary Surgery Hospital in China. All the patients' information were recorded and validated. The study received ethical approval from the site.

PIVKA-II levels were examined by the ARCHITECT PIVKA-II assay( Abbott,USA), which is a two-step sandwich immunoassay, using chemiluminescent paramagnetic microparticle technology for quantitative determination of PIVKA-II.

**Results**

Distributions of serum PVKA-II levels were significantly higher in HCC patients compared with cirrhosis patients. P value was <0.001 comparing HCC with cirrhosis group. In addition, we depicted ROC curve to evaluate the diagnostic performance of PIVKA-II as a biomarker . As a result, our curve showed the area under the ROC curve (AUROC) for PIVKA-II to be 0.87(95% confidence interval, CI: 0.82 - 0.91) in all HCC patients. Under different cut-off values , the sensitivity and specificity varied. When the sensitivity reached 100.0%, the specificity was only 5.1% at the cut-off of 11.9 mAU/ml. When it was set at 40.0 mAU/ml(the most common used cut-off value), the sensitivity was 84.2% with specificity of 95.5%. If the specificity was set-off at 100.0%, the sensitivity was 52.0% and the cut-off value was 256.4 mAU/ml.

**Conclusions**

The preliminary data supports the clinical application of PIVKA-II in HCC diagnosis in China population. However, the detailed clinical diagnosis strategy is still under investigated.

## A-140

### Association between SHBG & hs-CRP - A novel surrogate marker of Preeclampsia.

P. Udagani[1], V. C[2], V. KL[2], P. Goyal[1], M. KV[2]. *[1]Dr. Ram Manohar Lohia Hospital, New Delhi, New Delhi, India, [2]Bangalore Medical College and Research Institute, Bangalore, India*

**Background:**

Preeclampsia affects 3-5% of pregnancies. A woman in a developing country is seven times more likely to develop preeclampsia than in a developed country. 10-25% of these cases result in maternal death. In these disorders, placental ischemia and inflammation occur due to impaired trophoblastic invasion in uterine spiral artery. Inflammatory mediators released from ischemic placenta into the circulation lead to insulin resistance which further leads to endothelial dysfunction and hypertension. Research has proved that SHBG levels follow insulin trajectories.

**Objectives:**

a. To evaluate levels of inflammatory markers High sensitive C- reactive protein (hs-CRP) and Sex Hormone Binding Globulin (SHBG) in maternal circulation in normal and pre-eclamptic pregnancies.

b. To study the possible relation between maternal hs-CRP and SHBG in normal and pre-eclamptic pregnancies.

**Methods:**

A case control study of 60 subjects (30 preeclamptic primigravida and 30 age matched healthy pregnancies). Serum SHBG was measured by chemiluminescence and hs-CRP by immunoturbidimetric method.

**Inclusion Criteria**

a. Preeclamptic primigravida of gestational age above 20 weeks.

b. The diagnosis of preeclampsia was made according to the criteria by ACOG

1. Blood pressure higher than 140/90 mmHg

2. Edema

3. Proteinuria >300mg/24 hour or 1+dipstick method after 20th weeks of gestation.

**Exclusion criteria**

a. Patients with history of Gestational Diabetes Mellitus, Essential Hypertension, Diabetes Mellitus and other Cardio-Vascular diseases

b. Patients with history of Hypothyroidism, Inflammatory Diseases or patient on Corticosteroid Therapy.

**Reference Intervals:**

SHBG - 216-724mmol/l in third trimester

hs-CRP- <0.5mg/dl

**Results**

Serum SHBG levels were significantly decreased in women with preeclampsia when compared with controls (p value < 0.001). Levels of hs-CRP increased significantly in women with preeclampsia when compared with controls ( p value < 0.0001) . SHBG correlated significantly negatively with markers of severity of disease like SBP,DBP and uric acid. hs-CRP correlated significantly positively with SBP,DBP , uric acid. SHBG and hs-CRP had a significant (p=0.001) negative correlation with each other.

By ROC analysis, hs-CRP has significant cut off points of > 0.26 with sensitivity of 90 and specificity of 73.33 that is if the value is > 0.26 then there is significantly high risk disease.

SHBG has significant cut off point ≤ 525.4 with sensitivity of 80 and specificity of 60.

In univariate logistic regression for disease as dependent variable, with the increase in SHBG by 1 unit, risk disease significantly decrease by 5% with p value 0.06.

With increase in hs-CRP by 0.01 unit, risk of disease significantly increases by 88.19% with p value 0.005.

**Conclusion**

Increased levels of hs-CRP and decreased levels of SHBG are considered as supportive diagnostic tools in preeclampsia along with conventional markers. Since women with preeclampsia have a 1.5 to 2 times higher risk of developing heart disease later in life, hs-CRP and SHBG can also serve as indicators of future risk of cardiovascular diseases in these patient groups. Insulin resistance appears to be a potential mechanism linking preeclampsia and prospective risk of cardiovascular diseases.

## A-141

### A Prospective, Observational Study of the Clinical Decision Impact of the Prostate Health Index (*phi*) Test in a Urology Practice Setting

J. S. White[1], B. V. Shenoy[1], R. F. Tutrone[2], L. I. Karsh[3], W. J. Harmon[4], D. L. Broyles[5], T. E. Roddy[5], L. R. Lofaro[5], M. A. Reynolds[5]. *[1]Carolina Urology Partners, Huntersville, NC, [2]Chesapeake Urology, Baltimore, MD, [3]Urology Center of Colorado, Denver, CO, [4]Urology San Antonio, San Antonio, TX, [5]Beckman Coulter, Carlsbad, CA*

Objective

Data are limited on the clinical utility and cost effectiveness of new tests to detect PCa, and additional evidence is needed to determine their impact on physician decision making and patient care. The objective was to determine the impact of *phi* on patient management, by comparing physician responses to a questionnaire pre- and post-*phi* testing. The impact on decision to biopsy and proportion of negative biopsy results obtained was also evaluated.

Methods

An IRB approved observational study was conducted at four large urology group practices using a two-part questionnaire completed by the urologist. Patient recommendations were recorded before and after the *phi* score. A historical control group was queried from electronic medical records for eligible patients who did not receive a *phi* test. 502 men were prospectively, consecutively enrolled, and 575 additional men were included in the control arm, after exclusions. Subjects had PSA concentrations between 4-10 ng/mL, were ≥50 years of age, had a non-suspicious DRE, no prior suspicious or positive prostate biopsy, and were referred for biopsy based on elevated PSA. The PSA, free PSA, and [-2]proPSA concentrations were determined using the Beckman Coulter Access 2 Immunoassay Analyzer. *phi* was calculated as ([-2]proPSA/free PSA) x $PSA^{½}$.

Results

*The phi* score impacted physician decisions in about 74% of cases (Figure 1), including performing biopsies when the *phi* score was elevated and identifying biopsies that may have been unnecessary or that could be delayed. In instances where the biopsy decision was unchanged, questionnaire responses showed *phi* helped confirm decisions and made discussions with patients easier.

Figure 1.

| | *phi* score group | | | | | |
|---|---|---|---|---|---|---|
| **Did *phi* score impact decision to Biopsy or Monitor patient?** | **0-26.9 N (%)** | **27.0-35.9 N (%)** | **36.0-54.9 N (%)** | **55+ N (%)** | **Total** | **Percent of Total** |
| **Yes**, *phi* score impacted physician's decision | 81 (22.1) | 85 (23.2) | 130 (35.6) | 70 (19.1) | 366 | **73.5** |
| **No**, *phi* score did not impact physician's decision | 35 (26.5) | 34 (25.8) | 44 (33.3) | 19 (14.4) | 132 | **26.5** |
| **Total** | 116 (23.3) | 119 (23.9) | 174 (34.9) | 89 (17.9) | 498 *four missing | |

| Pre-*phi* Decision Biopsy | | | | Pre-*phi* Decision Monitor | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Post-*phi* Decision Biopsy N = 169 | | Post-*phi* Decision Monitor N = 145 | | Post-*phi* Decision Biopsy N = 70 | | Frequency and type of testing *unchanged* N = 18 | | Frequency and type of testing *changed* N = 96 | |
| *Yes* *N = 84 (50 %)* | No N = 85 | *Yes* *N =145 (100%)* | No N = 0 | *Yes* *N = 70 (100%)* | No N = 0 | *Yes* *N = 17 (94 %)* | No N = 1 | *Yes* *N = 50 (52 %)* | No N = 46 |

Conclusions

Results demonstrate that *phi* positively impacts patient care. The biopsy rate was significantly lower, and the percentage of GS ≥ 7 was significantly higher, post-*phi* testing versus controls, demonstrating a reduction in unnecessary biopsies and a selective shift toward identifying clinically significant PCa.

## A-142

### Serum Oxidative and Antioxidative Parameters in Obstructive Sleep Apne Syndrome Patients

H. Erman[1], A. Erek Toprak[1], E. Kilic[2], I. Yilmaz[1], N. Genc Kahraman[1], A. Kanbay[1]. *[1]Medeniyet university Medeniyet medical faculty, Istanbul, Turkey, [2]Bezmialem university bezmialem medical faculty, Istanbul, Turkey*

**Background:** The purpose of this study was to evaluate markers of systemic oxidative stress and antioxidant capacity in subjects with severity of OSAS.

**Methods:** A total of 106 OSA patients were included in the study (18 controls, 14 with mild, 14 with moderate, and 60 with severe OSA). Patients were grouped according to apneahypopnea index (AHI) as mild, moderate and severe OSA. Patients with AHI<5 served as control group. Known risk factors for oxidative stress, such as age, sex, obesity, smoking, hypelipidemia, and hypertension, were investigated as possible confounding factors. Plasma arylesterase, total oxidative stress (TOS), total antioxidant capacity (TAC), total thiol, catalase (CAT) levels were measured for all patients.

**Results:** The mean age was 52.49 ±12.9 years and 40.6 % (43/106) of the study population was female. Plasma arylesterase, TOS, TAC, total thiol, and CAT plasma values were not different between mild, moderate, severe OSA groups and controls ($p > 0.05$). Catalase levels were significantly lower in women patients with severe OSA compared to healthy women controls ($p < 0.05$). There was a negative correlation between AHI and serum total thiol levels ($r= -0.289$, $p<0.05$) in severe OSA groups.

**Conclusion:** The present prospective study provides evidence that OSA might be associated with decreased antioxidant burden possibly via catalase way.

## A-143

### Where we Stand on Omission of Biopsy for a Diagnosis of Celiac Disease

S. Jamshed, M. R. Al-Turkmani, L. V. Rao. *University of Massachusetts Medical School, Department of Pathology, Worcester, MA*

**Background:**

Duodenal biopsy has remained the gold standard for a Celiac disease (CD) diagnosis for decades. However, improved diagnostic accuracy of serological testing and potential risks associated with endoscopy in children has allowed omission of biopsy to become the standard of care in Europe under the 2012 ESPGHAN Guidelines. The diagnostic algorithm allows symptomatic children with screening tissue transglutaminase IgA (tTG-IgA) titer more than 10x upper limit of normal to undergo EMA and HLA testing. To date, this is the first widely accepted alternative to biopsy. Our aim is to retrospectively examine the performance of tTG-IgA at our institution to assess the feasibility of shifting towards a non-invasive diagnosis for CD in a specific pediatric population in the United States.

**Methods:**

Consecutive tTG-IgA serology was obtained on children aged 2 to 18 years from November 2015 to October 2016. Serology was performed using an immunoassay on QUANTA-Lite® tTG-IgA from INOVA Diagnostics and positive results reported when ≥4 U/ml. Patients with IgA deficiency and lab results/biopsy from outside institutions were excluded. Biopsy findings were graded according to Marsh-Oberhuber classification.

**Results:**

Total tTG-IgA serology performed yielded 2723 results. The distribution and characteristics of patients are summarized in Table 1. The sensitivity, specificity and PPV of tTG-IgA is 86.7%, 99.4% and 84.7%, respectively. According to 2012 ESPGHAN guidelines, duodenal biopsy may have been omitted in 38% of patients depending on EMA and HLA status.

**Conclusion:**

We report high sensitivity and specificity of tTG-IgA at our institution, and is comparable to the literature. These results strengthen the notion that this simple screening test, when paired with appropriate symptomatology, can reliably identify a select population in whom EMA and HLA may be warranted, and biopsy omitted. In order to initiate a non-invasive transition, more emphasis is necessary on helping clinicians better understand the performance of celiac serology testing.

Table 1.

| | **Duodenal Biopsy** | |
|---|---|---|
| **tTG-IgA** | **Positive** | **Negative** |
| **Positive** | **78**<br>52 - Titer >10x ULN (67%)<br>35 - GI symptoms<br>32 - Marsh 3 lesion | **14**<br>5 – Titer >10x ULN (36%) |
| **Negative** | **12**<br>3 with Marsh 2 (25%):<br>1 - EMA+<br>1 - HLA-DQ2+<br>1 - Dermatitis Herpetiformis<br>9 with Marsh 1 (75%):<br>3 - Intestinal infection<br>2 - Peptic injury<br>2 - Crohn's disease<br>1 - Hyperthyroidism<br>1 - Dysmotility | **2619** |

## A-144

### Comparison of Active B12 with Total B12 and Methylmalonic Acid in patients with cognitive impairment

J. Boyd[1], Z. Ismail[2], A. Chin[1], C. Naugler[1], H. Sadrzadeh[1]. *[1]Calgary Laboratory Services, Calgary, AB, Canada, [2]University of Calgary, Calgary, AB, Canada*

**Background:** Patients with vitamin B12 deficiency are at risk for developing neurological abnormalities and methylmalonic academia. Although serum methylmalonic acid (MMA) can be used to confirm B12 deficiency, this test is not readily available in most laboratories. Instead, total B12 measurements are used as a surrogate, although it does not correlate well with MMA. Active B12 assays, which only measure B12 bound to transcobalamin, have been suggested to be a better marker of B12 deficiency than total B12. In this study, we evaluated the performance of active B12 compared to total B12, MMA and neurological evaluation.

**Methods:** This study was approved by our institutional ethics committee and patients were recruited at neurology clinics. Once consent was obtained, patients received neurological evaluation as per standard of care. Patients also had blood drawn for total B12, active B12 and MMA testing. Total B12 was measured using a Roche Cobas e601 platform. Active B12 was measured using Abbott Active B12 reagent on an Abbott Architect. MMA was measured by LC-MS/MS.**Results:** To date, 62 patients have been recruited into the study. These patients are a mixture of those with normal cognition, cognitive impairment and dementia. Preliminary results showed total B12 correlated well with active B12 (y=0.4254x-49.941; R2=0.6642). However, total B12 and MMA as well as Active B12 and MMA showed poor correlation. This relationship was maintained when only samples with MMA greater than 250 pmol/L were included. Data from neurological assessments of each patient is being collated and will be presented at the meeting.

**Conclusion:** Our preliminary results showed that B12 deficiency can be assessed by either active B12 or total B12. However, active B12 may not be a suitable surrogate for MMA analysis. Clinical information will be correlated with biochemical analysis and the results will be present completely in July.

## A-145

### Genetic Risk Prediction for Developing Age-related Macular Degeneration and Disease Progression

K. Gawlik, J. Lopez, S. Chang, R. Vairavan, F. Kureshy. *AutoGenomics Inc., Carlsbad, CA*

**Purpose:** Age-related macular degeneration (AMD) remains the leading cause of irreversible visual loss among the elderly in developed countries. The earlier asymptomatic form of AMD is characterized by the formation of confluent drusen in the macula, while advanced AMD includes geographic atrophy (GA) and choroidal neovascularization (CNV). It is likely that the management of patients with AMD will be influenced by assessment of genetic risk. The aim of this study was to develop a separate class prediction algorithm for AMD lifetime risk assessment and disease progression from intermediate AMD to either GA or CNV based on individual genetic profiles.

**Methods:** DNA samples were genotyped using the AutoGenomics INFINITI platform. AMD panel (for research use only) included ABCA1(rs1883025); APOE(rs429358, rs7412); ARMS2(rs10490924); C3(rs2230199); CCDC109B(rs17440077); CETP(rs3764261); CFB(rs4151669, rs522162); CFH(rs1048663, rs1061170, rs10737680, rs1329428, rs2274700, rs3766405, rs412852); CFI(rs10033900); COL8A1(rs13095226); HTRA1(rs11200638); LIPC(rs493258, rs10468017); LPL(rs12678919); TIMP3(rs9621532) and VEGFA(rs3025000, rs943080). Class prediction model building and testing were performed using TreeNet software (Salford Systems, San Diego, CA). The binary logistic regression analysis with a 10-fold cross-validation method was applied in the algorithm development.

**Results:** We genotyped the selected 25 SNPs in 893 cases with advanced AMD (consisted of 206 GA and 687 CNV), 265 cases with intermediate AMD and 384 controls. Progression was defined as transition from intermediate to advanced AMD, either GA or CNV, in the worse eye during a follow up visit. We selected only patients with intermediate AMD who did not progress within 7 years to advanced stages and considered them as non-progressors. For model building we applied a 'shaving' technique, in which the predictors are ranked from top to bottom (the most important to the least important) at every step when the bottom predictor is removed and the model is rebuilt. This technique allowed us to choose three distinct models with the best performance and further evaluate. As a result, we developed three class prediction algorithms: 1) for AMD lifetime risk assessment in AMD patients versus controls based on 13 SNPs; 2) for progression to GA in GA progressors versus non-progressors based on 9 SNPs; 3) for progression to CNV in CNV progressors versus non-progressors based on 10 SNPs. The ROC of the model for AMD risk for the test set was 0.75 with 70% sensitivity and 68% specificity. The ROC of the model for progression to GA for the test set was 0.63 with 53% sensitivity and 72% specificity. The ROC of the model for progression to CNV for the test set was 0.55 with 55% sensitivity and 52% specificity.

**Conclusions:** Our results suggest that the INFINITI AMD panel can be successfully used for the AMD lifetime risk assessment as well as disease progression. In addition, risk of developing AMD and disease progression can be further improved when the panel is combined with environmental factors such as age, smoking and other variables. The proposed approach to AMD risk determination, separate for lifetime risk and disease progression, might provide a useful clinical tool and be potentially applied toward more personalized care.

## A-146

### HE4 as a diagnostic and follow-up biomarker in non- small cell lung cancer

U. Kumbasar[1], Z. G. Dikmen[2], Y. Yılmaz[1], B. Ancın[1], E. Dikmen[3], R. Dogan[1]. *[1]Hacettepe University Medical Faculty Department of Thoracic Surgery, Ankara, Turkey, [2]Hacettepe University Medical Faculty Department of Medical Biochemistry, Ankara, Turkey, [3]Hacettepe University Medical Faculty Department of Thoracic Surgery, Ankara, Turkey*

**Abstract**

**Background**

Early detection of non-small cell lung cancer (NSCLC) cases is crucial since nearly one third of them are unresectable during diagnosis and also the recurrence rates are high following treatment. Human epididymis protein 4 (HE4) is predominantly expressed in a variety of normal human tissues including epididymis, epithelial cells of proximal airways and the female genital tract. HE4 has emerged as one of the most promising diagnostic and prognostic biomarker in epithelial ovarian cancer. High levels of HE4 have also been detected in lung, breast and pancreas cancers. Thus, in this study we aimed to evaluate both the diagnostic performance and the post-resection progress of serum HE4 in patients with NSCLC.

**Methods**

Thirty-one patients who had benign lung disease (group 1) were compared with the same number of patients with resectable NSCLC (group 2). Blood samples were collected in serum separator tubes and were centrifuged at 1500g for 10 minutes (Rotanta 460). HE4 levels were measured by chemiluminescent microparticle immunoassay at ARCHITECT *i*2000SR immunoassay analyzer (Abbott Diagnostics) at the time of diagnosis and following surgery - at post-operative 1st month. The cut-off limit for serum HE4 was considered 70 pmol/L.

**Results**

The serum HE4 in NSCLC group [median: 89.70 (58.10-397.00)] pmol/L was significantly higher than the benign group [median: 42.60 (28.10-198.90)] pmol/L, respectively ($p< 0.001$). HE4 levels in NSCLC group significantly decreased from 89.70 (58.10-397.00) pmol/L to 71.50 (41.70-232.30) pmol/L following pulmonary resection

($p<0.001$). In advanced stages (stage III and IV) the decrease in serum HE4 levels [25.30 (10.50-164.70)] pmol/L was significant than the decrease in early stages (stage I-II, [14.60 (14.20-133.60)] pmol/L ($p=0.025$). According to ROC analysis, the area under the curve (AUC) of HE4 was 0.921 (95% CI, 0.843-0.998), ($p< 0.001$). Both the sensitivity and specificity of HE4 as a biomarker was found 87.1%.

**Conclusion**

Our data demonstrated that HE4 is a potential biomarker for the diagnosis of NSCLC with high sensitivity and specificity. We believe that HE4 levels could also be used to monitor NSCLC recurrences and treatment response during follow-up.

## A-147

### Serum parathyroid hormone levels and calcium oxalate crystals in urine

J. D. Santotoribio, C. Cañavate-Solano, J. F. Cuadros-Muñoz, M. Mayor-Reyes, S. Perez-Ramos. *Puerto Real University Hospital, Cadiz, Spain*

**Background:** Renal lithiasis is the formation of kidney stones in the urinary tract, a common disease, affecting 1-15% of the world's population. The majority of kidney stones are calcium oxalate (70%). Calcium oxalate crystallizes when its concentration is supersaturated in the urine. The increase in the concentration of calcium oxalate may be due to the increase in the urinary concentration of solutes such as calcium. Parathyroid hormone (PTH) increases renal calcium reabsorption, decreasing urinary calcium and increasing serum calcium. A decrease in serum PTH levels may increase calcium oxalate crystals in urine by decreasing renal calcium reabsorption and increasing its concentration in the urine. The aim was to determine the association between serum PTH levels and the presence of calcium oxalate crystals in the urine.

**Methods:** We selected patients who were measured serum PTH and urinary sediment during the years 2014 to 2016. Serum PTH was measured by electrochemiluminescence immunoassay in modular E-170 (Roche Diagnostic®). Urine sediment was measured in analizer FUS-100 (DIRUI®). Patients were classified into two groups according to the urine sediment: patients with calcium oxalate crystals in the urine and patients without crystals. Statistical analysis was performed using the software MedCalc®.

**Results:** We studied 883 patients with ages between 14 and 92 years (median = 66 years), 478 women and 405 men. Forty-two patients had calcium oxalate crystals in the urine (group 1) and 850 had no crystals (group 0). The statistical analysis is shown in the following table:

| Group | n | **PTH** median (range) |
|---|---|---|
| **0** | 841 | 81.4 pg/mL (6.9-697.5) |
| **1** | 42 | 44.9 pg/mL (17.8-91.9) |
| **Mann-Whitney U test** | p<0.0001 | |
| **Odd Ratios** | 0.9804 | |

Serum PTH levels were lower in the group of patients with calcium oxalate crystals than in the group of patients without crystals. Mann-Whitney U test showed statistically significant differences between the two groups with serum PTH levels.

**Conclusions:** Serum PTH levels are associated with the formation of calcium oxalate crystals in urine. High serum PTH levels may be a protective factor for renal lithiasis.

## A-148

### Serum Growth Arrest Specific Protein 6(Gas-6) Levels in Patients with Schizophrenia

F. Gerin[1], A. Erek Toprak[1], H. Erman[1], S. Duruyen[1], A. Bas[2]. *[1]Istanbul Medeniyet University, Istanbul, Turkey, [2]Istanbul University Cerrahpasa Medical School, Istanbul, Turkey*

**Background:** We have investigated serum growth arrest-specific protein 6 (GAS-6) levels from patients with schizophrenia divided into acute phase remission phases as well as control group.

**Methods:** This study was conducted in Psychiatry Department of Istanbul University Cerrahpasa Medical Faculty. The patients who were diagnosed with schizophrenia after regular psychiatric examination according to DSM-IV criteria (n=22) as well as control subjects were included in the study. Schizophrenia patients with acute phase and remission phase were evaluated by Positive and Negative Syndrome Scale (PANSS) and Clinical global Impression Scale (CGI-S). The serum GAS-6 levels of schizophrenia patients during acute phase and remission phase were compared with the serum GAS-6 levels of healthy controls. Serum GAS-6 levels were measured by commercial ELISA development kit (R&D).

**Results:** No difference was found in serum GAS-6 levels among the three groups; schizophrenia with acute phase, schizophrenia with remission phase, and controls. There were no correlations between serum GAS-6 levels and PANSS and CGI scores.

**Conclusion:** To reach a definitive data and better interpretation about the relationship between GAS-6 and schizophrenia, future studies with larger groups of patients with schizophrenia subdivided by drug naïve and treated with antipsychotics/other treatment modalities and controls are needed.

## A-149

### Serum Gas 6 and Omentin Levels and relationship with Flow mediated dilatation , Aortic distensibility , Left ventricule mass index and disease severity in Patients with Psoriasis

A. Erek Toprak, A. Karadag, H. Erman, F. Aksu, T. Ustunbas, O. Kostek, I. Yilmaz, M. Caliskan. *Istanbul Medeniyet University, Istanbul, Turkey*

**Background:** *Growth arrest-specific gene 6 (*GAS6*) encodes a vitamin K-dependent protein secreted by* endothelial cells, vascularsmoothmuscle cells,and adipocytes *that regulates inflammation, insülin resistance, angiogenesis, and atherosclerotic plaque formation. The level of GAS6 expression is associated with plaque stability and stroke.* Changing in aortic distensibility (AoD) may reflect the features of the atherosclerotic process. Omentin, a novel adipokine secreted by the stromal vascular cells of adipose tissue, is considered associated with vascular, metabolic, and various chronic inflammatory diseases. Psoriasis is an autoimmune, inflammatory and chronic disease.This study aimed to determine the association between serum Gas6 and omentin levels with BMI, insulin resistance, flow mediated dilatation , Aortic distensibility and Left ventricule mass index in Patients with Psoriasis

**Methods:** The study was conducted on 30 healthy subjects (mean age: 41 ± 10 years) who were free of coronary risk factors and 52 patients with psoriasis(mean age: 41 ± 10 years).Body mass index were calculated. Circulating glucose, cholesterol , insülin, HbA1c, CRP, Gas6 and omentin levels analysed and insulin resistance were determined.Gas 6 and omentin were anaysed with ELISA method with commercially available kits(Biovendor ,Czech Republic) Transthoracic echocardiography was used to measure the Beta index , aortic distensibility (AoD), left ventricular mass index.Flow mediated dilation was measured with high resolution B mode ultrasound machine (Toshiba, aplio XU) with a 7.5 MHz linear transducer.

**Results:** Patients and control group were similar about age, body mass index, waist circumference and sex distribution.Insulin resistance (HOMA-IR) was high significantly in psoriasis patients.Levels of serum Gas6 levels were significantly higher (p<0.001) in patients group whereas omentin were significantly lower(p.0.001). Aort distensibility and beta index were low in patients group(p:0.01 and p:0.02 respectively). Also serum Gas 6 levels were positive and significantly correlated with HOMA-IR (p:0.020,r:283) and left ventricule mass index(p<0.01,r:689). In addition, serum omentin levels were were positive and significantly correlated withwaist circumference(p:0.01, r:0.600). Disease severity(PASI psoriasis area and severity index) was not correlated with any parameter in the study.

**Conclusion:** Circulating Gas6 and omentin levels and aort distensibility and beta index are significantly different in two groups. Serum Gas 6 levels are strongly correlated with insülin resistance status and left ventricule function status. Gas 6 may play a role assessing insülin resistance and left ventricule function status in patients with psoriasis.

## A-150

### Risk assessment of opioid addiction with a multi-variant genetic panel involved in the dopamine pathway

S. Chang[1], R. Hudspeth[1], R. Vairavan[1], F. Tennant[2]. *[1]AutoGenomics Inc, Carlsbad, CA, [2]Veract Intractable Pain Clinic, West Covina, CA*

**Background:** More than 116 million people worldwide are struggling with chronic pain and most require prescription drugs. In the US, opioid prescription misuse and heroin use accounted for the majority of accidental overdose deaths in 2014. Genetic factors play a key role in opioid prescription addiction, but are generally not evaluated in clinical practice. Currently, there is no objective way for practitioners to identify pain patients in medical management who are at risk to abuse or become addicted to prescribed medication or to identify those pain patients who will require high dosages or an unusual regimen of medication. The purpose of this study is to assess a risk of developing prescription opioid addiction with a multi-variant addiction panel involved in the mesolimbic dopamine system.

**Methods:** We genotyped samples for 16 single nucleotide polymorphisms (SNPs) involved in the brain reward pathways from 70 patients diagnosed with prescription opioid/heroin addiction and 68 normal control patients with a multiplexed film-based microarray technology. The addiction panel targets 16 mutations: 5-HTR2A (rs7997012), 5-HTTLPR (rS25531), COMT (rs4680), DRD2 (rs1800497), DRD1 (rs4532), DRD4 (rs3758653), DAT1 (rS6347), DBH (rs1611115), MTHFR (rs1801133), OPRK1 (rs1051660), GABA (rs211014), OPRM1 (rs1799971), MUOR (rs9479757), GAL (rs948854), DOR (rs2236861) and ATP-BCT (rs1045642). The genotyping data were subjected to a class predication model building with 10-fold cross validation for testing. A risk score from 1 to 100 was further computed with a score over 52 representing an elevated risk of addiction.

**Results:** The receiver operating characteristic (ROC) for the model was 0.78 with 76% sensitivity (95% CI: 84 - 85) and 72% specificity (95% CI: 60 - 82). PPV is 74% and NPV is 74%. Fifty-Three of 70 (75.7%) addicts and 19 of 68 (27.9%) normal controls showed an addiction risk score over 52. (X2=31.55;df=1;P<.05).

**Conclusion:** The prediction algorithm with this multi-variant genetic panel can be used for prescription opioid addiction risk assessment. By identifying patients with high risk to prescription opioid addiction along with mutation status of cytochrome p450 genes involved in therapeutics, it may provide information to physicians to improve therapeutic decisions in pain management and prevent abuse and addiction.

## A-151

### Performance Evaluation of the VITROS® NephroCheck® Test*

G. Ogbonna, S. Jackson, S. Clark, J. Parsells, S. Phonethepswath. *Ortho Clinical Diagnostics, Rochester, NY*

Acute kidney injury (AKI) is a complex disorder with a high mortality due to comorbidities and management challenges, especially in the critically ill patient. The VITROS® NEPHROCHECK® Test quantitatively measures Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) to generate an AKI risk index (AKIRISK™ Score). Patients with an AKIRISK™ Score less than 0.30 are at low risk of developing moderate to severe AKI within 12 hours of assessment while those with values ≥ 0.30 are at increased

risk. We have evaluated the performance of the VITROS NEPHROCHECK Test on the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System. The test is linear across the range of 0.4 to 29.7 ng/mL for TIMP-2; and 5.6 to 463.0 ng/mL for IGFBP-7 resulting in an AKIRISK™ Score range of 0.002 to 13.8. Limits of Blank were determined to be 0.164 ng/mL and 0.456 ng/mL for TIMP-2 and IGFBP-7, respectively. Limits of Quantitation were determined to be 0.392 ng/mL for TIMP-2 and 1.393 ng/mL for IGFBP-7 respectively, resulting in an AKIRISK Score of 0.002. A 20-day precision study with pooled patient samples at mean AKIRISK Scores of 0.10, 0.32, 0.38, 2.24, 3.46, 6.72, and 8.16 resulted in within-laboratory percent coefficients of variation (%CV) of 10.9%, 10.6%, 12.3%, 11.3%, 8.7%, 6.9% and 6.9% respectively on the VITROS 3600 Immunodiagnostic System. Similar results were obtained from the VITROS 5600 Integrated System. Potential endogenous interfering substances likely to be present in urine including acetoacetate, acetone, ammonia, albumin, creatinine, hemoglobin, myoglobin, urea and uric acid were tested and shown not to interfere in the assay. The accuracy of the test was evaluated with 145 patient specimens spanning the assay measuring range against the Astute Medical NEPHROCHECK Test System (Astute) and the following linear regression statistics were obtained: VITROS 3600 = 1.07*Astute + 0.03; (r) = 0.96; and VITROS 5600 = 1.03*Astute + 0.05; (r) = 0.96. Studies were performed to determine the clinical utility of the VITROS NEPHROCHECK Test using samples (n=339) collected from the intended use population and healthy cohorts (n=399). VITROS results were assessed based on AKIRisk Score of 0.30 against clinical outcome. Presence or absence of acute kidney injury was determined by clinical adjudication. A sensitivity of 87.27% (95% exact confidence interval: 75.52% to 94.73%) and specificity of 49.30% (95% exact confidence interval: 43.34% to 55.27%) with a negative predictive value of 95.24% (95% exact confidence interval: 90.43% to 98.06%). The assay demonstrated good performance and showed high negative predictive value indicating utility in identifying patients at risk of developing acute kidney injury within 12 hours of assessment. (* under development)

## A-152

### Alkaline phosphatase reference interval in children and adolescents in Brazil

P. Araujo[1], E. Mello Ribeiro[1], L. Vieira Neto[2], M. Miguens Castelar Pinheiro[1], Y. Schrank[1], D. Valente Gomes[1], M. Freire[1], R. Fontes[1]. *[1]DASA, RJ, Brazil, Rio de Janeiro, Brazil, [2]UFRJ, RJ, Brazil, Rio de Janeiro, Brazil*

**Background:** The interpretation of laboratory tests requires reference intervals (RI) that may vary between different populations. For the diagnosis of hypophosphatasia, disease presenting with low serum alkaline phosphatase (ALP), lower limits of RI must be well determined. Since the ALP methodology in practice in our clinical laboratory does not provide lower reference levels for children and adolescents, our aim was to validate lower limits through CALIPER findings, adjusted to the Brazilian population. **Methods:** Serum specimens of 1950 children, from 1 to 18 years old, reporting ALP measurement, between April 2015 and March 2016, were retrospectively assessed. The same subgroups proposed by CALIPER study and the Clinical and Laboratory Standards Institute (CLSI) guideline have been used to validate obtained results. ALP was measured by the Roche/Hitachi platform Cobas. Inclusion criteria were patients with normal results for liver function, bone metabolism, kidney function, and blood counts. Exclusion criteria were hospitalization, low weight, use of drugs that could interfere in the ALP measurement, and patients in which ALP dosing was requested more than 3 times. The percentage of girls and boys excluded were 13,9% and 12,8%, respectively, therefore 1690 patients were selected. Data normal distribution was analyzed with Kolmogorov-Smirnov test. Outlying observations were calculated using Dixon test. RI was defined as 2.5-97.5%. The RI obtained in this study was considered valid if less than 10% of patients were out of CALIPER RI, and if the difference between minimum and maximum value for each age group and sex was less than 25% between both studies. **Results:** The obtained RI results and 90% confidence interval (CI) of ALP are shown in Table 1. **Conclusion**: According to CLSI, the results of this study have been enabled for use as ALP RI when compared to CALIPER study, for the Brazilian children and adolescents' population.

Table 1- Data of patients selected, and results of Brazilian study compared to CALIPER study.

| Age group (years) | Gender group (n) | Total number of patients (n) | Patients excluded (n/%) | Patients selected (n) | ALP RI (U/L) | ALP CI | Outsiders (n/%) | CALIPER RI (U/L) | Difference* (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1-9 | Both | 322 | 32/11.3 | 282 | 149-301 | (134-319) | 24/7.45 | 135-320 | +10.0/-6.0 |
| 10-12 | Both | 232 | 24/11.5 | 208 | 127-326 | (122-401) | 19/8.1 | 122-400 | +4.0/-18.5 |
| 13-14 | Girls | 199 | 26/13.0 | 173 | 62-212 | (51-246) | 4/2.0 | 52-243 | +19.1/-12.7 |
| 13-14 | Boys | 197 | 12/6.0 | 185 | 129-437 | (101-454) | 18/9.1 | 109-449 | +18.3/-12.7 |
| 15-16 | Girls | 248 | 33/13.3 | 215 | 52-120 | (45-122) | 24/9.6 | 46-110 | -11.5/+9 |
| 15-16 | Boys | 250 | 39/15.6 | 211 | 78-268 | (62-334) | 9/3.6 | 77-317 | +1.3/-15.4 |
| 17-18 | Girls | 252 | 43/17 | 209 | 45-97 | (40-99) | 25/9.9 | 41-82 | +9.7/+18.2 |
| 17-18 | Boys | 250 | 43/17.2 | 207 | 40-129 | (38-141) | 16/6.4 | 50-142 | -9.1/-9.1 |

Legend: ALP: alkaline phosphatase; Patients selected: Total number of patients minus patients excluded; ALP RI: Reference interval for ALP determined in this study; CI: 90% confidence interval; Outsiders: number of subjects tested in the present study that fell out 10% above or below the original reported limits; RI: reference interval; * Percent difference to plus (+) or minus (-) the results obtained in this study compared to CALIPER study. The first percentage refers to the inferior reference value difference and the second value refers to superior reference value difference; CALIPER: Canadian Laboratory Initiative in Pediatric Reference Intervals.

## A-153

### Effect of meal on clinical chemistry and hormone assays in a mixed group of patients

S. Sinha, P. Goyal, A. Chitkara, P. Udagani, S. Verma, R. Kumar. *Dr. Ram Manohar Lohia Hospital, New Delhi, New Delhi, India*

**Background :** The leading causes of preanalytic variability are related to patient preparation, blood drawing, sample transportation and preparation. Studies documenting the effect of meals on clinical chemistry and hormone studies have been limited in scope.

**Objective** : The purpose of this study was to determine the effects of a preanalytical variable – meal on the serumclinical chemistry parameters and hormone levels.

**Methodology** : Subjects were randomly chosen from among patients presenting for blood testing at the biochemistry laboratory of Dr.R.M.L.Hospital. Whole blood samples were taken after overnight fasting and 2 hours after meals. Serum was extracted and processed on Vitros Fusion 5.1 microslide system (clinical chemistry) and VitrosEci chemiluminescence system (hormones). Paired t-test was used to analyse results.

**Results** : Among clinical chemistry parameters (n=98), creatinine, alanine transaminase, alkaline phosphatase, total proteins, albumin, total cholesterol, HDL-cholesterol, calcium, phosphate and iron levels showed a significant decrease after meal whereas urea, uric acid, aspartate transaminase and triglyceride levels increased. Hormone assays (n=19) revealed decrease in levels of TSH, PSA, Free T4, Free T3, estrogen, progesterone, FSH, LH, prolactin and testosterone while there was an increase in levels of vitamin D and PTH.

**Conclusion** : Meals can bias clinical chemistry and hormone reporting and must be carefully considered during routine lab testing to reduce lab errors.

| Test | baseline | postprandial | mean % diff | p value |
|---|---|---|---|---|
| Clinical chemistry (n=98) | | | | |
| urea | 25 ± 10.8 | 26 ± 10.9 | 4.1 | <0.01 |
| creatinine | 0.7 ± 0.3 | 0.7 ± 0.3 | -1.0 | 0.28 |
| uric acid | 5.1 ± 1.53 | 5.1 ± 1.49 | 2.6 | 0.018 |
| sgot | 32 ± 15.7 | 32 ± 16.8 | 4.7 | 0.07 |
| sgpt | 39 ± 20.9 | 36 ± 20.2 | -4.5 | <0.01 |
| ALP | 98 ± 65.9 | 93 ± 67.9 | -3.9 | <0.01 |
| T.Protein | 7.8 ± 0.6 | 7.6 ± 0.5 | -2.7 | <0.01 |
| Albumin | 4.5 ± 0.4 | 4.4 ± 0.4 | -2.4 | <0.01 |
| T.Chol | 176 ± 39.5 | 170 ± 36.7 | -2.4 | 0.011 |
| HDL-C | 46 ± 12.6 | 43 ± 11.7 | -4.9 | <0.01 |
| Triglycerides | 115.5 ± 72.8 | 135 ± 63.1 | 9.6 | <0.01 |
| Ca | 9.6 ± 0.5 | 9.5 ± 0.5 | -1.9 | <0.01 |
| P | 4 ± 0.5 | 3.8 ± 0.5 | -5.7 | <0.01 |
| Fe | 85 ± 48.6 | 78 ± 42.2 | -7.9 | <0.01 |
| Hormone assays (n=19) | | | | |
| TSH | 2.34 ± 6.4 | 2.34 ±3.9 | -20.0 | 0.19 |
| PSA | 0.04 ± 0.5 | 0.02 ± 0.4 | -28.1 | 0.15 |
| FT4 | 1.19 ± 0.3 | 1.17 ± 0.2 | -1.9 | 0.22 |
| FT3 | 3.55 ± 0.8 | 3.25 ± 0.7 | -2.5 | 0.41 |
| Estr | 96.42 ±131.9 | 105.14 ±121.1 | -6.3 | 0.51 |
| Prog | 2.78± 8.4 | 2.61 ± 3.7 | -33.6 | 0.23 |
| FSH | 8.58 ±23.9 | 10.9 ± 19.0 | -12.8 | 0.13 |
| LH | 7.42 ± 17.2 | 9.86 ± 14.9 | -6.6 | 0.33 |
| Prolactin | 10.5 ± 17.3 | 12.9 ±11.9 | -7.8 | 0.49 |
| Testo | 0.71 ±6.9 | 0.61 ± 6.9 | -11.1 | 0.62 |
| PTH | 71.3 ± 80.1 | 100.4 ±90.1 | 3.7 | 0.68 |
| Vitamin D | 10.8 ± 8.4 | 14.6 ±8.8 | 1.2 | 0.75 |

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Endocrinology/Hormones

## A-154

**Protective Role of C-peptide against Metabolic Memory**

M. P. Bhatt. *Gandaki Medical College Teaching Hospital, Pokhara, Nepal*

**Background:** The therapeutic approach to glycemic control alone has been generally ineffective in preventing diabetic vasculopathy. Thus, an alternative therapeutic approach is necessary to target both hyperglycemia and diabetic complications.

**Methods:** Recently, we investigated biological roles of C-peptide, suggesting a new therapeutic strategy that may become available to protect against hyperglycemic memory (HGM) using human umbilical vein endothelial cells and diabetic mice.

**Results:** HGM induced apoptosis by persistent generation of intracellular ROS and sustained formation of NO and abetic complications. We elucidated the role of C-peptide in inhibiting $ONOO^-$, and nitrotyrosine. These HGM-induced intracellular events are normalized by treatment with C-peptide, but not by insulin alone. C-peptide also inhibited upregulation of metabolic memory-related proteins, p53 and mitochondrial adaptor $p66^{shc}$ after glucose normalization. Further, C-peptide replacement therapy prevented persistent generation of ROS and $ONOO^-$ in the aorta of diabetic mice whose glucose levels were normalized by the administration of insulin. C-peptide, but not insulin, prevented HGM-induced endothelial apoptosis in the diabetic aorta.

**Conclusion:** This study highlights a promising role for C-peptide in preventing hyperglycemic memory and diabetic complications.

## A-155

**Easy as 1, 2, 3? Trimester-specific TSH Reference Intervals in a Well-Characterized Population Using the Beckman Coulter 3rd IS Immunoassay**

S. P. Wyness[1], L. Prust[2], R. H. Christenson[3], J. A. Straseski[4]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Beckman Coulter Inc., Chaska, MN, [3]University of Maryland School of Medicine, Baltimore, MD, [4]Department of Pathology, University of Utah, Salt Lake City, UT*

**Background:** Lack of harmonization of thyroid stimulating hormone (TSH) immunoassays has led to variable reference intervals (RIs) and disagreement within the scientific community. This is concerning, as a clear understanding of what is "normal" is necessary to diagnose disease. Thyroid function changes throughout pregnancy make establishing RIs in this population additionally complex. Commercial immunoassays also use different reference standards, further confounding comparisons between studies.

**Objective:** The aim of the current study was to establish TSH RIs for Beckman Coulter immunoassays using well-characterized samples from pregnant and non-pregnant individuals. These assays utilize the more recent WHO 3rd international standard (IS).

**Method:** 1571 healthy U.S. subjects ≥18 years (y) were prospectively enrolled in an 8-center study. Subjects had no personal or family history of thyroid disease and were not using prescription medications. 164 subjects with thyroid peroxidase >9 IU/mL or thyroglobulin antibodies >4 IU/mL were excluded. RIs were established for women in their first (≤13 weeks), second (14-26 weeks) and third (≥ 27 weeks) trimesters of pregnancy, as well as men and non-pregnant women. Subjects included 318 women in first trimester pregnancy, mean age 27 y (SD 5.1), 84% Caucasian, 22% Hispanic, 16% Other; 362 second trimester, mean age 28 y (SD 5.6), 84% Caucasian, 32% Hispanic, 16% Other; 334 third trimester, mean age 27 y (SD 5.5), 84% Caucasian, 27% Hispanic, 16% Other; and 393 men and non-pregnant women (198 male, 195 female), mean age 41 y (SD 15.9), 77% Caucasian, 23% Hispanic, 23% Other. One third-trimester subject was excluded using Reed-Dixon rule. TSH was measured using the Access TSH (3rd IS) assay on Beckman Coulter Immunoassay Analyzers (UniCel DxI 800 and Access 2).

**Results:** The central 95th percentile RIs for the UniCel DxI 800 (Access 2) were determined to be 0.15-3.25 µIU/mL (0.15-3.33 µIU/mL) in first trimester pregnancy, 0.37-4.05 µIU/mL (0.37-4.20 µIU/mL) in second trimester, 0.48-4.41 µIU/mL (0.48-4.62 µIU/mL) in third trimester, and 0.50-3.92 µIU/mL (0.51-4.15 µIU/mL) for males and non-pregnant females. Upper and lower reference limits were contained within corresponding 95% confidence intervals between assays.

**Conclusion:** Similar to other methods and reports, TSH RIs increased as pregnancy progressed. While these trimester-specific upper RI limits were higher than some reports, there is considerable variation in the assays, standardization materials, populations, and statistics used in the literature. The male and non-pregnant female 95th percentile RI was consistent with previously published results using alternate methods, including those proposed in the 2002 NACB laboratory medicine practice guidelines. This highlights the importance of establishing RIs for individual assays and the critical need for TSH assay standardization.

## A-156

**Cystatin C As A Marker Of GFR In Type 2 Diabetic Nephropathy.**

P. Gyawali[1], B. Jha[2], K. B. Raut[2]. *[1]Kathmandu University School of Medical Sciences, Kavrepalanchok, Nepal, [2]Institute of Medicine, Kathmandu, Nepal*

**Background:** Diabetes is the most common cause of CKD worldwide. Growing body of evidence suggests, serum cystatin C as a superior marker than serum creatinine for assessment of renal function and in detecting early decline in renal function in diabetic nephropathy.This study examined the adequacy of the cystatin C as a marker of GFR for the assessment of nephropathy in the Nepalese patients with type 2 diabetes.

**Methods:** 101 patients diagnosed with type 2 diabetes, were categorised into different stages of nephropathy based on urine protein to creatinine ratio (PCR). Serum cystatin C level was measured using latex turbidimetry (Giesse diagnostic), reference level 0.59-1.03mg/L. Serum creatinine was measured using modified Jaffe method with the reference level male (80-115µmol/L) and female (53-97µmol/L). Analytes were measured in Biotecnica 1500 chemistry auto-analyzer. GFR was estimated using MDRD equation and cystatin C based CKD-EPI (2012) equation. SPSS ver.20, t-test, one-way ANOVA, Pearson's correlation and ROC were used for data analysis and interpretation.

**Results:**Cystatin C was elevated in 49 patients and serum creatinine was elevated in 38 patients out of 101 patients. Cystatin C level increased significantly with the progression of nephropathy ($p < 0.01$). The mean serum cystatin C level in different stages of nephropathy were 0.78± 0.21mg/L (PCR <15mg/mmol), 0.95± 0.33mg/L (PCR 15-50mg/mmol) and 1.96± 0.91mg/L (PCR >50mg/mmol). Serum cystatin C level correlated significantly with urine PCR and serum creatinine ($r=0.516$, $p < 0.01$) and ($r = 0.90$, $p < 0.001$) respectively. A significant ($p < 0.001$) inverse correlation was observed between serum cystatin C and serum creatinine with eGFR (MDRD) ($r=-0.89$, $r=-0.81$) respectively. ROC analysis showed that the AUC was marginally better for serum cystatin C [(0.959) 95% CI: 0.925-0.993] than serum creatinine [(0.952)95%CI: 0.915-0.989] to detect eGFR<60ml/min/1.73m$^2$ ($p < 0.001$). To detect eGFR <90ml/min/1.73m$^2$ AUC for cystatin C was 0.82 (95% CI:0.734-0.906) and for serum creatinine was 0.88 (95% CI: 0.806-0.954) ($p < 0.001$). The best cut off value of serum cystatin C to detect eGFR<60ml/min/1.73m$^2$ and<90ml/min/1.73m$^2$ was 0.993mg/L(sensitivity92%, specificity 82%) and 0.775 mg/L (sensitivity 76%, specificity 84%) respectively.

**Conclusion:**Serum cystatin C is useful alternative or adjunct to creatinine as a marker of GFR for assessment of renal function in type 2 diabetic nephropathy.

## A-157

**Development of a New Biochip Based Immunoassay Unaffected by DHEA-S interference for the Accurate Measurement of Serum Progesterone**

S. Marshall, D. McAleer, T. McConnell, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background:** Accurate and reliable measurement of serum progesterone has important clinical implications as this hormone plays a significant physiological role in pregnancy**.** Progesterone levels are used by In Vitro Fertilisation (IVF) clinicians when deciding if implantation is feasible. A threshold, varying between 0.9-1.2ng/mL, is used to determine if the endometrium is receptive to implantation and if progesterone levels are found to be above this threshold fresh embryo transfer is postponed. Immunoassays used by many fertility clinics to assess the specific concentration of circulating progesterone are optimised for measurement of higher progesterone levels and lack the specificity and sensitivity required to identify small changes in these

lower progesterone concentrations, which may impact patients undergoing IVF. The current study aimed at developing a new biochip based immunoassay for the specific measurement of progesterone at low concentrations in serum without interference with Dehydroepiandrosterone Sulphate (DHEA-S), which is prescribed for IVF preparation.

**Methods:** A direct competitive chemiluminescent immunoassay on a biochip platform with the fully automated Evidence Evolution was utilized. Assay sensitivity was determined as Limit of Blank (LOB), Limit of Detection (LOD) and functional sensitivity in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2. Repeatability was determined following CLSI protocol EP05-A3: 2 runs per day in duplication for 20 days (n=80). A DHEA-S concentration of 20000ng/mL was used to determine interference, which was calculated as both cross reactivity and percentage interference in accordance with CLSI guidelines EP07-A2 and compared to two other CLIA systems. A correlation study was conducted by analyzing 44 serum samples and compared with an ECLIA assay.

**Results:** The analytical evaluation showed LOB, LOD and functional sensitivity values of 0.017ng/mL, 0.073 ng/mL and 0.122 ng/mL respectively. Repeatability was expressed as CV (%) for samples at the following concentrations; 1.130, 12.742, 46.020 ng/mL and was 5.5%, 5.5% and 6.9% respectively. When DHEA-S interference was evaluated, the biochip based assay showed -6.4% interference at 0.345 ng/mL of progesterone in comparison to other commercially available CLIAs, which showed 82.5% interference at 0.540 ng/mL of progesterone and 211% interference at 0.7 ng/mL of progesterone. In the correlation study, linear regression on the resulting data generated an r value of 0.981 for samples in the range of 0.39-53.5 ng/mL.

**Conclusion:** The results show that this new biochip based immunoassay for the determination of progesterone in serum, applied to the Evidence Evolution, a high throughput, random access with STAT capabilities, fully automated analyser, exhibits specificity, accuracy and precision for low concentrations. This device is a valuable and reliable analytical tool for the measurement of progesterone levels during IVF as it does not suffer interference from DHEA-S, which is frequently prescribed to patients preparing for IVF. Moreover as the biochip platform offers flexibility to incorporate multiple assays on the biochip surface, other steroids hormones can be simultaneously determined thus increasing the information to facilitate clinical understanding.

## A-158

### Autoimmune thyroid disease: Hashimoto s Thyroiditis is associated with low levels of Vitamin D in adults patients.

M. RIBERI[1], M. Cid[2], A. Castillo[1]. *[1]LABORATORIO CASTILLO-CHIDIAK, CORDOBA, Argentina, [2]Universidad Nacional de Cordoba, CORDOBA, Argentina*

Background. Autoimmune thyroid diseases (AITD) are common autoimmune disorders. Hashimoto's thyroiditis (HT) is one of the main clinical presentations of AITD and is characterized by lymphocytic infiltration of the thyroid parenchyma. The clinical hallmarks of HT is hypothyroidism, common findings are high serum concentration of thyroid stimulating hormone (TSH) and positive anti-thyroid peroxidase antibodies (ATPO). Evidence suggests that low levels of 25-hydroxy Vitamin D (Vitamin D) may contribute to the development of autoimmune disease; however, the relationship between Vitamin D deficiency and Hashimoto's thyroiditis is still controversial. The objective of this study is to investigate the association between serum TSH levels, positive ATPO and levels of Vitamin D in healthy and HT patients in the local population. Methods. The study was conducted on 190 patients drawn in our clinic between August and November 2016. The mean subject age was 56 ± 17 years old and the male/female ratio was 28 (14.7% male):162 (85.3% female). Pregnant women and patients with abnormal parathyroid hormone levels were excluded. All blood samples were collected in Spring to minimize the impact of seasonal fluctuations of Vitamin D concentrations. We measured TSH, FT4, ATPO and Vitamin D concentrations in healthy and hypothyroids patients. The cut off for positive ATPO was > 37 UI/mL, the normal reference intervals for TSH and FT4 were 0.40 to 4.00 µU/ml, and 1.00 to 1.80 ng/dl, respectively. Deficiency for Vitamin D was defined as serum concentrations below 30 ng/mL. TSH, FT4, ATPO and Vitamin D concentrations were determined using a chemiluminescent microparticle immunoassay (CMIA) on the Advia Centaur XP (Siemens, Germany). Data obtained for all measurements of Vitamin D was analyzed with Welch´s Test. TSH and FT4 in both groups was analyzed using the Mann Whitney U test. A p value < 0.05 represented a significant difference. Data was expressed as mean ± error of the mean (SEM). Results. TSH serum concentrations were significantly increased in hypothyroid patients compared with control patients (4.22 ± 0.51 µU/ml vs 2.20 ± 0.11 µU/ml, p < 0.05). Patients with elevated ATPO had lower concentrations of Vitamin D than the control group (19.13 ± 0.68 ng/mL vs 22.61 ± 0.64 ng/mL, respectively, p < 0.05). FT4 concentrations showed no significant difference between hypothyroid group and control group (1.27± 0.19 vs 1.28 ± 0.11, p < 0.05). Conclusions. Results from the present study support the idea that Hashimoto's thyroiditis is associated with female gender, positive ATPO, high levels of TSH, and Vitamin D deficiency. We observed that serum Vitamin D concentration is significantly lower in HT patients in comparison to the control group. This suggests Vitamin D deficit may be one of the risk factors for HT development. Importantly, low levels of Vitamin D were observed in control group. We recommend supplementation with Vitamin D in general population.

## A-159

### Adrenocortical dysfunction among HIV infected Patients: Correlation between duration of HAART and development of dysfunction.

Z. U. Ibrahim[1], I. A. Yahaya[1], A. O. A[2]. *[1]Aminu Kano Teaching Hospital, Kano, Nigeria, [2]Lagos University Teaching Hospital, Lagos, Nigeria*

**BACKGROUND:** The use of Highly Active Anti-retroviral Therapy (HAART) has greatly reduced the morbidity and mortality associated with HIV infection and thus increasing the life expectancy of HIV infected patients. Long term complications (endocrine and metabolic) associated with the use of these drugs are becoming increasingly apparent, especially adrenal gland dysfunction. However, few studies have reported the influence of HAART on development of adreno-cortical dysfunction in the area of study.

**OBJECTIVE:** To determine the effect of duration of HAART on the development of adreno-cortical dysfunction among HIV infected patients.

**MATERIALS AND METHODS:**

Two hundred and forty subjects (made up of 80 HAART treated, 80 HAART naïve HIV patients and eighty HIV negative controls) were recruited for the study. Baseline saliva samples were analyzed for cortisol using ELISA kits sourced from Salimetrics Europe Limited. A short synacthen test was conducted on subjects with low baseline salivary cortisol (<2.6 nmol/L) and a late night salivary cortisol measurement was performed on subjects with high baseline salivary cortisol (>43.0 nmol/L).

**RESULTS:** There was a significant correlation between duration of HAART and development of adreno-cortical dysfunction among HIV positive patients (p=0.003).

Out of twenty two subjects found to have adreno-cortical dysfunction, sixteen subjects (72.8%) had HAART for >2 years duration while six (27.2%) had HAART for <2 years duration.

**CONCLUSION:** Treatment with HAART for >2 years duration influences development of adreno-cortical dysfunction among HIV patients.

**KEY WORDS:** HIV, HAART, Adreno-cortical dysfunction.

## A-160

### Correlation of Thyroid Function and Biochemical Parameters in Obese Subjects of Western Nepalese

S. K. JHA[1], N. K. Yadav[1], U. Jha[2], P. K. GS[1], D. R. Pokharel[1], M. Sigdel[1], P. S. Shukla[1]. *[1]Manipal College of Medical Sciences, Pokhara, Nepal, [2]Pokhara University, Lekhnath, Nepal*

**Background:** Thyroid dysfunction is endocrine disorder affecting about 300 million people worldwide and over half are presumed to be unaware of their condition. It estimated that 0.2% of death in Nepal results from endocrine disorders of which Iodine deficiency has been a major cause.WHO estimation suggests that one billion people are overweight or obese, and predicts that number will increase 1.5-fold by 2015 worldwide. A moderate elevation of TSH concentrations, which is associated with triiodothyronine (T3) values in or slightly above the upper normal range, is frequently found in obese humans. Obesity in Nepal is bringing new challenges in connection with rapid urbanization and modernization. The positive association between obesity and the risk of developing type II diabetes has been repeatedly observed, both in cross-sectional studies and in prospective studies.

**Aims and objectives:** The present study was done to evaluate Thyroid function test in obese and also to show their correlation with demographic, anthropometric and biochemical characteristics of the study subjects.

**Materials and Methods:** It was a hospital based case control study including 77 obese (having BMI ≥ 25.0) and 50 control. All subjects having no any known thyroid and other chronic illness. All the demographic and anthropometric data were collected using a preformed set of questionnaire and biochemical data were obtained from the laboratory analysis of the patients' blood samples. Statistical analysis was done with SPSS version 17.

**Results:**A statistical significant difference was noted between controls and cases-obese with respect to BMI (p<0.000), waist circumference (p<0.002) and HDL (p<0.044). In this study we found overall of 5.2 % prevalence in obese cases with Thyroid disorders and among this 25% with Primary Hypothyroidism, 75% with Sub clinical Hypothyroidism and no cases of hyperthyroidism. In this study, FT3, FT4 and TSH were found to be statistically insignificant while comparing the control with obese subjects. Similarly, study revealed mean±SD of FT3, FT4 and TSH in control and obese were found to be (2.44±0.69 and 2.44±0.54 with p-value 0.384), (1.00±0.26 and 1.01±0.23 with p-value 0.726) and (2.66±1.51 and 3.89±11.37 with p-value 0.100) respectively. Pearson's correlation analysis between serum FT3, FT4 and TSH with respect to baseline characteristics, biochemical parameters of the study subjects showed significant correlation (p>0.05) between FT4 with WHR in obese and no significant correlation (p>0.05) between other baseline characteristics. There was significant correlation (p>0.05) between FT4 with TC and LDL in obese and was no significant correlation (p>0.05) between other biochemical Parameters.

**Conclusion:** This study shows prevalence of hypothyroidism in obese subject thus thyroid function test should be done for screening of obese patients. More studies are required to find out the cause of Hypothyroidism. This information will greatly help the clinicians to rule out the disease and also help in prevention of complication associated with obesity.

## A-161

### assessment of thyroid disorders in rheumatoid arthritis patients

P. Regmi[1], M. Pandey[2], S. Baral[3], M. Khanal[1]. *[1]Nepal Medical College & Teaching Hospital, Kathmandu, Nepal, [2]Kantipur Dental College & General Hospital, Kathmandu, Nepal, [3]Bhaktapur Hospital, Bhaktapur, Nepal*

Background:

Rheumatoid Arthritis (RA) patients present with various autoimmune antibodies. And some studies have documented those patients with RA experience an increased occurrence of thyroid disorders of both the autoimmune and non-autoimmune types. Therefore, this study aims to identify thyroid disorders in RA patients.

Methods:

This study conducted in Nepal Medical College and Teaching Hospital recruited 85 patients (40 males and 45 females) with both Rheumatoid factor and Anti cyclic citrulinated peptide (anti-CCP) positive tests. After receiving written consent from patients, fasting blood sample was collected and free triiodo thyronine (fT3), free thyroxine (fT4), thyroid stimulating hormone (TSH), anti TPO and anti Thyroglobulin tests were performed.

Results:

The mean±SEM fT3, fT4 and TSH values of the RA patients were 3.58±0.11pg/ml, 1.19±0.06 ng/dl and 5.26±1.31 mIU/L respectively. Among the participants, 77% were euthyroid, 14% had subclinical Hypothyroidism, 3.5% had overt hypothyroidism, 2% had primary hypothyroidism and 2% had low fT3. Thyroid antibodies were detected in 15 of 85 (17%) patients with RA. 5 of these patients had subclinical hypothyroidism, 2 had overt hypothyroidism, one had low fT3 while the others were euthyroid. Elevated Anti-CCP was associated with presence of subclinical or overt hypothyroidism (P=0.003) and anti CCP positive cases presented with high likelihood of having hypothyroidism (P=0.004). While no significant association and likelihood found in presence of thyroglobulin antibody and presence of thyroid disorder.

Conclusion:

We recommend that thyroid function tests should better be included in the clinical evaluation of patients with RA.

## A-162

### COMPARATIVE STUDY OF LIVER ENZYMES IN UNCOMPLICATED TYPE 2 DIABETICS AND APPARENTLY HEALTHY INDIVIDUALS AT THE UNIVERSITY COLLEGE HOSPITAL IBADAN, NIGERIA

T. D. Ogunleye, O. I. Famuyiwa. *University College Hospital, Ibadan, Nigeria*

**Background:** Type2 Diabetes mellitus is of major public health concern worldwide. Previous studies have shown that individuals with type 2 diabetes have higher incidence of liver function test abnormality than individuals without diabetes. There is however scarcity of information on liver enzymes in type 2 diabetics in our community. This study therefore investigated the plasma levels of AST, ALT and GGT in type 2 diabetics attending the endocrinology clinic at the University College Hospital, Ibadan, Nigeria.

**Methods:**The laboratory records of liver function tests of uncomplicated type 2 diabetics and apparently healthy individuals from January to November 2016 of our laboratory was compiled. The liver enzymes investigated were Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma Glutamyl Transferase (GGT). Cobas C311 was used for the analysis of our assays. Levels 1 and 2 quality control material produced by Roche was always included in our daily work. The Reference Range of AST employed in our laboratory was 0- 37 IU/l, that of ALT was 0-40 IU/L while that of GGT was 7- 50 IU/L. IBM version 20 was employed for statistical analysis.

**Results:** Age range of type 2 diabetics of this study was 42-85 years with a mean of 63.32 ± 10.91 years while the age range of apparently healthy individuals was 40-89 years with a mean of 61.40 ± 11.82 years , p = 0.378. About 11.5% (7/61) of type 2 diabetics had elevated AST as compared to 6.7% apparently healthy individuals (4/60), p = 0.529. Also 4.9% (3/61) of type 2 diabetics had elevated ALT as compared to 5.0% (3/60) of apparently healthy individuals, p= 1.000. Moreover 35.6% (21/59) of type 2 diabetics had raised GGT compared to 27.1% (15/59) in apparently healthy individuals, p= 0.428. Mean value of AST in type 2 diabetics was 24.67 ± 12.41 while that of apparently healthy individuals was 24.08 ± 11.57, p = 0.788. On the other hand mean value of ALT in type 2 diabetics was 21.95 ± 19.63 and that of apparently healthy individuals was 18.80± 15.44, p= 0.329.Also the mean GGT levels in type 2 diabetics was 51.12 ± 32.73 and that of apparently healthy individuals was GGT 42.02 ± 24.01 , p= 0.088.

**Conclusion:**

Elevated AST, ALT and GGT do not seem to be a feature in patients on treatment for type 2 diabetics at the University College Hospital, Ibadan, Nigeria. Glycaemic control should be strongly advocated in diabetics patients in Ibadan, Nigeria.

## A-163

### The utility of Glycated Hemoglobin (GHb) test in the screening and diagnosis of Type 2 Diabetes mellitus.An outpatient clinics survey in Chennai s.An outpatient clinics survey in Chennai

D. S. Sheriff, S. S. D. Sakthi Dasan, G. K. Guna. *Melmaruvathur Adhisparasakthi Institute of medical Sciences and Research, Melmaruvathur, India*

**Aim:** To assess HbA1c performance against a single FastingBlood Glucose (FBG) for diagnosis of undiagnosed Type 2 diabetes(T2D) and impaired fasting glycaemia(IFG) among general medical outpatients in Chennai. **Methods:** Participants aged ≥20 years were cross-sectionally surveyed from August to October 2014. All participants underwent testing for HbA1c and FBG. The HbA1c sensitivity, specificity and predictive values in the diagnosis ofT2D and IFG were computed and their Pearson's correlation and scatter diagrams determined. **Results:** A total of 291 participants(74.2% women)with a mean age of 50.1±11.0years provided data for the current analysis. HbA1c at cut-off of ≥ 6.5% (48mmol/mol) had a sensitivity and specificity for T2D of 100%(15.81-100.00) and 86.3%(86.16-89.92) respectively. Similarly, for IFG, the sensitivity and specificity was 100%(2.5-100) and 36.3%(30.3-42.6) respectively. The Positive Predictive Value (PPV) was 4.8%(0.58-16.16) and 0.6%(0.02-3.45) for T2D and IFG screening respectively. The Negative Predictive Value (NPV) was 100% in both cases of T2D and IFG screening.HbA1c had a modest,positive correlation (r) with FBG for the overall population ($r$ =0 .536, $p$< 0.001); for women, ($r$=0.578, $p$<0.001) and for men ($r$=0.336, p =0.003). **Conclusion:**HbA1c had high sensitivity but widely varying specificity, high proportion of discordant results and poor prediction of T2D and IFG in this setting. Although, HbA1c correlation with fasting glucose was modest, both tests are required toimprove diagnostic reliability in asymptomatic T2D screening program.

## A-164

### Serum leptin level in Hypothalamic Amenorrhea

S. Deshar. *Tribhubhan University Teaching Hospital, kathmandu, Nepal*

**ABSTRACT Background** Researches support functional hypothalamic amenorrhea (FHA) is weight-loss, stress, and exercise-related condition which results from aberrations in pulsatile gonadotropin-releasing hormone (GnRH) secretion causing impairment of the gonadotropins (FSH/LH). The final consequences are complex hormonal changes manifested by profound hypoestrogenism. A sensitive marker of nutritional status, leptin is known to correlate with fat mass and to respond to

changes in caloric intake. Leptin is an adipocyte-secreted hormone that plays a key part in energy homoeostasis. Studies in animals and human beings have shown that low concentrations of leptin are fully or partly responsible for starvation-induced changes in neuroendocrine axes, including low reproductive, thyroid, and insulin-like growth factor (IGF) hormones.**Objectives** This study aimed to determine serum leptin level in hypothalamic amenorrhea, to correlate serum leptin level with BMI and to compare serum leptin with other types of secondary amenorrhea viz; hyperprolactinemia, PCOS and hypothyroidism.**Methods** It is a single center, cross sectional, observational study. A total of 90 participants from gynecology OPD were enrolled in this study within 10 months. 81 cases were of secondary amenorrhea who had amenorrhea more than 3 months duration excluding pregnancy. They were divided into hypothalamic amenorrhea (42), hyperprolactenimia (19), hypothyroid (6), PCOS (14) and 9 eumenorrheic cases were age, height and weight matched with FHA. SPSS ver. 20.0 was used to analyze the data. T test and ANOVA were used to find mean differences and Pearson's correlation was used to establish the correlation between study variables.The p value less than 0.05 is considered significant. **Results** Mean age of study population was 25.3±5.2 years. Among all 38% of study population was of age group 20-25 years. Within the secondary amenorrhea group 47% were of hypothalamic amenorrhea followed by 21% hyperprolactinemia, 16% PCOS and 10% hypothyroidism. The weight and BMI of the hypothalamic amenorrhea cases were found to be significantly lower than other causes of amenorrhea ($p<0.001$). The mean serum leptin level was found to be lower in hypothalamic amenorrhea compared to other causes of amenorrhea and eumenorrheic control group (3.019±1.1 ng/ml vs. 9.315±4.2 ng/ml vs; 5.60±2.40 ng/ml, $p =0.001$). While in PCOS; BMI and serum leptin level were higher. Likewise serum TSH, LH, FSH, estrogen and testosterone were also found lower in hypothalamic amenorrhea compared to other types of amenorrhea ($p<0.001$). The cut off value of serum leptin in hypothalamic amenorrhea was found to be 4.45ng/ml from other causes of amenorrhea and control group. There were positive correlations between serum leptin and BMI, LH, FSH, TSH, estrogen and testosterone ($p<0.001$). **Conclusion** This study showed that serum leptin, weight and BMI level is significantly lower in hypothalamic amenorrhea than other types of amenorrhea and normal eumenorrheic control. The positive correlations between leptin and gonadotropins, estrogen, testosterone and TSH reflect the reproductive role of leptin in the HPG axis. Thus, leptin may act as the critical link between nutritional adequacy and the reproductive system, indicating whether adequate energy is present for normal reproductive function. **Key Words** Hypothalamic amenorrhea, Serum leptin, BMI, Nutrition, Gonadotropins

## A-165

### Thyroid-Related Testing Utilization: A Multi-Center Benchmark Study

D. C. Lin, J. A. Straseski, R. Schmidt. *Department of Pathology, University of Utah, Salt Lake City, UT*

**Background:** Test utilization improvements require better knowledge of practice variation. Thyroid tests are some of the most commonly performed laboratory tests, yet little is known is known about the thyroid test ordering patterns. The objective of this study was to analyze practice variation in thyroid-related testing and to determine the impact of laboratory utilization management programs on testing patterns. **Methods:** 82 sites across the United States participated in the study. A survey was conducted to collect annual thyroid-related test volume data and utilization management activities. The thyroid-related tests examined included thyroid stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4), free triiodothyronine (FT3), total triiodothyronine (TT3), triiodothyronine uptake (T3U), and reverse triiodothyronine (rT3). Annual complete blood count (CBC) volumes were also collected to normalize TSH test volume (TSH/CBC), which served as a comparator for thyroid workup rates across sites. Individual thyroid testing volumes were normalized to that of TSH to compare thyroid test selection patterns. Quality of thyroid test ordering was assessed using the following test volume ratios: FT4 to T4-related tests (both FT4 and TT4) ratio, and T3U to TSH ratio. We also collected data on laboratory utilization management activities at each organization. **Results:** The thyroid workup rate (TSH/CBC) was higher for outpatient (0.26), relative to inpatients (0.03). Significant variation in test selection patterns were observed across sites for all tests. Based on the median values, 14 FT4, 3 TT4, 4 FT3, 2 TT3, 0.1 T3U, and 0.1 rT3 tests were ordered for every 100 TSH tests ordered. Approximately 90% of the T4-related orders were FT4 rather than TT4. T3-related orders (FT3 and TT3) were roughly evenly distributed between FT3 and TT3. While most of the organizations had implemented test utilization management activities to varying degrees, there was a weak relationship between the extent of these activities and the quality of thyroid test ordering. For instance, high quality thyroid test ordering would be suggested by a high FT4 to T4-related tests volume ratio, and a low T3U to TSH test volume ratio. FT4/T4 was positively correlated with utilization management activities ($r=0.38$) but the association was not statistically significant ($p = 0.15$). T3U/TSH had a statistically significant negative correlation with utilization management activities ($r = -0.54$, $p = 0.03$). **Conclusion:** The test ordering patterns for analytes such as FT4 were consistent with guideline recommendations in the literature, e.g., the preferred use of FT4 over TT4 during workup. However, based on our sample, there still appears to be wide variation in thyroid-related test ordering patterns in the United States. As such, better implementation of more stringent test utilization management activities may be beneficial. Together, these results suggest that there remains much room for improvement in thyroid test utilization for a number of organizations, and that clearer guidelines may be warranted.

## A-166

### Development of a New Biochip Array Applied to the New Random Access Fully Automated Evidence Evolution Analyser for the Simultaneous Measurement of TSH, Free T4 and Free T3

D. Vance, S. Lindsay, T. McConnell, D. McAleer, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background**

Thyroid function tests are indicated in the diagnosis and management of thyroid disorders and most commonly Thyroid Stimulating Hormone (TSH), Free Thyroxine (T4) and Free Triiodothyronine (T3) are measured. TSH is secreted from the pituitary gland and it has been suggested to be the most sensitive indicator of hypo- or hyperthyroidism. TSH regulates thyroidal secretion of the thyroid hormones T4 and T3, which in turn exert a negative feedback on the pituitary and hypothalamus. A multi-analytical tool allowing the simultaneous measurement of these three hormones is therefore advantageous in clinical settings. This study reports the development of a new biochip array for the multiplex measurement of TSH, FT4 and FT3 from a single sample and applied to the first high throughput, random access with STAT capability, fully automated biochip analyser, Evidence Evolution. This application represents a new multi-analytical tool in the investigation of thyroid function.

**Methods**

Simultaneous chemiluminescent competitive and sandwich immunoassays were developed and applied to the biochip analyser Evidence Evolution, the capture antibodies being immobilised on the biochip surface at discrete test sites. Functional sensitivity was assessed along with repeatability precision using serum based precision material. Serum patient samples (n=53) were assessed and the results compared with commercially available methods.

**Results**

The biochip assay showed a functional sensitivity value of 0.01 µIU/mL for TSH. Repeatability assay precision values for low, medium and high levels of TSH, FT3 and FT4, expressed as CV (%) were 3 %, 3 % and 8 % CV for TSH, 4.7 % 3.8 % and 6.9% for FT3 and 2.9 % 2.6 % and 4.9% for FT4. R values of 0.99 for TSH, 0.98 for FT3 and 0.97 for FT4 were obtained following regression analysis of the results after the assessment of the 53 serum samples with the biochip assay and another commercially available methods.

**Conclusion**

The results show applicability of the newly developed biochip array for Evidence Evolution for the reliable simultaneous quantitative determination of high sensitive TSH, alongside FT3 and FT4 from a single serum sample. This multi-analytical approach will aid in the efficient diagnosis and management of patients with thyroid disorder. The new Evidence Evolution platform also incorporates STAT sample and random access capabilities.

## A-167

### Method-Specific Reference Intervals for Thyroid Function Tests during the Third Trimester of Pregnancy

S. L. La'ulu[1], S. P. Wyness[1], I. Fawson[2], J. A. Straseski[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Department of Pathology, University of Utah, Salt Lake City, UT*

**Background:** The American Thyroid Association recommends trimester- and method-specific reference intervals (RI) for markers of thyroid function during pregnancy. Study objectives were to establish RIs for thyroid stimulating hormone (TSH) and free thyroxine (FT4) during the 3rd trimester of pregnancy using the Roche cobas e602. This expands upon our previously reported RIs for 1st and 2nd trimesters.[1,2]

**Methods:** Surplus maternal serum screen specimens were collected from 157 subjects ranging from 15-43 years of age (median=26 years), with gestational age of 27-40 weeks (median=28.3 weeks). TSH and FT4 testing were performed using the Roche cobas e602. Thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) autoantibodies were measured using the Beckman Coulter DxI. TgAb and/or TPOAb positive subjects were excluded from analyses (>4.0 and >9.0 IU/mL, respectively). The central 95% nonparametric RI for TSH was determined, and then FT4 RIs were determined using subjects within this TSH RI. Results were compared to previously determined RIs using self-reported healthy, non-pregnant subjects, and data from 1st and 2nd trimesters.[1,2] The RI for pregnant subjects was considered significantly different if the reference limits did not fall within the 90% confidence intervals (CI) of comparison group.

**Results:** TSH and FT4 RIs are summarized (Table). When comparing RIs from 3rd trimester subjects to non-pregnant subjects, the lower reference limit for TSH was not found to be significantly different; whereas the upper reference limit was significantly lower (*). For FT4, both the lower and upper reference limits for 3rd trimester subjects were significantly lower than non-pregnant individuals. Additionally, significant differences were observed between the three trimesters.

**Conclusions:** Significant differences for TSH and FT4 RIs were observed between the 3rd trimester of pregnancy and non-pregnant individuals, and between the three trimesters. This supports guidelines recommending trimester- and method-specific RIs for thyroid function tests.

[1]Silvio et al. ClinBiochem 2009

[2]Wyness et al. ClinChimActa 2011

| Analyte/ Population | n | 2.5th percentile (lower limit) | 90% CI (2.5th) | 50th percentile (median) | 97.5th percentile (upper limit) | 90% CI (97.5th) |
|---|---|---|---|---|---|---|
| **TSH (mU/L)** | | | | | | |
| 3rd trimester | 145 | 0.39 | 0.16 – 0.52 | 1.48 | 3.84* | 2.84 – 5.47 |
| Non-pregnant | 134 | 0.36 | 0.01 – 0.72 | 1.94 | 4.77 | 4.14 – 5.29 |
| **FT4 (ng/dL)** | | | | | | |
| 3rd trimester | 139 | 0.70* | 0.64 – 0.75 | 0.96 | 1.22* | 1.17 – 1.31 |
| Non-pregnant | 128 | 0.89 | 0.80 – 0.93 | 1.22 | 1.58 | 1.50 – 1.65 |

## A-168

### Level of Prostaglandin, tumor necrosis factor - alpha and prolactin in fertile and infertile women in Calabar, Nigeria

I. K. ISONG, D. C. Okpokam, Z. A. Okhormhe, C. A. O. Usoro. *University of Calabar, Calabar, Nigeria*

**Abstract**

**Background:** Infertility is defined as the inability of a couple to achieve conception despite frequent unprotected, well timed sexual intercourse for a year duration. **Aim:** To identify and ascertain non invasive sensitive techniques of determining the levels of prostaglandin F2α and E2, tumor necrosis factor-α (TNF- α) and prolactin in fertile and infertile women.

**Method:** One hundred and two (102) volunteers infertile women, aged 20-45 years were recruited from the University of Calabar Teaching Hospital. They were further divided into three groups based on their prolactin levels; namely those with normal ovarian hormones value (normal); (n=32), those with prolactin values between 25-60ng/ml (moderate) (n=46) and those with prolactin values greater than 60ng/ml (high); (n=24). Sixty (60) apparently healthy, age matched women were also selected to serve as the control group. Sera samples was obtained and used for prolactin, prostaglandin E2 and F2α and Human TNF-α using Enzyme immunosorbent assay.

**Results:** There was no significant difference ($p>0.05$) in the prostaglandin E2 and tumor necrosis factor-α between the infertile women with normal hormonal profile and the controls. However, the prolactin and prostaglandin F2α was significantly higher ($p<0.05$) in the infertile women with normal ovarian profile than the control. The prolactin values were significantly higher in the high group compared to the normal ovarian profile and moderate group. There was a strong positive correlation. ($r=0.584$; $p<0.05$) between $PGE_2$(pg/ml) and $PGF_{2\alpha}$(pg/ml).

**Conclusions:** From our study we observed that Prostaglandin F2a, Tumor necrosis factor alpha and Prostaglandin E2 and F2 ratio is high in infertile subjects. Our findings may also provide some new insights to understand the physiopathology or pathogenesis of infertility and tumor necrotic factor and prostaglandins and may hold new therapeutic potential.

## A-169

### STATUS OF VITAMIN-D IN RELATION TO GLYCEMIC INDICES > LIPID PROFILE IN POST-MENOPAUSAL WOMEN WITH TYPE 2 DIABETES MELLITUS

A. O. Aljohi[1], M. Abudawood[2], H. Tabassum[2], S. Ansar[2], F. Aljaser[2], S. Sobki[1], M. ALI[3]. *[1]Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [2]King Saud University, Riyadh, Saudi Arabia, [3]Mumtaz Degree and P.G. College, Hyderabad, India*

**Background:** Abnormal vitamin D level and glucose homeostasis are two of the most chronic medical conditions leading to osteoporosis and cardiovascular disease following menopause transition in females. Vitamin D deficiency is the most commonest health problem among postmenopausal women worldwide. Low levels of vitamin-D could be associated with elevated risk of cardio metabolic disorders comprising cardiovascular disease and type 2 diabetes. Besides enduring multiple complications of chronic hyperglycaemia, diabetic patients tend to be soft targets of deadly cardiovascular disease (CVD) due to dyslipidemia. The aim of the present study was to evaluate and compare vitamin D status in relation to glycemic indices ,lipid profile between premenopausal and postmenopausal women with type 2 diabetes (T2DM).

**Methods:** : In this cross sectional study, 600 women with T2DM were divided in pre-menopausal (n = 300) and post-menopausal (n = 300) group. Levels of fasting blood glucose , HbA1C, lipid profile parameters, i.e., total cholesterol (TC), triglycerides (Tg), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and vitamin D were measured in pre and postmenopause women and analysed by SPSS software. Comparison between the groups was done by one way ANOVA followed by Holm-Sidak test .

**Results:** The mean ages of premenopausal and postmenopausal were 43.16 ± 4.2 and 59.59±10.08 years, respectively. Levels of HbA1C, FBG, TC, Tg and LDL-C increased significantly ($p<0.001$) in postmenopause women compared to premenopausal women. In contrast to these parameters, serum levels of HDL-C, Vitamin D decreased significantly in T2DM postmenopause diabetic women compared to premenopausal diabetic women. Vitamin-D was negatively correlated with age,HbA1C,LDL-C at $p<0.05$. This study had shown that dyslipidemia in postmenopausal diabetic women had higher prevalence of high Tg, TC, and LDL-C than the pre-menopausal women, indicating that they were more prone to cardiovascular diseases.

**Conclusion:** Dyslipidemia observed in postmenopause women accompanied with decreased vitamin-D increases the risk factors in Type 2 Diabetes.

## A-170

### Validation of optimized saliva immunoassays for Testosterone, Progesterone and Cortisol.

M. Herkert[1], T. Dudek[1], B. Uelker[1], C. E. Geacintov[2]. *[1]DRG Instruments, Marburg, Germany, [2]DRG International, Springfield, NJ*

Between 95 and 99% of a hormone in the bloodstream is bound to carrier proteins, and only the unbound fraction freely diffuses into tissues, including the salivary gland. Therefore, saliva is a clinically informative, biological fluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases. It is easily collected and stored and ideal for early detection of soluble biomarkers, because both diurnal and monthly profiles of hormone levels parallel traditional serum patterns. Here we present validation data that confirm that the analytes testosterone, progesterone and cortisol can be measured with good precision and sensitivity from oral fluid. Furthermore, results perfectly correlate to mass spectrometry results. All assays have a total assay time of 1.5 hours, and need 100 µl of saliva sample. Spiking recovery and linearity were proven to be in the range of 100 +/- 15%. Salivary Testosterone (SLV-3013): Measurement of testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones, including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism, and virilization due to tumors, polycystic ovaries, and adrenogenital syndromes. Assay characteristics are: Measuring range: 2.63 (LoD) - 1000 pg/ml. LoQ: 10.1 pg/ml. Mean intra-assay precision: 4.7%, mean inter-assay precision: 7.6%. Method comparison showed very good correlation to LC-MS/MS ($r = 0.9904$; $y = 1.015 x - 2.8203$) and normal ranges were determined for men (age-dependent) and women. Salivary Progesterone (SLV-5911): The steroid hormone Progesterone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy. Assay characteristics

are: Measuring range: 1.1 - 2400 pg/mL. Mean intra-assay precision: 6.3%, mean inter-assay precision: 9.2%. Method comparison showed very good correlation to LC-MS/MS (r=0.997; y = 0.9612x - 11.071) and normal ranges were determined for women in follicular and luteal cycle phase as well as men. Salivary Cortisol (SLV-2930): Cortisol shows a diurnal rhythm with highest concentrations in the morning and steady decrease to very low levels 12 hours later. Cortisol secretion increases in response to any stress in the body, whether physical (such as illness, trauma, surgery, or temperature extremes) or psychological. Moreover, elevated cortisol levels and lack of diurnal variation have been identified with Cushing's disease and in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, Addison's disease) and in ACTH deficiency. Assay characteristics are: Measuring range: 0.09 - 30 ng/mL. Mean intra-assay precision: 3.9%, mean inter-assay precision: 7.4%. Method comparison showed very good correlation to LC-MS/MS (r=0.999; y = 1.032x +0.111) and normal ranges were determined for men and women at morning, noon and evening.

## A-171

### Mean platelet volume and diabetes in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

C. B. Maluf, M. H. S. Diniz, S. M. Barreto, R. C. P. dos Reis, P. G. Vidigal. *School of medicine. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

**Background:** Diabetes Mellitus (DM) is associated with higher risk of atherothrombosis, and 80% of patients with DM died because of thrombosis whose principal trigger is endothelial dysfunction and platelet hyperactivity. Under physiological conditions, the number of platelets is inversely proportional to mean platelet volume (MPV), to keep a constant level of platelet mass. Studies in DM patients show that the balance between platelet production and depletion is lost, and they tend to have higher MPV values without difference in the platelet count. Our purpose in this study was to investigate whether diabetes and pre- diabetes are independently associated with MPV, an easily obtained marker of platelet size and platelet activity. **Methods:** We used the baseline data (2008-2010) of 3115 civil servants (aged 35-74 yr) from a university and enrolled in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Venous blood sampling was performed after 12- to 14- hour-fasting, using tubes containing ethylenediaminetetraacetic acid (EDTA). The time between sampling and exam procedure was strictly controlled to be within 2 hours, and blood samples were kept at room temperature until the measurements. Presence of DM were classified using fasting plasma glucose (FPG; ≥126 mg/dL [7.0 mmol/L]), 2-hour plasma glucose (PG) during an oral glucose tolerance test (OGTT) (2h PG OGTT; ≥200 mg/dL [11.1 mmol/L]), and glycated hemoglobin (HbA1c; ≥6.5%; [48.0 mmol/mol]). DM was also defined by the self-reported information or use of insulin or hypoglycemic medication identified in the baseline survey of the ELSA study. Pre-diabetes was classified by the presence of impaired fasting glucose (IFG) (FPG ≥ 100 mg/dL [5.6 mmol/L] to 125 mg/dL [6.9 mmol/L]), and/or impaired glucose tolerance (IGT) (2h PG OGTT ≥140 mg/dL [7.8 mmol/L] to 199 mg/dL [11.0 mmol/L]), and/or HbA1c ≥5.7% (39 mmol/mol) to 6.4% (46 mmol/mol), according to ADA. Multiple linear regression analysis was used to estimate the independent association of the diabetes and pre-diabetes with the MPV after adjusting for sex, age, platelet count, and hypertension. All the variables entered in the multiple regression analysis using the forward approach. Statistical assumptions to perform multiple linear regressions were checked by residual analysis. **Results:** MPV (adjusted $r^2$=0.143; p=0.01), was independently associated with diabetes and pre-diabetes, compared to normoglycemic subjects. The set of variables included in the multivariate model remained explained about 14% of the variability of the MPV evaluated. Diabetes had higher β (0.207) that than pre-diabetes (β=0.110) in the model to estimate the independent association with MPV. **Conclusion:** In this large cohort of free living Brazilians, ours results showed that increased MPV is independently associated with the presence of diabetes and pre-diabetes, suggesting an early change in initial increase of the glucose levels. Platelets from diabetic patients are an accelerated rate of renewal, so higher MPV values may act as a marker of the production of bigger, denser, and more reactive platelets in DM type 2. Whether this condition is the cause or consequence of atherothrombotic cardiovascular events in diabetics remains unclear.

## A-172

### Biomarker changes in adult men with low testosterone (low-T)

M. Samoszuk[1], S. Haitjema[2], M. de Groot[2], W. van Solinge[2], M. Li[1], F. Adair[1], I. Hoefer[2]. *[1]Beckman Coulter, Inc., Brea, CA, [2]University Medical Center., Utrecht, Netherlands*

**Background:** Androgens such as testosterone are known to have effects on many organs and systems, such as prostate, bone marrow, bone turnover, muscle, and metabolism. However, it is not known if men with androgen deficiency (low-T) have consistent or characteristic abnormalities in the biomarkers that measure the functions of the organs and systems that are influenced by androgens. The purpose of this retrospective study was to compare the mean levels of various biomarkers in men with low-T (n=1752) and in men with normal T (n=9617).

**Methods:** The Utrecht Patient Oriented Database (UPOD) contains all health care data and measurements from all patients admitted to the University Medical Center Utrecht in the Netherlands. We extracted data from male patients over 40 years old who presented for evaluation of possible low-T and who had a laboratory measurement of total testosterone levels in combination with a measurement of one or more of the following biomarkers on the same day: free testosterone (n=6264), uric acid (n=308), estradiol (n=1016), prostate specific antigen (PSA, n=2897), sex-hormone binding globulin (SHBG, n=7126), luteinizing hormone (LH, n=4422)), creatinine (n=6781), bone alkaline phosphatase (BAP, n=3421), creatine kinase (n=167), LDH (n=2829), hemoglobin A1c (n=2249), and 25-hydroxy-vitamin D (n=856). Measurements from patients having a diagnosis of prostate cancer were excluded. Analyses were stratified based on serum testosterone levels classified into lowest (<4.5), low (4.5-7), and normal (>= 7 mmol/L). Differences between testosterone strata were assessed with the Kruskal Wallis test.

**Results:** Compared to men with normal levels of T, the men with the lowest levels of T had significantly (p<0.001) lower means of free testosterone (51 versus 300 pmol/L); PSA (0.49 versus 0.94 micrograms/L); SHBG (29 versus 35 nmol/L); luteinizing hormone (1.5 versus 3.6 IU/L); and estradiol (40 versus 89 pmol/L). In comparison to men with normal levels of T, men with low levels of T also had statistically (p<0.001) higher mean levels of LDH (217 versus 198 U/L); BAP (81 versus 75 U/L); and hemoglobin A1c (41 versus 39 mmol/mol). Mean uric acid levels in men with the lowest T levels were also higher than in men with normal T (0.41 versus 0.34 mmol/L, p=0.02).

**Conclusion:** Our results indicate that low T in adult men is associated with significant changes in various biomarkers that measure the functions of organs and systems that are influenced by androgens, such as prostate, bone, and the endocrine system. This finding is important because it may lead to improved diagnosis and treatment of low-T by identifying those men who have objective evidence of physiologic changes produced by androgen deficiency that may warrant therapy.

## A-173

### Relation between thyroid hormone levels and hypertension in Kumasi, West Africa

P. Nsiah[1], E. K. Agyare[2], F. A. Yeboah[3], B. A. Eghan[3], O. K. Opare-Sem[3]. *[1]University of Cape Coast., Cape Coast, Ghana, [2]Florida A&M University, Tallahassee, FL, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

**Background:** Hypertension is a major threat to the health of adult individuals in West Africa however, the management and control of hypertension has been poorly managed. Thyroid disorders have been implicated in high blood pressure (BP) of patients with essential hypertension. Currently, there are no data on the frequency with which hypertension may be associated with and caused by thyroid disorders, the treatment of which may restore the elevated BP to normal in individuals in West Africa. The aim of the study is to study whether there is an association between thyroid disorders and hypertension

**Methods:** The study was conducted at Komfo Anokye Teaching Hospital, in Kumasi, West Africa. Participants were randomly selected and their participation was voluntary. Informed consent was obtained from each individual and the study was approved by the University of Science and Technology Medical School. Participants with renal disease, lipid disorders, electrolyte imbalances and diabetes were excluded. Blood pressure was measured with a sphygmomanometer after 30 minutes of rest with the participants and individual with a systolic and/or diastolic blood pressure exceeding 160/95 mm Hg was defined hypertensive. Serum triiodothyronine ($T_3$), thyroxine ($T_4$), and thyroid stimulating hormone (TSH) were determined by enzyme-linked immunosorbent assay technique.

**Results:** Based on the serum levels of T4 and TSH, no statistically significant difference ($T_{4\ non\text{-}HPT}$ vs $T_{4\ HPT}$, $p = 0.18$; $TSH_{non\text{-}HPT}$ vs $TSH_{HPT}$, $p = 0.4$) was observed between non-HPT ($T_4$ = 70.4 ± 13.9 ng/ml; TSH = 1.9 ± 0.9 µIU/ml) and HPT ($T_4$ = 81.1 ± 23.9 ng/ml; TSH = 1.7 ± 1.1 µIU/ml). However, mean serum $T_3$ level of HPTs was statistically greater than that of non-HPTs ($T_{3\ HPT}$ 1.6 ± 0.7 ng/ml and $T_{3\ HPT}$ 1.2 ± 0.5 ng/ml, $p < 0.03$). In univariate analysis, correlation between systolic, but not diastolic, BP and serum level of either $T_3$ or $T_4$ was significant (r = 0.30, $p = 0.001$ and r = 0.25, $p = 0.002$ respectively). We did not observe any level of significance between systolic BP and TSH (r = -0.13 and $p = 0.124$). Similar correlation was observed between diastolic BP and serum level of $T_3$ or $T_4$ (r = 0.31, $p = 0.001$ and r = 0.28, $p = 0.001$ respectively). As expected, no significant difference between diastolic BP and TSH was noticed (r = -0.12 and $p = 0.136$). In our multivariate analysis, TSH again did not significantly influence systolic or diastolic BP while $T_3$ or $T_4$ strongly influence both systolic and diastolic BP. Relating thyroid hormone levels across the age groups, $T_3$ and $T_4$ levels of HPT were found to be higher than that of non-HPT in all corresponding age groups except TSH which was higher in 41-50 year group of HPT than non-HPT group. There was no significant difference between the ages of both non-HPT and HPT participants (non-HPT = 50.6 ± 11.7 and HPT = 51.0 ± 12.4 years).

**Conclusion:** These results suggest a close association between $T_3$ and $T_4$ and hypertension.

## A-174

### The Study of Trimester-Specific Thyroid Stimulating Hormone and Free Thyroxine Reference Intervals with Chinese Women by Experimental and Statistical Methods

Y. Zhai[1], L. Han[1], Z. Zhao[2], Z. Cao[1]. *[1]Beijing Obstetrics & Gynecology Hospital, Beijing, China, [2]National Institutes of Health, Bethesda, MD*

**Background:** As a result of physiological and metabolic changes during pregnancy, thyroid hormones can be affected significantly throughout entire three trimesters. For example, two pregnancy-related hormones—human chorionic gonadotropin (hCG) and estrogen, are well known to cause increased thyroid hormone levels in the blood. To support thyroid disease diagnosis in pregnancy, the objective of this study was to establish trimester-specific thyroid stimulating hormone (TSH) and free thyroxine (FT4) reference intervals (RIs) in Chinese women by experimental and statistical methods.

**Methods:** A total of 1205 pregnant women were recruited from Jan 2016 to Dec 2016 at our hospital according to the following exclusion criteria: Patients who are with a personal or family history of thyroid disease, with a goiter, have more than one fetus, or pregnancy complications. Those initially selected patients were further tested for TSH, FT4 and thyroid peroxidase antibody (aTPO), performed on the chemiluminescent platform Siemens ADVIA Centaur® XP. Only patients tested negative for aTPO were included in reference interval establishment. Besides, linear regression was carried out between FT4 and log transformed TSH to see if there is a linear correlation. Lastly, to validate the Hoffmann indirect method for the derivation of TSH and FT4 RIs, 10044 outpatients who came to our institute in 2016 for thyroid function screening in their first trimester (1-13 week) were included. The reference change value (RCV) was calculated for determining the statistical significance of the differences between the calculated RIs by Hoffmann method and the observed RIs in this study.

**Results:** According to the CLSI recommendation, RIs for both TSH and FT4 were determined as 2.5$^{th}$ percentile to 97.5$^{th}$ percentile on the data distribution. The TSH and FT4 trimester-specific RIs were shown as follows: 0.59-3.56 mIU/L, 11.8-18.4 pmol/L (n=188, 1st trimester); 0.79-4.60 mIU/L, 11.6-17.5 pmol/L (n=133, 2nd trimester); 0.65-4.20 mIU/L, 9.6-15.1 pmol/L (n=157, 3rd trimester).. When compared pairwise with Mann-Whitney test, both TSH and FT4 levels were statistically significant between 1st and 2nd, 1st and 3rd, 2nd and 3rd (not for TSH). The RIs of TSH and FT4 determined by Hoffmann method for first trimester outpatient pregnant women were 0.33-3.96 mIU/L and 11.7-17.5 pmol/L respectively. There is no significant difference between observed and calculated RIs for first trimester pregnant women in this study. No linear relationship was observed between FT4 and logTSH in any trimester-specific population.

**Conclusion:** We have established trimester specific RIs for thyroid function test in a Chinese population using both experimental and statistical methods. The results of the two methods are comparable. The similar approach can be applied to evaluate and verify the trimester specific RIs for other analytes.

## A-175

### Performance Evaluation of the ADVIA Centaur Androstenedione Assay

L. Parker[1], J. Driscoll[1], C. Higgins[1], M. Stranz[1], K. Garvey[1], J. Lyons[1], Z. Zhao[1], Y. Zheng[1], P. Donovan[2]. *[1]Siemens Healthcare Diagnostics, Newark, DE, [2]Siemens Healthcare Diagnostics, Walpole, MA*

**Background:** Androstenedione is a 19-carbon steroid that serves as a precursor for testosterone and estrone. It is most commonly used in conjunction with other steroid assays to evaluate the function of the adrenal glands and ovaries or testes and to determine the cause of symptoms of androgen excess.

A new ADVIA Centaur® Androstenedione (ANDRO) assay for the measurement of androstenedione in human serum and plasma is being developed by Siemens Healthineers. The studies below describe preliminary performance of the assay on the ADVIA Centaur® Immunoassay System.

**Methods:** The ADVIA Centaur ANDRO assay is a fully automated competitive immunoassay using direct chemiluminescent technology. Reagents include a biotinylated sheep monoclonal antibody coupled to streptavidin-coated paramagnetic particles in the solid phase and a newly developed acridinium ester in the Lite reagent. The assay requires 20 µL of patient sample or calibrator, which is incubated with solid phase and Lite reagent. Competition for solid phase binding occurs between androstenedione in the sample and the Lite reagent. Separation follows, and the amount of signal generated is inversely proportional to the concentration of androstenedione in the sample. The time to first result is 18 minutes.

**Results:** LoQ studies and linearity evaluation of the ADVIA Centaur ANDRO assay demonstrated an assay range of 0.30 to 10.00 ng/mL; with automated dilution, the measuring interval was extended to 50.00 ng/mL. The assay correlated well with LC-MS/MS, and equivalent performance was obtained using serum, lithium heparin, and EDTA plasma tube types. The assay showed ≤10% interference for all interferents tested and ≤1% cross-reactivity for all endogenous and most exogenous cross-reactants evaluated. Within-lab precision was <9% CV (with 95% confidence) across the assay range. Stability data demonstrated a calibration interval and onboard stability of 20 days and 16 days, respectively.

**Conclusions:** The ADVIA Centaur ANDRO assay demonstrates good precision and correlates well to LC-MS/MS.

*Information about this device is preliminary. Safety and effectiveness for the uses discussed have not been established. The device is under development and not commercially available. Future availability cannot be ensured.

## A-176

### Critical values in the endocrinology laboratory;our experience

M. Viale, A. Kozak, M. Balzaretti, G. Rubino, E. Miler, M. Serra, P. Fainstein Day. *Hospital Italiano de Buenos Aires, CABA, Argentina*

**Background:** Critical Values(CV) are results of diagnostic tests that express a medical situation wich may put the patient's life at risk if nothing is done properly and on time. Many clinical situations in Endocrinology could generate results of CV in laboratory parameters. According to available literature and in conjunction with specialized professionals of our staff we defined the following Endocrine disorders that could compromise patients life: Myxedematous Coma,Thyroid Storm, Acute Adrenal Crisis, Acute Abdomen in Assisted Fertilization and Trophoblastic disease.

Once the professional staff of the laboratory chooses to determine a program of CV, they must clearly define a policy which should include the list of tests, the mechanisms and people responsible for notifying the CV when they occur.The frequency of the CV, is highly variable and depends on the type of population served and other characteristics of each institution.OBJECTIVE: To evaluate the frequency of CV in our laboratory along a year after we defined policy of them regardless of whether they are inpatients or outpatients. Also report the time of clinical evolution in the Electronic Health Records (EHR).

**Methods:** In order to develope a documented system for CV we define the following list of serum determinations: Thyrotropin(TSH)>100.0uUI/mLTotalThyroxine(T4)>20.0ug/mL; Free T4<0.4 and >4.0ng/dL; Estradiol(E2)>4000pg/ml; BHCG>500000mU/mL performed in Architect i2000(Abbott) and Cortisol at 8 pm without corticosteroids:<5 ug/dL in Immulite 2000(Siemmens) and we determine the frequency of them. Both fully automated and *chemiluminescent* analyzers. The records in the EHR were divided into four groups according to the time of delay in the evolution differentiating between inpatients outpatients.

**Results:** Total number per year (TN/Y) of Cortisol is n=2619, number of CV per year (CVn)=93 (3.6%); E2 TN/Y=7029 CVn=15(0.2%); BHCG TN/Y=4697 CVn=2(0.04%);TSH TN/Y=95013 CVn=58(0.06%);T4L TN/Y=31920 CVn=20(0.06%);TotalT4 TN/Y=23286 CVn=15(0.06%).The frequency of the total CVs per year is: Cortisol (41%) followed by TSH(37%), T4(7%), E2(7%), T4L(6%), T3(1%) y BHCG(1%). The percentage of clinical evolutions in the EHR within the first hour of recording the CV (inpatients/outpatients)(30.1%/9.8%); between 1 and 6 hours(21%/5.6%); between 6 and 24 hours (7.7%/0.7%) and more than 24 hours (18.2%/1.4%).

**Conclusion:**Cortisol was the most frequent parameter we found and it should be the first to be include in the list.TSH despite being the most requested determination in our laboratory was not the most common CV probably due to extensive knowledge of this pathology.

The policy of CV, rather than a rule or a tool for continuous improvement in the clinical laboratory is a right for patients and the circuit is closed when the doctor records and takes corrective action.The CV reporting process is an important laboratory resource to maximize clinical benefits. Due to insufficient information of CV in the Laboratory of Endocrinology our intention is to provide our experience to improve the quality of them.

## A-177

### The functional SNP and expression of IL15 gene are associated with the development of autoimmune thyroid disease.

Y. Arakawa[1], M. Watanabe[1], K. Takemura[1], N. Inoue[2], Y. Hidaka[2], Y. Iwatani[1]. *[1]Osaka University, Osaka, Japan, [2]Osaka University Hospital, Osaka, Japan*

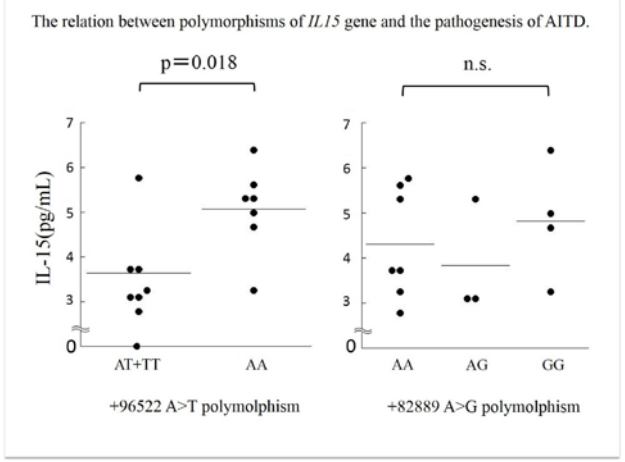
[**Background**]There are considerable differences in the prognosis of autoimmune thyroid diseases (AITDs) including Graves′ disease (GD) and Hashimoto′s disease (HD). It has been known that the genetic producibilities of some cytokines and immune modulators are associated with their prognosis.

IL-15 is a proinflammatory cytokine and produced by several cells such as monocytes and activated CD4+ T cells. In various autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and HD, higher serum levels of IL-15 have been reported, suggesting that IL-15 may be associated with the onset of autoimmune diseases.

**[Methods]**To clarify the association between the genetic producibility of IL-15 and the pathogenesis of AITDs, we genotyped +96522 A>T and +82889 A>G polymorphisms in the *IL15* gene using 127 patients with HD, including 55 patients with severe HD and 48 patients with mild HD; 130 patients with GD, including 52 patients with intractable GD and 44 patients with GD in remission; and 79 healthy volunteers.

**[Results]** Both the *IL15* +96522 A allele and AA genotype were more frequent in patients with severe HD than in those with mild HD. The serum levels of IL-15 were higher in individuals with the *IL15* +96522 AA genotype than in those with the T allele, and they were also higher in patients with severe HD than in those with mild HD. On the other hand, the mRNA levels of IL-15 were not significantly different among individuals with each genotype of both SNPs. After incubation with recombinant human IL-15, the proportions of Th17 cells in $CD4^+$ cells were increased, and those of Treg cells in $CD4^+$ cells were maintained.

**[Conclusion]**Our study indicates that the *IL15* +96522A>C polymorphism correlates with the severity of HD, most likely by increasing Th17 cells.




## A-178

### Evaluation of the analytical performance of Tosoh G11 for $HbA_{1c}$ determination

Y. Chung, S. Yun, Y. Cho. *Korea University, Seoul, Korea, Republic of*

Background: Glycated hemoglobin($HbA_{1c}$) is a key biomarker for the monitoring of glycemic balance in diabetic patients. It can be measured by various methods, including ion-exchange high-pressure liquid chromatography (HPLC), boronate affinity chromatography, immunoassay method and capillary electrophoresis. The aim of this study is to evaluate the performance of new system, Tosoh G11 ( ion-exchange HPLC) in comparison to two other used system (Tosoh G8 and Biorad D-100) in routine testing.

Methods: 40 samples of whole blood in Anam Korea University hospital were collected from during January 2017. We evaluated analytical performance of new device, Tosoh G11. Within-run precision test was determined by 20 assays from same sample(quality control samples with different $HbA_{1c}$ values: two different levels, high and low, each sample being analyzed 20 times) on the same day. Between-day precision test was determined by daily measurement of $HbA_{1c}$ during 5 days, using two different quality control samples. The correlation with two other systems (Tosoh G8, biorad-D-100)was assessed by analyzing 40 samples. A test for linearity was investigated by preparing six different samples. Carry-over test was done by 4 high and low value samples each. Reference range analysis was done by CLSI C28-A3 that less than 10% of more than 20 samples must be in reference range provided by instructor.

Results: In within-run precision test, mean and coefficients of variation (CVs) for low and high value samples were 4.87%, 9.73%, and 0.97%, 0.57% respectively. In between-day precision test, CVs were less than 0.68%. The comparison of $HbA_{1c}$ values obtained using Tosoh G11 and Tosoh G8 showed a good correlation, with the following equation for the linear regression line: $y = 0.9664x + 0.2463$, and a coefficient of correlation, $R^2 = 0.9982$. In addition, Tosoh G11 and Biorad D-100 also showed a good correlation, with following equation for the linear regression line: $y = 1.0335x - 0.1587$, $R^2 = 0.9941$. New device exhibited a good linearity for $HbA_{1c}$ values ranging from 3.4% to 18.8%. The equation of the linear regression line was $y = 0.9762x + 0.0136$ with a correlation coefficient, $R^2 = 0.9999$. Result of carry-over test was 0.00%, less than 1%. In reference range analysis, none of the 20 samples was rejected.

Conclusion: In conclusion, this new device, Tosoh G11 showed good analytical performance at high throughput. Thus, the results of this evaluation suggest that the Tosoh G11 is suitable for a routine use in clinical chemistry laboratories.

## A-179

### Evaluation of the Beckman Access Free T3 Assay reference interval following an assay formulation change

B. J. Larson, K. M. Ness, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

**Background:** Free triiodothyronine (fT3) is a second- or third-line test in the evaluation of hyperthyroidism. In our laboratory, fT3 measurements are performed using the Beckman Access Free T3 assay on the Unicel DxI platform. On March 2016, following the implementation of a new reagent lot, an increase in the frequency of elevated fT3 results was observed despite an acceptable lot evaluation. The average historical frequency of abnormal results was 16% using a reference interval of 2.0-3.5 pg/mL and increased to 40% with the new fT3 lot. Following discussions with manufacturer, it was concluded that the new reagent lot contained a different formulation design than our prior lots. This formulation change was introduced to improve the Access Free T3 reagent pack stability and resulted in the upward shift in fT3 concentrations. Although the manufacturer did not update the reference interval, the medical device recall letter indicated that laboratories should discontinue the use of the assay until the reference intervals were verified, adjusted or reestablished by the laboratory. The goal of this study was to establish the fT3 reference interval with the new fT3 reagent formulation.

**Methods:** Free T3 concentrations in serum from 129 individuals (71 (55%) male, 58 (45%) female) were determined. The participants were excluded if they have the following conditions: any thyroid disease, endocrine disorders, kidney disease or failure, liver disease, pregnancy, high iodine diet or hospitalization within the last 3 months. The following medications were also excluded: thyroid medications, amiodarone, lithium, glucocorticoids, propranolol, phenytoin, carbamazepine, furosemide, and hormone replacement (estrogen, testosterone). Samples were tested for thyroid stimulating hormone (TSH), Free T4, thyroperoxidase antibody

(TPO), and thyroglobulin antibody to assure normal thyroid status. The central 95th percentile reference interval and the confidence intervals were calculated using quantile regression methods (SAS QUANTREG).

**Results:** Verification of the manufacturer's reference interval of 2.1-3.9 pg/mL with a small sample was unacceptable with only 80% (23/29) of results within the reference interval. A new reference interval was established with 129 individuals. The calculated central 95th percentile reference interval was 2.8-4.4 pg/mL. The 95$^{th}$ confidence intervals were 2.7-2.9 and 3.9-5.0 for the 2.5$^{th}$ and 97.5$^{th}$ percentiles, respectively. Retrospective evaluation of the new reference interval using fT3 results (n=4362) obtained with the new reagent lot showed a decrease of abnormal results from 40% to 20% which was in alignment with the historical frequency.

**Conclusions:** We were unable to verify the manufacturer's reference intervals using the new fT3 assay formulation. We established a new reference interval for the Beckman Access Free T3 assay to account for the upward shift in fT3 concentrations observed with the formulation. With the implementation of this reference interval the frequency of abnormal results decreased to match historical frequencies.

## A-180

### Free Thyroxine Concentrations in the Hypothyroidism Treated Versus Untreated Populations

R. Benirschke, T. Theparee, J. Dohnal, H. Lee. *Northshore University Health System, Evanston, IL*

**Background:**

Thyroid stimulating hormone (TSH) and free thyroxine (FT4) are integral tests for assessing thyroid function and guiding therapy. Although a normal TSH is the primary endpoint for patients being treated for hypothyroidism, FT4 is often also measured. Unfortunately, reference intervals for FT4 are not applicable to treated patients and abnormal results create confusion both for clinicians and patients. Although it is known that FT4 is generally higher in treated patients than their untreated counterparts, few studies detail the extent of these elevations and none on a large scale.

**Objectives:**

To assess how FT4 concentrations differ in patients being treated for hypothyroidism versus those who are not.

**Methods:**

Paired TSH and FT4 results between February 16$^{th}$, 2016 and September 26$^{th}$, 2016 were extracted from the electronic medical record. Additional data included age, gender, thyroid medications, pregnancy status, and all other thyroid related tests including free triidothyronine (FT3), total thyroxine (TT4), total triiodothyronine (TT3), anti-thyroid peroxidase antibody (ATPO), anti-thyroglobulin antibody (ATG), thyroid stimulating immunoglobulin (TSI), and free thyroxine by dialysis (FT4D). With the exception of FT4D and TSI, all testing was performed on the Roche Cobas 8000. Unfiltered data included 24,297 unique clinical encounters for 19,898 patients. All data analyses and figures were created with R Statistical Package Version 3.3.1 and R Studio Version 0.99.902.

**Results:**

Two populations were designed to compare how patients with a normal TSH (0.30-5.60 uIU/mL) differ biochemically depending on whether or not they are receiving medications for hypothyroidism. The reference population (P1) includes patients that are not pregnant, have no detectable thyroid related autoantibodies, and are not being treated for hyper- or hypothyroidism (8,179 encounters of 7,972 patients). The treated population (P2) includes patients that are not pregnant and have a current prescription for a hypothyroidism treatment (5,985 encounters of 5,113 patients). For P1, FT4 (ng/dL) had a mean (μ) of 1.17, a median (M) of 1.16, and a standard deviation (s) of 0.19. For P2, FT4 had a μ=1.39, M=1.40, and s=0.27. The central 95$^{th}$ percentile for both populations was calculated parametrically and non-parametrically and gave similar results with 0.79-1.55 for P1 and 0.86-1.94 for P2. FT3 (pg/mL) was also measured in a subset of these patients. Here the reference population (692 encounters of 667 patients) had a μ=2.86, M=2.90, and s=0.45 and the treated population (613 encounters of 547 patients) had a μ=2.69, M=2.6, and s=0.62. FT4 in this subset maintained the relationship seen in the original populations.

**Conclusion:**

FT4 concentrations in patients being treated for hypothyroidism are shifted higher relative to their untreated counterparts. This shift is not accompanied by a corresponding shift in FT3. Although TSH is the primary guide for therapy, this data provides information to clinicians as to when a FT4 above the reference interval in a treated patient may warrant further investigation and testing.

## A-181

### Siemens IMMULITE® 2000-2000 XPi TSI Assay in the differential diagnosis of Graves' disease (GD)

J. T. Adams, D. M. Goertz. *Pathology Associates Medical Laboratories (PAML), Spokane, WA*

**Background:** Graves' disease (GD) is the most common cause of hyperthyroidism in the United States. Differential diagnosis of GD can be complicated by non-specific symptoms and antibodies that reveal overlapping etiologies, so a quantitative assay differentiating between thyroid *stimulating* and *inhibiting* antibodies can be essential for accurate diagnosis and treatment. In December 2016, Pathology Associates Medical Laboratories (PAML) of Spokane, WA, implemented the Siemens IMMULITE® 2000-2000 XPi TSI, the first automated and semi-quantitative thyroid *stimulating* immunoglobulin (TSI) assay in the US.

**Methods:** PAML shared 40 serum samples with a nationally recognized, CAP certified laboratory. Accuracy, precision, reportable range studies, and verification of the reference interval were performed. Siemens TSI Calibration Verification Material was utilized for calibration verification. Along with the lab to lab correlation, we also validated a secondary instrument. Lastly, manufacturer claims were evaluated comparing IMMULITE TSI to thyroid stimulating hormone receptor antibody (TRAb) on the Cobas e411. Statistical analyses were performed with EP Evaluator 11.

**Results:** Our data showed good correlation (R = 0.9955, slope = 0.934, and bias = -6.7%). Internal instrument to instrument correlation showed good performance as well (R = 0.9988, slope = 1.005, and bias = 2.07%). Intra-assay precision was 0.0-5.0%, and inter-assay precision was 8.1-8.8%. Accuracy, reportable range, and linearity of TSI were evaluated on both the primary and backup analyzers. On both instruments, the assay was accurate within the allowable systematic error (10%) and allowable total error (20%). The maximum deviation for a mean recovery from 100% was 6.1% and 7.6%, respectively. The results were linear and passed reportable range acceptance criteria. For the verification of reference interval: 39 of 40 clinical samples, were within the reference interval of <0.1 IU/L (97.5% agreement). Qualitative comparison of IMMULITE TSI to TRAb on the Cobas e411 showed a Positive agreement = 100%, Negative agreement = 88.9% and Overall agreement = 73.3% (n=36).

**Conclusion:** The validation showed good accuracy, precision and linearity; therefore, TSI was adopted by PAML in December 2016. As an early adopter of this new assay, we offer clients an improved turn-around time, reduced sample volume requirement, a more clinically sensitive (98.3%) and specific (99.7%) method over the previous bioassay, and increased diagnostic confidence because this thyroid marker is diagnostic of Graves' disease. Further studies are required to determine how TSI levels correspond to long term thyroid disease management, and to determine the utility of monitoring GD patients through serial TSI testing.

## A-182

### Quantitative Determination of Thyroid Stimulating Hormone (TSH) in Human Serum by Lumipulse® *G* TSH-III Assay

N. Benina[1], S. Gannon[1], C. Feldman[1], L. Zhu[1], S. Raju[1], M. Nguyen[1], K. Falcone[1], K. McMullen[1], D. Ziegler[1], N. White[1], E. Eklund[2], J. Hunsaker[3], P. Wheeler[4], J. Genzen[3], G. Lambert-Messerlian[2], D. Grenache[3], K. Donaldson[4], R. Radwan[1], J. Young[1], D. Dickson[1], Z. Vucetic[1]. *[1]Fujirebio Diagnostics, Malvern, PA, [2]Women & Infants Hospital of Rhode Island, Providence, RI, [3]ARUP Laboratories, Salt Lake City, UT, [4]Prescient Medicine, Hershey, PA*

**Background:** TSH (thyroid-stimulating hormone) is a pituitary hormone that acts on the thyroid gland to produce and release thyroxine (T4), and triiodothyronine (T3); the hormones that stimulate metabolism. TSH concentrations in blood closely reflect changes in thyroid function and are routinely used for evaluation of patients suspected of having an excess (hyperthyroidism) or deficiency (hypothyroidism) of thyroid hormones.

**Methods:** The Lumipulse G TSH-III is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative measurement of TSH in specimens on the LUMIPULSE G1200 System by a two-step sandwich immunoassay method. TSH specifically binds to an anti-human TSH monoclonal antibody (mouse) coated on particles and forms immunocomplexes. After washing, an Alkaline phosphatase (ALP)-labeled anti-human TSH monoclonal antibody specifically binds to the TSH immunocomplexes, completing the sandwich. The amount of TSH is derived from the luminescence signals generated by adding the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). The calibrators for the Lumipulse G TSH-III assay are traceable to in-house reference calibrators

whose values have been assigned to the 3rd International Standard, 2003 (code: 81/565) by the National Institute for Biological Standards and Control (NIBSC). All of the validation studies were performed according to respective CLSI guidelines.

**Results:** The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)/Functional Sensitivity (FS) of the Lumipulse G TSH-III assay on the LUMIPULSE G1200 System were 0.001, 0.002 and 0.006 µIU/mL, respectively. The Lumipulse G TSH-III assay demonstrated linearity in the range from 0.001 to 227.804 µIU/mL. There was no high-dose hook effect observed for samples containing up to ~3,100 µIU/mL of TSH. A twenty day precision study of 6 human serum-based panels assayed in duplicate at two separate times of the day (n = 80 for each sample) demonstrated within-laboratory (total) precision of ≤ 6.4%. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 9 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum albumin, immunoglobulin G, biotin, human anti-mouse antibody, and rheumatoid factor) and 17 commonly used therapeutic drugs. Cross-reactivity of the Lumipulse G TSH-III assay with other substances (5000 mIU/mL FSH, 200,000 mIU/mL hCG, 100 ng/mL hGH and 1000 mIU/mL LH, respectively) that are similar in structure to TSH demonstrated no cross-reactivity. A comparison of Lumipulse G TSH-III with an FDA-cleared predicate device was analyzed using weighted Deming regression. For the 141 tested specimens (Concentrations range from 0.026 to 84.299 µIU/mL), the slope, y-intercept, and correlation coefficient (r) were 0.97, -1.051 µIU/mL, and 0.9838, respectively. Finally, reference intervals as defined by 2.5th and 97.5th percentiles of the population were established for Lumipulse G TSH-III in 119 euthyroid adults (0.392-3.762 µIU/mL); 89 hyperthyroid adults (0.021-2.086 µIU/mL) and 110 hypothyroid adults (0.036-47.725 µIU/mL).

**Conclusion:** The data demonstrate that the Lumipulse G TSH-III assay on the automated LUMIPULSE G1200 System is sensitive, accurate and precise for routine quantitative determination of TSH in serum specimens.

## A-183

### Accuracy-based proficiency testing for testosterone measurement - a follow-up study 2016

Z. Cao[1], J. C. Botelho[2], R. Rej[1], H. Vesper[2], R. Astles[2]. *[1]Wadsworth Center, New York State Department of Health (NYSDOH), Albany, NY, [2]Centers for Disease Control and Prevention (CDC), Atlanta, GA*

**Background:** Accurate measurement of testosterone is important in patient care and public health. Although proficiency testing (PT) can monitor and aid in improving quality performance of clinical laboratories and commercial products, PT providers often use altered, in contrast to authentic, human specimens as a matrix. As a result, laboratory performance is often assessed against its peer group mean/median but does not evaluate absolute accuracy of the analytical system. We conducted accuracy-based PT for testosterone, using commutable samples, as a follow up to our previous accuracy-based PT done during Sept 2012-Jan 2013 (data not shown).

**Methods:** Five samples were prepared using single-donor authentic human serum and distributed to NYSDOH-certified laboratories. The samples were analyzed for testosterone using 16 different analytical systems. The target values were determined using the CDC reference measurement procedure.

**Results:** Sixty-five laboratories reported results. Eight of 16 analytical systems had ≥ 3 participants and only their results were examined for analytical system mean and bias of total testosterone (Table). All 65 laboratories' results were evaluated against a single criterion (target ± 25.1%), the minimal requirement for total allowable error based on biological variability. The percentages of results that met the criterion for samples 1 to 5 were 35.4%, 98.5%, 89.2%, 96.9%, 83.1%, respectively. We defined obtaining results for at least 4 of 5 samples within ± 25.1% as "passing." Of all 65 participating laboratories, 87.7% had passing scores. The passage rates for 8 analytical systems are listed in the table (first column from left). Only one analytical system, which had obtained CDC Hormone Standardization (HoST) certification until 2013, had biases < 5% for samples 2-5, with concentrations seen in hypogonadism and normal adult male.

**Conclusions:** Our results indicate that efforts in improving assay accuracy and precision for testosterone assays remain relevant and necessary.

| Sample ID | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Analytical System (n), Passage rate % | Mean ng/dL (Bias, %) | | | | |
| CDC target | 43.5 | 160 | 294 | 457 | 534 |
| Abbott Architect i System (4), 100% | 55.2 (26.8) | 169.5 (6.0) | 294.2 (0.1) | 505.0 (10.5) | 596.2 (11.6) |
| Beckman Coulter Access2 (7), 100% | 79.8 (83.4) | 155.4 (-2.9) | 307.2 (4.5) | 436.1 (-4.6) | 474.4 (-11.2) |
| Beckman Coulter UniCel DxI 600 (9), 78% | 81.5 (87.3) | 152.9 (-4.4) | 288.8 (-1.8) | 410.5 (-10.2) | 429.0 (-19.7) |
| Beckman Coulter UniCel DxI 800 (6), 100% | 82.2 (89.0) | 151.5 (-5.3) | 285.3 (-2.9) | 401.6 (-12.1) | 427.2 (-20.0) |
| Roche Cobas e601 (3), 100% | 65.0 (49.4) | 165.3 (3.3) | 305.9 (4.0) | 479.0 (4.8) | 521.7 (-2.3) |
| Siemens ADVIA Centaur (15), 73% | 53.4 (22.7) | 144.7 (-9.6) | 249.9 (-15.0) | 423.8 (-7.3) | 422.1 (-21.0) |
| Siemens Immulite2000 (8), 100% | 39.8 (-8.5) | 148.3 (-7.3) | 329.6 (12.1) | 438.8 (-4.0) | 414.3 (-22.4) |
| Tosoh Bioscience (4), 75% | 59.7 (37.2) | 148.9 (-7.0) | 357.3 (21.5) | 533.8 (16.8) | 617.3 (15.6%) |

## A-184

### Performance Evaluation of a Total Inhibin ELISA and Reference Intervals in Female and Male Populations

J. A. Erickson[1], D. G. Grenache[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, [2]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

**Background:** Inhibins are dimeric glycoproteins secreted primarily by the granulosa cells in the ovaries and Sertoli cells in the testes. The hormone consists of an α-subunit linked with either a βA-subunit or a βB-subunit, resulting in heterodimers designated as inhibin A and inhibin B, respectively. Several forms are present in the circulatory system including mature and partially processed αβ-dimers, and inactive free α-subunits. The measurement of inhibins is clinically useful in the diagnosis and prognosis of granulosa cell and mucinous tumors of the ovary. It has been demonstrated that granulosa cell tumors secrete inhibin A, B and the free α-subunit while mucinous tumors primarily secrete the free α-subunit. The purpose of this study was to assess the performance characteristics of and to validate the Ansh Labs (Webster, TX) Total Inhibin ELISA. Additionally, a reference limit for postmenopausal women was verified and reference intervals for premenopausal women and men were established.

**Methods:** Deidentified residual serum specimens sent to ARUP Laboratories for routine testing, as well as serum specimens obtained from healthy volunteers, were used for this study. Total inhibin was measured according to the test kit manufacturer's protocol. The performance characteristics evaluated were analytical sensitivity, linearity, method comparison, precision and analyte stability. Reference limit and interval studies were performed with serum specimens obtained from healthy volunteers. The University of Utah's Institutional Review Board approved this study.

**Results:** The analytical sensitivity was as follows: Limit of blank, 0.3 pg/mL; limit of detection, 2.0 pg/mL; limit of quantitation, 9.0 pg/mL (parametric analysis of 60 zero calibrator, 60 approximately 3 pg/mL and 40 approximately 9 pg/mL measurements; allowable error, 20%). Linearity was established by combining serum specimens with high and low total inhibin concentrations at different ratios to create as set of 9 specimens, each of which were tested in triplicate. Linear regression analysis produced a slope of 1.02, intercept of -15.8 and $r^2$ of 0.997. A method comparison study (n = 40) with another lab using the same total inhibin assay, generated a slope of 1.06, intercept of -6.6, and r of 0.993. Precision was determined from two serum pools of differing total inhibin concentrations tested over 20 days, four replicates per pool per day. Repeatability and within-laboratory CVs were 3.7 and 7.8% at 34.4 pg/mL, and 2.8 and 4.1% at 373.9 pg/mL, respectively. Total inhibin was stable for 12 hours at room temperature, 7 days (min) at 4-8 °C, 3 months (min) at -20 °C, and over a minimum of 3 freeze/thaw cycles. A postmenopausal reference limit of 10 pg/mL was verified (n = 21, 97.5th percentile). Reference intervals were established as 2-300 pg/mL for premenopausal females and 50-190 pg/mL for males (n = 125 each, nonparametric analysis, 95th percentile).

**Conclusions:** The Ansh Labs Total Inhibin ELISA demonstrates acceptable performance for quantifying total inhibin in human serum. Reference intervals have been established for both premenopausal females and males, and a reference limit verified for postmenopausal females.

## A-185

### Unexpected high values of LH: high molecular weight forms (macro LH)?

M. F. M. C. Pinheiro[1], D. M. V. Gomes[1], T. T. Tachibana[2], J. G. H. Vieira[2], A. M. Vangelotti[3], S. V. L. Argolo[1], V. L. G. Leal[3], M. C. Freire[1]. *[1]DASA, Rio de Janeiro, Brazil, [2]Laboratório Fleury, São Paulo, Brazil, [3]Instituto Estadual de Diabetes e Endocrinologia, Rio de Janeiro, Brazil*

**Background:** Several pre-analytical and analytical interference factors that could influence hormone tests and hamper its interpretation have been described. Autoantibodies can cause interference in immunoassays for a number of analytes including insulin, growth hormone, thyroid hormones, prolactin, TSH and most rarely, for luteinizing hormone (LH). **Methods:** We present the case of a female patient, 45 years of age, with a diagnosis of primary hypothyroidism, Hashimoto thyroiditis, when she was 30 years old. She had also multinodular goiter, submitted to total thyroidectomy 12 years ago. The diagnosis was benign, folicular adenoma. She had regular menses. The patient never used any LH-stimulating drug, nor had ever received LH or HCG injections. **Results:** The laboratory evaluation showed a constantly high LH value (>200.0 IU/L, ECLIA, Roche), with FSH levels ranging from 3.2 to 26.5 IU/L; estradiol, 28 to 495 pg/mL; prolactin, 14.3 to 29.7 ng/mL. LH was also measured by ICMA, Advia (Siemens) and Unicel (Beckman), with values of 74.6 and 23.7 IU/L, respectively. Serial dilution showed parallelism with the curve obtained with a standard LH preparation. Antibodies against thyroperoxidase were present in high concentrations, 653 KU/L (reference levels < 35 KU/L). Her serum was subjected to gel-filtration chromatography on a Superdex 200 colunn (0.9 x 30 cm; Pharmacia) calibrated with the Pharmacia high-molecular-weight calibrators, and the elution showed that almost all of the LH eluted as a high-molecular-weight form ($M_r$ >250000). Recovery after precipitation with polyethylene glycol was very low, LH 3.7 IU/L, consistent with a macro LH. **Conclusions:** The etiology of this phenomenon is probably a complex of LH with immunoglobulin (Ig)-G, particularly with anti-LH autoantibodies. The relationship with autoimmune diseases (Hashimoto thyroiditis) remains to be defined. This condition must be considered in the event of a finding of unexpectedely high LH values, non-coincident with the patient clinical context.

## A-186

### Analysis of Anti-Müllerian Hormone Levels in Adult Chinese Women: A Multicenter Reference Intervals Study

x. cheng, S. Yu, K. Zhang, P. Li, J. Han, L. Qiu. *peking union medical college hospital, Peking Union Medical College, beijing, China*

**Background:** Anti-Müllerian hormone (AMH) plays an important role in ovarian reserve assessment and individualized in vitro fertilization (IVF) treatment. Due to the surge in the numbers of women delaying childbearing until older ages and patients with reproductive disorders or premature ovarian failure in Chinese populations, it is urgent to obtain an accurate, representative AMH reference interval for adult Chinese women.

**Methods:** From May to September 2013, sera from 1,169 apparently healthy adult females from five regional representative cities in China (Beijing, Hangzhou, Guangzhou, Dalian and Urumqi) were collected, and we used a Beckman DxI800 automated chemiluminescence immunoassay analyzer to detect AMH levels. A multiple regression analysis was used to investigate the effects of region, sex, age, Body Mass Index (BMI), Systolic Blood Pressure (SBP), exercise on AMH. We evaluated 5 candidate regression models to describe the decline of AMH with age and established AMH reference intervals in different age groups.

**Results:** The main factor affecting AMH levels was age (B = -0.756, P<0.001). Regions, BMI, SBP and exercise had no significant effects on AMH levels. The linear, quadratic and cubic models could either provide the best fit regression model to describe the decline of AMH with age ($R^2$=0.40). The AMH reference intervals for adult Chinese women aged 19~24 years, 25~29 years, 30~34 years, 35~39 years, 40~44 years , 45~49 years and ≥50 years were 0.74~16.06, 0.67~11.64, 0.50~9.99, 0.09~8.33, 0.04~4.09 , 0.01~1.46 and 0.00~0.18 ng/ml, respectively.

**Conclusion:** This study used an AMH chemiluminescence reagent newly developed by Beckman to establish AMH reference intervals for adult Chinese women in different age groups. The results have important reference value for the clinical application of AMH as a biomarker.

## A-187

### Audit Of Oral Glucose Tolerance Testing

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

**Background:** In the Singapore Ministry of Health diabetes diagnostic algorithm, oral glucose tolerance tests are restricted to patients with fasting plasma glucose concentrations of 6.1 - 6.9 mmol/L. This guideline was published in 1999 and is the standard of care in Singapore. This study examined whether indeed this algorithm is used in a 1400 bed general hospital and whether the results could be classified into the accepted categories of impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM). **Methods:** Details of all oral glucose tolerance tests performed from 2013 - 2015 inclusive were extracted from the laboratory information system. An oral glucose tolerance test involves collection of fasting and 120 min plasma glucose samples following a 75g oral glucose load. **Results:** In 3 years, 1125 oral glucose tolerance tests were performed of which 254 (22.6%) had fasting glucoses of 6.1-6.9 mmol/L. The final categorisation for these cases was: 45 IFG, 87 IGT and 121 DM. Comparing the results of the fasting glucose and 120 min glucose for the other cases, there was 81% concordance with 155 fasting glucose <7.0 / 120 min glucose >=11.1 mmol/L and 22 fasting glucose >=7.0 / 120 min glucose <11.1 mmol/L. There were 16 cases with fasting glucose >10.0 mmol/L. There were 76 cases with fasting glucose <=4.5, of which none had 120 min glucose >=11.1 mmol/L. **Conclusion**: Most oral glucose tolerance tests did not meet the Singapore Ministry of Health criteria justifying the performance of an oral glucose tolerance test. In 20% of these cases, the fasting glucose and 120 min glucose results led to conflicting categorisation. Better clinician education and triage of requests is needed to reduce inappropriate requests and diagnostic confusion. As a first step, deciding not to proceed with an oral glucose tolerance test if the fasting glucose concentration <=4.5 mmol/L would reduce unnecessary testing without any potential diagnostic data loss.

## A-188

### Relationships between Vitamin D Status, Androgens and Determinants for Severity and Progression in Some Prostate Diseases

B. Hadziev[1], B. Galunska[1], D. Gerova[2], P. Kossev[3], A. Hinev[3], D. Svinarov[4]. *[1]Department of Biochemistry, Molecular medicine and Nutrigenomics, Medical University, Varna, Bulgaria, [2]Department of General Medicine and Clinical Laboratory, "St. Marina" University Hospital, Medical University, Varna, Bulgaria, [3]Department of Surgery, Clinic of Urology, "St. Marina" University Hospital, Medical University, Varna, Bulgaria, [4]Alexander Hospital, Medical University, Sofia, Bulgaria*

**Background:** The hypothesis that androgens can cause prostate growth and accelerate prostate cancer is nowadays replaced by the saturation concept. Recently, the antiproliferative effect of calcitriol provoked intensive research on the role of vitamin D in prostate growth and tumor aggressiveness. We aimed to investigate the relationships between vitamin D status, androgens and determinants associated with the severity and progression of benign prostate hyperplasia (BPH) and prostate cancer (PCa).

**Methods:** One hundred twenty five men with clinical suspicion for prostate disease evoked by elevated serum PSA levels and/or abnormal digital rectal examination, consented to enter the study. Patients with acute prostate inflammation, systemic infection, cardio-respiratory failure, diseases contraindicating surgical treatment, and vitamin D supplementation were excluded. According to the biopsy results, patients were divided into two groups: 37 with BPH and 88 with PCa. Tumors were graded by the Gleason grading system. PCa patients were divided into three risk groups (RG) according to EAU guidelines (RG1-low; RG2-intermediate; RG3-high) and by the tumor grade (Gleason score<7:Gl1-low; Gleason score=7: Gl2-intermediate; Gleason score>7: Gl3-high). Total testosterone (TT), free testosterone (FT), dehydroepiandrosterone sulfate (DHEAS), androstendione, and sex-hormone binding globulin (SHBG) were assayed by verified ELISA methods. Free androgen index (FAI) was calculated as TT/SHBG. Vitamin D status was evaluated by the serum levels of 25-hydroxyvitamin D (25OHD) measured by a validated LC-MS/MS method. Prostate specific antigen (PSA) was measured by a standard chemiluminescent immunoassay. GraphPad Prism v.6.00 was used for data analysis: t-test and one-way ANOVA to find mean differences and Spearman's test to establish the correlation of tested parameters.

**Results:** Vitamin D deficiency (<50nmol/L) was more frequent in PCa than in BPH patients (69.4% versus 43.2%). Among measured androgens TT and FT revealed a significant decrease in the PCa group versus BPH, while DHEAS was increased

in PCa patients. A significant negative relationship between DHEAS and PSA (r=-0.33, p=0.05), and a tendency for a decrease of 25OHD with the increase of PSA (r=-0.29, p=0.09) were established in BPH. Significant negative correlation with 25OHD was detected only for FAI (r=-0.42, p=0.03). A weak, but significant negative correlation was established between PSA and TT (r=-0.23, p=0.03), FT (r=-0.28, p=0.009), and DHEAS (r=-0.22, p=0.04) in PCa. Stratification by the cut-off value (50nmol/L) for 25OHD showed significantly higher PSA levels for the vitamin D deficient group (20.91±22.31ng/ml vs 27.70±4.28ng/ml, p<0.001). Increase of risk and the tumor aggressiveness was associated with decrease in 25OHD and FAI: a negative correlation with tumor grade and risk was found for 25OHD (r=-0.19, p=0.08; r=-0.23, p=0.05 respectively), and for FAI (r=-0.27, p=0.01; r=-0.25, p=0.03 respectively). ROC curve analysis showed highest discriminative value between BPH and PCa for 25OHD (AUC±SE= 0.68±0.05; CI=0.57 - 0.78; p=0.002).

**Conclusion:** Significant differences between PCa and BPH were observed for all tested steroids. Association with risk and tumor grade, and eventual discriminative potential between BPH and PCa was found for 25OHD.

## A-189

### Development of Novel Specific and Sensitive ELISAs for Proglucagon-Derived Peptides

A. Kumar[1], B. Kalra[1], S. Mistry[1], A. S. Patel[1], V. Budhavarapu[1], T. Chatterji[1], G. Savjani[1], R. Krishhna[2], D. D'Alessio[2]. *[1]Ansh Labs, Webster, TX, [2]Division of Endocrinology, Metabolism and Nutrition, Department of Medicine, Duke University School of Medicine, Durham, NC*

**Objective:** The aim of this study was to develop well characterized sensitive and specific ELISAs to quantitate Glucagon, Oxyntomodulin (OXM), and Glucagon-like peptide 1 (GLP-1) in biological fluids. **Relevance**: Proglucagon, (PG) a 160aa peptide is cleaved from preproglucagon and the later is encoded by the glucagon gene (GCG) in humans. PG is a precursor of Glucagon, OXM, GLP-1 and several other peptides. These peptides arise by differential processing of PG. Glucagon, corresponding to PG residues (33-61aa), is formed in the alpha cells of the pancreas. Oxyntomodulin is a 37aa peptide hormone secreted by the gut endocrine L-cells post-prandially and shares identical amino acid sequence in the N-terminal to glucagon, with an extension of 8aa peptide in the C-terminus. Prohormone convertase 1/3 cleaves Proglucagon precursor into Oxyntomodulin, GLP-1/2 and GRPP upon nutrient ingestion. Oxyntomodulin is known to bind both the GLP-1 receptor and the glucagon receptor, but with lower affinity compared to GLP-1 and glucagon. Oxyntomodulin has been studied as a weight loss agent in obese patients via suppression of food intake and increase in energy expenditure. Glucagon has been studied for the treatment of hypoglycemia and glucagon receptor antagonists are under development for the treatment of type 2 diabetes. GLP-1 and GLP-2 receptor agonists appear to be promising therapies for the treatment of type 2 diabetes and intestinal disorders, respectively.

**Methodology:** Specific monoclonal antibody based ELISAs for glucagon (AL-157), oxyntomodulin (AL-139), and GLP-1 (AL-172) have been developed to measure their respective analyte in ≤50uL of the plasma. The glucagon assay is standardized to NIBSC code 69/194 v3.0 preparation and the other assays were gravimetrically calibrated to their corresponding pure peptides. These ELISAs were validated for their specificity to the Proglucagon fragments, specimen stability, and their circulating levels (fasting and non-fasting) in matched serum and plasma. Monoclonal antibody based ELISAs for GRPP, GLP-2, and MPGFs has also been developed and will be presented in the poster.

**Validation:** Glucagon, OXM, and GLP-1 ELISAs with a dynamic range of 20-300pg/mL, 3-300pg/mL, 15-600 pg/mL are highly specific to glucagon, OXM, and GLP-1, respectively. These assays did not cross-react to GRPP, Glucagon, OXM, GLP-1, and GLP-2 when assayed in their individual ELISAs. Proglucagon KO serum samples (n=3) in the OXM assay were non-detectable, whereas a concentration of 103-246pg/mL was observed in the wild type mice (n=3). Median levels of Glucagon, OXN when studied in fresh/2-8°C/1FT/2FT drawn in EDTA plasma (no DPP-4) were 75.8/82.9/84.4/83.8pg/mL and 353.8/342.4/389.3,409.9pg/mL, respectively. Median GLP-1 level (2 FT) on the same subjects was 235.2pg/mL. Fasting/non-fasting (n=5) median Glucagon, OXN, and GLP-1 levels were 85.1/84.6, 215.3/645.9, 215.7/269.3pg/mL, respectively.

**Conclusions:** Whole portfolio of easily accessible and standardized assays for Proglucagon-derived peptides are available to reliably quantitate these important endocrine and local regulators in physiological and pathophysiological studies for metabolic disorders.

## A-190

### Performance Evaluation of the ADVIA Chemistry Enzymatic Hemoglobin A1c Assay*

J. E. Jones, J. Gisiora, J. Dai. *Siemens Healthineers, Newark, DE*

**Background:** According to the World Health Organization, an estimated 422 million adults were living with diabetes globally in 2014. Early diagnosis of diabetes is critical for the management of the disease. The longer a person lives with undiagnosed and untreated diabetes, the worse his or her outcome will likely be. Glycemic states can be measured by fasting blood glucose, serum fructosamine, or glycated hemoglobin (HbA1c). HbA1c is a better indicator of mean blood glucose level. HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β-chain of HbA whereby a labile Schiff base is formed and converted into the more stable ketoamine (irreversible) via an Amadori rearrangement. A new enzymatic HbA1c assay (A1c_E) has been developed for use on the automated random-access ADVIA® Clinical Chemistry Systems. The objective of this study was to evaluate the performance of this new A1c_E assay on the ADVIA Clinical Chemistry Systems.

**Methods:** The first step of the reaction is to hemolyze the red cells with the pretreatment solution and convert hemoglobins to methemoglobin. The first reagent (R1) is added to form azido-methemoglobin, and the protease in R1 hydrolyzes glycated hemoglobin to form fructosyl-valine-histidine. The second reagent (R2) containing fructosyl peptide oxidase is added to convert the fructosyl-dipeptide to $H_2O_2$ (a byproduct of the enzymatic oxidation reaction) that reacts with the chromagen, 10-carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)-phenothiazine (DA-67), in the presence of horseradish peroxidase. The performance evaluation in this study included precision, linearity, correlation with the NGSP reference method, and total error assessment. Data were collected on ADVIA Clinical Chemistry Systems (1800, 2400, and XPT), which use the same reagent packs, calibrators, and commercial controls.

**Results:** The precision (within-lab %CV) of the new A1c_E assay using two levels of commercial controls and five whole-blood pools ranging from ~4.50 to ~12.00% HbA1c (n = 80) on the ADVIA Clinical Chemistry Systems across three lots was ≤1.3% (repeatability) and ≤1.9% (within-lab). The analytical range of the assay was 3.8–14.0% HbA1c. The assay correlated well with the NGSP: ADVIA 1800 A1c_E assay = 1.03 [NGSP] − 0.204 (r = 0.994, n = 163; sample range: 3.70–14.60% HbA1c). The assay demonstrated a %TE ≤3.92 on the ADVIA 1800 Clinical Chemistry System.

**Conclusions:** The A1c_E assay on the ADVIA Clinical Chemistry Systems from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method.

*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

## A-191

### Performance Evaluation of Free Thyroxine (FT4) and Thyroxine (T4) Assays[1] on the Atellica Immunoassay Analyzer[2]

A. Wang, E. Saharig-Romero, M. Wu, X. Zhao, K. Morocho, J. Freeman. *Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

**Introduction:** Quantitative measurements of free thyroxine and thyroxine are important for the detection, diagnosis, and treatment of thyroid disease. The prototype Atellica™ IM FT4 and T4 assays[1] (Siemens Healthcare Diagnostics Inc.) are intended for the quantitative measurement of FT4 and T4 using the Atellica Immunoassay (IM) Analyzer.[2] The purpose of this study was to evaluate the analytical performance of the Atellica IM FT4 and T4 assays with serum samples.

**Methods:** The Atellica IM FT4 and T4 assays[1] are "competitive" immunoassays utilizing direct chemiluminescent technology. They use the same reagent formulations as the ADVIA Centaur® FT4 and T4 assays (Siemens Healthcare Diagnostics Inc.). For the Atellica IM FT4 assay, free T4 in the patient sample competes with acridinium ester-labeled T4 in the lite reagent for a limited amount of biotinylated polyclonal rabbit anti-T4 antibody. Biotin-labeled anti-T4 is bound to avidin that is covalently coupled to paramagnetic particles in the solid phase. For the Atellica IM T4 assay, T4 in the patient sample competes with T4, which is covalently coupled to paramagnetic particles in the solid phase, for a limited amount of acridinium ester-labeled monoclonal mouse anti-T4 antibody in the lite reagent. The Atellica IM T4 assay requires an ancillary reagent that contains a releasing agent to free up the bound T4. Performance testing included precision and assay comparison studies. The assay comparison study was conducted according to CLSI EP09-A3, with Deming regression of patient sample results compared with results observed from the ADVIA

Centaur Immunoassay System. For assay precision, each sample was evaluated in duplicate twice a day for 20 days according to CLSI guideline EP05-A3.

**Results:** The Atellica IM FT4 assay comparison yielded a regression equation of y = 1.011x − 0.099 ng/dL, with r of 0.997, versus the FT4 assay on the ADVIA Centaur XP System with 119 serum samples ranging from 0.45 to 11.6 ng/dL. The Atellica IM T4 assay comparison yielded a regression equation of y = 1.048x − 0.347 µg/dL, with r of 0.993, versus the T4 assay on the ADVIA Centaur XP System with 141 serum samples ranging from 0.3 to 30 µg/dL. The Atellica IM FT4 assay 20-day precision study yielded repeatability of 1.2 to 4.7% CV and within-lab precision of 2.2 to 6.8% CV over a sample result range of 0.4 to 10.7 ng/dL. The Atellica IM T4 assay 20-day precision study yielded repeatability of 1.8 to 7.2% CV and within-lab precision of 3.9 to 12.6% CV over a sample result range of 1.4 to 26.3 ng/dL.

**Conclusion:** The Atellica IM FT4 and T4 assays tested on the Atellica IM Analyzer demonstrated analytical performance capable of providing accurate and precise measurements of free thyroxine and thyroxine.

[1] In development. The performance characteristics of this device have not been established.

Future availability cannot be guaranteed. [2]Not CE-marked. Not available for sale. Future availability cannot be guaranteed

## A-192

### OPTIMIZING CORTISOL COLLECTION AFTER CORTICOTROPIN STIMULATION: HOW MANY TIMES SHOULD WE COLLECT CORTISOL SAMPLES FOR APROPRIATE EXCLUSION OF ADRENAL INSUFFICIENCY?

Y. Schrank, M. D. Freire, P. B. M. C. Araújo, D. M. V. Gomes, M. F. M. C. Pinheiro, R. Fontes. *DASA - Brazil, Rio de Janeiro, Brazil*

**Background:** The last guidelines on diagnosis and treatment of primary adrenal insufficiency published in 2016 by the endocrine society recommends diagnostic testing to exclude primary adrenal insufficiency (PAI) in acutely ill patients with otherwise unexplained symptoms or signs suggestive of adrenal insufficiency. The society recommends the short corticotropin test (250 mcg) as the "gold standard" diagnostic tool to establish the diagnosis of the disease. Samples for cortisol determination is collected at basal, 30 or 60 min after iv corticotropin stimulation. Peak cortisol levels below 500 nmol/L (18 mg/dL) at 30 or 60 minutes indicate adrenal insufficiency. Objectives: The aim of our study was to examine whether the cortisol stimulation test could be performed with fewer samples without compromising its diagnostic value. **Methods:** We performed a cross-sectional retrospective examination of 75 consecutive indivduals submitted to cortisol stimulation test with corticotropin in the context of adrenal insufficiency investigation. Corticotropin was applied intravenously at a dose of 250 mcg. Blood samples for cortisol were taken at time 0, 30 and 60 minutes. Serum cortisol concentration was tested with the Cobas analyzer electrochemiluminescence immunoassay. A test was considered responsive when peak cortisol at any time ≥ 500 nmol/L (18 mcg/dL). **Results:** Sixty three (84 %) of individuals submitted to the corticotropin test showed a positive response to the stimulus (peak cortisol at any time ≥ 500 nmol/L). Mean age was 35.4 ± 20.5, range 2-79 years, with female:male ratio of 2.6:1. Fifteen (24 %) of individuals were children under 18 year. Median cortisol values at time 0, 30 and 60 were respectively 10.7, 20.4 and 24.5 mcg/dL. All of our responsive individuals showed a peak cortisol response 60 minutes after corticortrophin. **Conclusions:** As cortisol peak happens 60 minutes after corticotropin stimulus and as the diagnosis of PAI is based on the peak stimulated serum cortisol concentration after the stimulus, it seems rational, cost-effective and more comfortable for the patient to collect cortisol only once, 60 minutes after corticotropin administration. We emphasize that such a procedure does not compromise the diagnostic accuracy of the test.

## A-193

### Retrospective analysis of the utility of anti-thyroglobulin antibody testing to assess thyroid autoimmunity

A. Chauhan, A. Beckman, A. Teklu, A. B. Karger. *University of Minnesota, Minneapolis, MN*

**Objective**: Antibodies to thyroperoxidase (anti-TPO) and thyroglobulin (anti-Tg) are associated with autoimmune thyroid diseases (AITD) – Hashimoto's thyroiditis and Graves' disease. While elevation of one or both antibodies is associated with AITD, clinical practice guidelines published in 2012 by the American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE) do not recommend measurement of anti-Tg levels in the assessment of patients with suspected or known AITD. Instead, the guidelines advocate for measurement of anti-TPO alone in certain cases of suspected autoimmune hypothyroidism. Despite this, in our practice we have observed that providers frequently order both anti-TPO and anti-Tg to assess patients with known or suspected AITD. Therefore, our objective was to retrospectively assess the diagnostic utility of anti-Tg testing by determining the concordance and discordance of results compared to anti-TPO.

**Methods**: The results of 1204 anti-thyroid antibody tests, performed between 4/1/2016 and 6/30/2016, were retrospectively reviewed. 708 test results represented 354 patients who underwent testing for both anti-Tg and anti-TPO. An additional 477 patients underwent anti-TPO testing alone, and 19 patients had anti-Tg testing alone. Anti-TPO and anti-Tg were measured on the Siemens Immulite 2000 (Siemens Healthcare Diagnostics) by chemiluminescent immunoassays. The reference interval for anti-TPO was < 35 IU/mL, and for anti-Tg was < 40 IU/mL.

**Results**: Out of 354 patients with both anti-TPO and anti-Tg testing, 78% of patients (n= 277) showed concordance for anti-Tg and anti-TPO. Among concordant cases, 11% of patients (n = 40) had elevation of both anti-Tg and anti-TPO, whereas the remaining 67% of patients (n = 237) had anti-ATG and anti-TPO within normal limits. 22% of patients (n = 77) had discordant test results. Out of the discordant cases, 18% (n = 62) had high anti-TPO and normal anti-Tg, whereas only 4% (n = 15) had high anti-Tg and normal anti-TPO. Therefore, testing of anti-TPO alone would have accurately identified the presence or absence of thyroid autoimmunity in 96% (n = 339) of all patients studied in our retrospective cohort.

**Conclusion:** While both anti-TPO and anti-Tg may be elevated in patients with AITD, our data support the ATA/AACE guidelines, which recommend reliance on anti-TPO testing alone for assessment of thyroid autoimmunity. This approach would have eliminated 339 unnecessary anti-Tg tests over a 3 month period, for a projected elimination of 1,356 anti-Tg tests per year. Another consideration is to offer anti-Tg as a reflex test when anti-TPO is negative; however in our cohort of 354 patients, 71% (n = 252) had normal anti-TPO levels, and therefore relying on reflex testing would result in a substantial number of anti-Tg tests still being performed. Additionally, introducing a reflex testing option might influence providers who routinely order anti-TPO alone to select anti-TPO with reflex to anti-Tg, thereby increasing anti-Tg testing. Therefore, we recommend that providers be educated on the low yield of anti-Tg testing, and that they utilize this test only in patients with negative anti-TPO and a strong clinical suspicion for autoimmune hypothyroidism.

## A-194

### Design Optimization for the ADVIA Centaur Anti-Müllerian Hormone Assay*

T. Chuang, J. Bogdanovic, A. Jacewicz, T. Tran, A. Penugonda, S. Sinha, A. Han, M. Sharma, H. Lee, M. Sommer, D. Hammer, E. Rotter-Wask, W. Bedzyk, D. Hovanec-Burns. *Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

**Background:** Measurement of anti-Müllerian hormone (AMH) in vitro has become a significant tool for the assessment of ovarian reserve and an aid in the evaluation of polycystic ovary syndrome. Considering intrinsic and extrinsic factors that may influence AMH levels, an assay that can produce reliable and reproducible results is highly desirable.[1-5] The objective of this study was to design and optimize an AMH assay* from Siemens Healthineers on the ADVIA Centaur® Immunoassay Systems.

**Methods:** A direct sandwich format was selected for the assay. Screening studies were conducted to optimize the performance of the solid phase and the detection ligand. The solid phase optimization included evaluation of commercially available magnetic latex particles (MLPs) precoated with streptavidin and in-house paramagnetic particles (PMPs) pre-coated with anti-fluorescein isothiocyanate antibody. Multiple acridinium ester (AE) labels were evaluated using the same MLP to identify a suitable detection ligand that produces optimal signal-to-noise ratio (S/N). Assay standards and controls were developed utilizing affinity-purified AMH from bovine tissue in protein buffer matrix. In-use stability of targeted AMH doses representing the lyophilized standards and control levels was evaluated at 2-8°C and −20°C after reconstitution. Fractional factorial design of experiment was used to identify the main factors affecting the standard curve slope and the magnitude of signal separation in the assay.

**Results:** The maximum S/N for the MLPs was 841 for Dynabeads M280, followed by S/N of 838 for Dynabeads M270, 589 for Dynabeads MyOne T1, 337 for Dynabeads MyOne C1, and 205 for Agilient LodeStars. All other MLPs and in-house PMPs reported S/N below 150. A double-zwitterionic AE was selected based on the highest S/N in comparison to other hydrophilic AE labels and robust performance during ambient temperature fluctuations study. The S/N obtained with the double-zwitterionic AE and Dynabeads M280 was 1.2-1.6 fold improvement compared

to the AE candidate with the lowest signal separation. The average recovery after reconstitution of lyophilized material containing targeted levels of AMH antigen at 14 days (2-8°C) and 30 days (−20°C) was 97% and 90%, respectively. The main factors affecting the S/N were sample volume, detection reagent volume, MLP concentration, and detection antibody concentration.

**Conclusion:** Highest S/N ratios were observed using streptavidin-coated M280 Dynabeads. The charge-neutral double-zwitterionic AE characteristics provided better signal separation in comparison to hydrophilic AEs with modified polyethylene glycol moieties. In-use stability study shows good antigen concentration recovery for up to 30 days. * Under development. The performance characteristics of this device have not been established. Not available for sale and its future availability cannot be guaranteed.

## A-195

### Thyroid function tests in Turkish geriatric population

Z. G. Dikmen[1], O. Portakal[2], E. Karaismailoglu[3], R. T. Dogrul[4], F. Sumer[4], B. B. Yavuz[4], F. Akbiyik[2]. *[1]Hacettepe University Medical Faculty Medical Biochemistry, Ankara, Turkey, [2]Hacettepe University Medical Faculty Medical Biochemistry, Ankara, Turkey, [3]Kastamonu University Medical Faculty Department of Biostatistics, Ankara, Turkey, [4]Hacettepe University Medical Faculty Department of Geriatrics, Ankara, Turkey*

**Background**

Subclinical hypothyroidism or hyperthyroidism is a common condition in the older population. The diagnosis of thyroid dysfunction remains challenging in older population, thus based on the measurement of thyroid function tests. To avoid misclassification and potential overestimation of thyroid dysfunction in geriatric patients, age specific reference ranges should be used. The aim of this study was to evaluate FT4, FT3 and TSH reference levels in participants aged ≥65 years.

**Methods**

FT4, FT3 and TSH, anti-thyroglobulin (antiTG) and thyroid peroxidase antibody (antiTPO) levels were measured by DxI 800 (Beckman Coulter Diagnostics, USA). The new set up TSH immunoassay was used in the study which shows better analytical sensitivity at low TSH concentrations, compared to the old method.

Individuals with antiTPO>9 IU/mL and antiTG>4 IU/mL were excluded and 122 individuals over 65 years old without any known thyroid disorder composed the study group.

The statistical analysis was performed by using IBM SPSS software, version 21 (SPSS Inc., Chicago, IL, USA) and MedCalc version 14.8.1 (Mariakerke, Belgium). Statistical significance was assumed when the p-value was <0.05. All results were expressed as mean±standard deviation (SD). Independent sample t test was used for the comparison of TSH, FT4 and FT3 values in gender and age group (65-75 and >76). Outliers were tested with the D'Agostino-Pearson test. The reference intervals were calculated with reference interval for normal distribution.

**Results**

The prevalence of antiTPO positivity was 8.3% and AntiTG positivity was 5.8% in our study group. In 2.5% of the individuals, both antibodies were out of the normal range. Age-specific geriatric reference ranges for TSH, FT4 and FT3 were determined after the exclusion of these individuals. At 2.5$^{th}$ lower limit (CI) and 97.5$^{th}$ upper limit (CI), the age-specific TSH range was 0.33 [0.28 – 0.39] mIU/mL and 3.99 [3.35 – 4.76] mIU/mL, mean±SD was 1.35 ± 0.79 mIU/mL, respectively. For FT4 mean±SD was 12.79±2.49 pmol/L, reference range was 7.86 [7.15 – 8.57] pmol/L and 17.85 [17.14 – 18.55] pmol/L. For FT3, mean±SD was 4.30±0.85 pmol/L, reference range was 2.57 [2.32 – 2,81] pmol/L and 6.02 [5.77 – 6.26] pmol/L. According to the Beckman Coulter system, TSH, FT4 and FT3 reference values for individulas between 18-65 years were 0.38-5.33 mIU/mL, 7.86-14.41 pmol/L and 3.8-6.0 pmol/L, respectively.

**Conclusion**

We observed an age dependent decline in TSH levels in individuals over 65 and also FT4 levels were higher in geriatric individuals when compared with the commercial assay reference range determined by Beckman Coulter. These differences may be due to the differences in the iodine status of the Turkish diet and environmental factors. Before therapy is initiated, thyroid function tests should be repeated in 6 to 12 months to exclude laboratory error or transient elevations.

## A-196

### The Use of Fructosamine in Cystic Fibrosis-Related Diabetes (CFRD) Screening

G. Lam[1], M. Doll-Shankaruk[2], J. Dayton[3], K. Rodriguez-Capote[3], T. Higgins[3], D. Thomas[3], M. Smith[1], N. Brown[1], W. Leung[1], M. Estey[3]. *[1]University of Alberta, Edmonton, AB, Canada, [2]Adult Cystic Fibrosis Clinic, Alberta Health Services, Edmonton, AB, Canada, [3]DynaLIFEDx, Edmonton, AB, Canada*

**Background:** Cystic fibrosis related diabetes (CFRD) is a disease of transient hyperglycemia, which if unrecognized and untreated results in irreversible decline in lung function and increased morbidity and mortality. Currently, CFRD is diagnosed with the oral glucose tolerance test (OGTT), as traditional markers of glycemic control, such as HbA1c and fasting glucose are unreliable in patients with CF. Given that compliance with the OGTT is poor, and screening thresholds are not based on relevant CF outcomes, such as impaired lung function, there is great interest in identifying an alternate screening test for CFRD. Serum fructosamine is a simple blood test that measures total glycated serum protein, and is used in clinical settings where HbA1c is unreliable. Here, we aim to determine whether serum fructosamine correlates with glycemic control and clinical outcomes in patients being screened for CFRD.

**Methods:** Twenty clinically stable adult patients undergoing annual screening for CFRD with the 75 g 2 hour OGTT were recruited for this study. Patients previously diagnosed with CFRD were excluded. A serum specimen was collected before commencing the OGTT, and fructosamine was measured using the Siemens fructosaminase-based method on the Advia 2400. Total protein was measured using the Siemens Biuret method, also on the Advia 2400. Fractional serum fructosamine (FSF) was calculated as fructosamine/total protein. Lung function was assessed by measuring the percent predicted forced expiratory volume in one second ($FEV_1$) by spirometry. Simple linear regression was performed in Microsoft Excel to assess the correlation between fructosamine and 2 hour OGTT results, FSF and 2 hour OGTT results, and FSF and $FEV_1$. Coefficients of determination were derived from Pearson correlation coefficients. ROC curve analysis was performed in MedCalc, and the Mann Whitney U test was used to assess statistically significant differences between groups.

**Results:** Based on the OGTT results, two patients (10%) had newly diagnosed CFRD, and three (15%) had impaired glucose tolerance (IGT). Serum fructosamine exhibited a significant positive correlation with 2 hour OGTT results ($r^2$=0.2389, p=0.029). Correction for total protein concentration resulted in a stronger correlation between FSF and 2 hour OGTT results ($r^2$=0.3201, p=0.009). ROC curve analysis suggested that FSF can reliably identify patients with an abnormal OGTT (AUC=0.840, p=0.0002), with a cutoff of ≥3.70 µmol/g exhibiting 100% sensitivity and 67% specificity. In addition, FSF exhibited a negative correlation with $FEV_1$ ($r^2$=0.3732, p=0.035). Patients with FSF ≥3.70 µmol/g has significantly lower $FEV_1$ (median 47%) compared to those with FSF <3.70 µmol/g (median 90%; p=0.015).

**Conclusion:** FSF correlated with both OGTT results and $FEV_1$, and reliably identified patients with abnormal OGTT results. This simple blood test shows potential as an effective tool in CFRD screening, and may greatly improve screening compliance.

## A-197

### Performance Evaluation of the ADVIA Centaur Intact PTH Assay* in Intraoperative Patients

B. Plouffe[1], J. McNair[2], S. Gafary[1], A. Carter[1], R. Christenson[2]. *[1]Siemens Heathcare Diagnostics Inc, Tarrytown, NY, [2]University of Maryland School of Medicine, Baltimore, MD*

**Background:** The ADVIA Centaur® PTH assay (Siemens Healthcare Diagnostics Inc.) is a two-site sandwich immunoassay using direct chemiluminometric technology. The first antibody in the Lite Reagent is a monoclonal mouse anti-human PTH (N-terminal) antibody labeled with acridinium ester. The second antibody is a biotinylated monoclonal mouse anti-human PTH (C-terminal) antibody that is bound to streptavidin-coated paramagnetic latex particles in the solid phase. The use of two monoclonal antibodies is expected to reduce lot-to-lot variability compared to assays using polyclonal antibodies. The assay is intended for use as an aid in the differential diagnosis of hyperparathyroidism, hypoparathyroidism, and hypercalcemia of malignancy, as well as intraoperatively in patients undergoing parathyroidectomy. **Objective:** To assess the performance of the ADVIA Centaur PTH assay in intraoperative patients. **Methods**: The study assessed the concordance of the ADVIA Centaur PTH assay with commercially available PTH assays approved for intraoperative use in the U.S. Sets of plasma samples were collected from patients

undergoing parathyroidectomy and shipped refrigerated to Siemens Healthineers, where they were tested in singleton. Each set included pre- and post-surgery samples. For the primary analysis, successful surgery was defined as surgery resulting in a 50% or greater drop in PTH level from pre-excision to the 10-minute post-excision test results, after the last parathyroid gland excision. Secondary analyses were also conducted, expanding the success criteria to include samples drawn at 10 minutes ±3 minutes from the last excision and adding the criterion that the final PTH measurement must fall in the normal range for the patient. Another analysis looked at each excision individually rather than on a per-surgery basis. **Results:** A total of 46 subjects were enrolled in the study. The primary analysis included the first 30 eligible subjects. These 30 subjects were diagnosed with primary hyperparathyroidism, aged 38 to 79, and four were males. Twenty-six of these subjects had local PTH tested on the IMMULITE® PTH assay (Siemens Healthcare Diagnostics Inc.) and four on a competitor assay. Twenty-nine of the 30 subjects had a successful surgery based on the local PTH results. The percent positive agreement and overall agreement of the primary endpoint were 100.00% (95% CI 91.5 to 100.00) and 96.7% (95% CI 86.4 to 99.3), respectively. For the secondary analyses, all positive agreements were greater than 90%, and all overall agreements were greater than 85%. **Conclusions:** The study showed acceptable concordance between the assays in this intraoperative intact PTH evaluation. *Under FDA review. Not available for sale in the U.S.

## A-198

### Fulvestrant Interference with Six Automated Estradiol Immunoassays and an LC-MS/MS Method: An Analytical and Clinical Investigation

C. Gherasim[1], W. E. Owen[2], J. A. Straseski[1]. *[1]Department of Pathology, University of Utah, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT*

**Background:** Fulvestrant is a structural estradiol (E2) analog and selective estrogen receptor downregulator (SERD) used to treat hormone positive (HR+) metastatic breast cancer (MBC) in postmenopausal women. E2 measurements may guide fulvestrant treatment as it is most effective in low E2 environments. The structural resemblance of fulvestrant to E2 raised concerns regarding interference with E2 testing but, to our knowledge, only a single case report has been documented. Unlike immunoassays, LC-MS/MS methods measure mass-to-charge ratios and are presumably not subject to the same interferences.

**Objectives:** Assess fulvestrant interference with six automated E2 immunoassays and an LC-MS/MS method, using spiked serum pools and MBC patient samples.

**Methods:** Fulvestrant interference was evaluated using an in-house LC-MS/MS E2 method and six commercial E2 immunoassays: ARCHITECT ci8200 (Abbott), DxI 800 (Beckman), cobas 8000 (Roche), Advia Centaur and Immulite 2000 (Siemens) and LIAISON XL (DiaSorin). Nine serum pools of different fulvestrant/E2 concentrations were prepared by adding 0.2-1% (v/v) fulvestrant stock (AstraZeneca) to pools of residual serum samples with comparable E2 concentrations as determined by LC-MS/MS. Interference studies were performed at three E2 concentrations (25ng/mL, 50ng/mL, 200ng/mL) and three fulvestrant concentrations (10,000pg/mL, 25,000pg/mL, 50,000pg/mL). Additionally, serum from five postmenopausal women undergoing fulvestrant treatment for MBC was collected prior to intramuscular dosing. Samples were measured on the same day in duplicate on all assays. Fulvestrant interference was determined as percent change and percent cross-reactivity.

**Results:** Biases of -17.4 to 68% percent were observed when comparing immunoassay and LC-MS/MS results for neat specimens at the lowest E2 concentration (25ng/mL), with LIAISON showing the least bias (-3.3%). The spiked pool with E2 concentrations representative for postmenopausal women (25ng/mL) treated with 25,000pg/mL fulvestrant (maximum reported in vivo concentration) showed the largest percent change (spiked vs. neat) for Centaur (544.7%) followed by LIAISON (148.1%), Immulite (140.4%), ARCHITECT (116.7%), cobas (81.1%) and DxI (39.4%). The magnitude of the interference was proportional to fulvestrant concentrations for all E2 immunoassays investigated and was significantly lower at high E2 concentrations. The E2 concentrations determined by LC-MS/MS in the five MBC patient samples ranged from 3.1-10.1 ng/mL, values below the functional sensitivity of 5/6 immunoassays investigated. The immunoassays measured E2 values of 117.6-193.9ng/mL (Centaur), 53.2–112.0ng/mL (Immulite), 47.0-72.0ng/mL (ARCHITECT), 28.4-48.9ng/mL (LIAISON), 10.3-31.1ng/mL (cobas)

and 5.0-37.0ng/mL (DxI) in the MBC patient samples, representing 49–5,611% difference from LC-MS/MS results.

**Conclusions:** These interference studies expand upon field safety notices issued by several vendors by including clinically relevant E2 concentrations and 3 different fulvestrant concentrations, performed on commercially available platforms on the same day. Centaur and Immulite were the most sensitive to fulvestrant interference, whereas DxI and cobas exhibited the smallest interference. The most significant interference was observed at the lowest E2 concentrations, where clinical decisions are most relevant for this patient population. Importantly, falsely elevated E2 concentrations compared to LC-MS/MS results were observed for all five MBC patient specimens using all six immunoassays, thus LC-MS/MS is the preferred method for this population. This study highlights the importance of characterizing method-specific interferences that may impact treatment decisions.

## A-199

### Performance Evaluation of Free Triiodothyronine (FT3) and Triiodothyronine (T3) Assays* on the Atellica Immunoassay Analyzer**

A. Wang, E. Saharig-Romero, M. Walczak, M. Wu, X. Zhao, J. Freeman. *Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

**Introduction:** Quantitative measurements of free triiodothyronine (FT3) and triiodothyronine (T3) are important for the management of thyroid functions. The prototype Atellica™ IM FT3 and T3 assays* (Siemens Healthcare Diagnostics Inc.) are intended for the quantitative measurement of FT3 and T3 using the Atellica Immunoassay (IM) Analyzer.** The purpose of this study was to evaluate the analytical performance of the Atellica IM FT3 and T3 assays with serum samples. **Methods:** The Atellica IM FT3 and T3 assays are "competitive" immunoassays utilizing direct chemiluminescent technology. They use the same reagent formulations as the ADVIA Centaur® FT3 and T3 assays (Siemens Healthcare Diagnostics Inc.). For the Atellica IM FT3 and T3 assays, the solid phase employs paramagnetic particles covalently coupled to a T3 analog that competes with free T3 in the sample for a limited amount of acridinium ester-labeled monoclonal mouse anti-T3 antibodies in the Lite Reagent. The ADVIA Centaur T3 assay requires an ancillary reagent that contains a releasing agent to free up the bound T3. Performance testing included precision and assay comparison studies. The assay comparison study was conducted according to CLSI EP09-A3, with Deming regression of patient sample results compared with results observed from the ADVIA Centaur Immunoassay System. For assay precision, each sample was evaluated in duplicate twice a day for 20 days according to CLSI guideline EP05-A3. **Results:** The Atellica IM FT3 assay comparison yielded a regression equation of $y = 0.988x - 0.06$ pg/mL, with r of 0.999, versus the FT3 assay on the ADVIA Centaur XP system with 139 serum samples ranging from 0.67 to 18.9 pg/mL. The Atellica IM T3 assay comparison yielded a regression equation of $y = 1.009x + 0.005$ ng/mL, with r of 0.988, versus the T3 assay on the ADVIA Centaur XP System with 137 serum samples ranging from 0.70 to 7.89 ng/mL. The Atellica IM FT3 Assay 20-day precision study yielded repeatability of 0.9 to 1.9% CV and within-lab precision of 1.3 to 3.3% CV over a sample result range of 2.40 to 19.74 pg/mL. The Atellica IM T3 assay 20-day precision study yielded repeatability of 1.5 to 5.4% CV and within-lab precision ranging of 6.4 to 10.0% CV over a sample result range of 0.5 to 8.0 ng/mL. **Conclusion:** The Atellica IM FT3 and T3 assays tested on the Atellica IM Analyzer demonstrated analytical performance capable of providing accurate and precise measurements of free triiodothyronine and triiodothyronine. * In development. The performance characteristics of this device have not been established. Future availability cannot be guaranteed.** Not CE-marked. Not available for sale. Future availability cannot be guaranteed.

## A-200

### A high-throughput test for diabetes care: an evaluation of the next generation Roche Cobas c 513 hemoglobin A1c assay

J. L. Gifford[1], T. Higgins[2], R. Krause[1], H. Sadrzadeh[1]. *[1]Calgary Laboratory Services, Calgary, AB, Canada, [2]DynaLIFEDX, Edmonton, AB, Canada*

**Objectives:** To evaluate the performance of the Roche Cobas c 513 (Roche Diagnostics, Basel, Switzerland), a next generation immunoassay analyzer, in comparison to the Roche Cobas Integra 800 CTS for HbA1c measurement.

**Introduction:** Diabetes mellitus is a condition that affects all age groups worldwide and can be readily diagnosed using laboratory methods. Monitoring patients with diabetes has always been a challenge for clinicians. Glycated hemoglobin A (HbA1c) levels in blood reflect the mean plasma glucose for the previous 3-4 months. As well, HbA1c has been shown to be the preferred marker for diabetes diagnosis and treatment. A rapid and accurate HbA1c method is of great importance to large clinical laboratories.

**Methods:** The c 513 (Roche Tina-Quant® HbA1c Gen.3 immunoassay) was evaluated against the Integra 800 CTS. Leftover EDTA whole blood specimens

containing different hemoglobin species as well as control materials from Roche and Bio-Rad (Bio-Rad, Hercules, California) were used in this study. The evaluation was performed according to CLSI guidelines. Accuracy was determined by measuring samples with %HbA1c values assigned by the College of American Pathologists. The lower limit of measurement for both hemoglobin and HbA1c was calculated as the mean plus 3 SD for a saline blank. The upper limit of linearity was verified using calibrator material or a high patient sample with appropriate dilutions. A method comparison between the c 513 and the Integra 800 CTS was performed by measuring 40 leftover specimens that span the analytical measuring range of the assay. The interference from hemoglobin variants was investigated by measuring samples with one hemoglobin variant on the c 513, the Integra 800 CTS, and the Bio-Rad Variant II Turbo 2.0 systems. To assess the effect of not mixing the specimens prior to analysis, unmixed samples (stored undisturbed at room temperature for up to 24 hours after the initial mixing) were measured.

**Results:** Within run precision was 0.5-0.7%CV for %HbA1c values of 5.6 and 10.6-10.8. Between run precision was 0.8-1.3%CV for %HbA1c values of 5.4, 9.1-9.3, and 13.8-14.4%HbA1c. Accuracy, determined using stored proficiency survey samples, demonstrated an average bias of -1.9%. The lower limits of the hemoglobin and HbA1c measurements were 0.19mmol/L and 0.019 mmol/L, respectively. The upper limit of linearity was 17.0mmol/L and 1.72mmol/L for the hemoglobin and HbA1c, respectively. The c 513 correlated well the Integra 800 CTS (coefficient=0.997, slope=0.93, and *y*-intercept=0.49). Overall, the effect of hemoglobinopathies on this assay was negligible except for specimens containing ≥10%HbF that demonstrated a negative bias. Over a 24 hour period, not mixing the specimens prior to analysis demonstrated a relative bias of -1.9 to 2.7%. The c 513 instrument can process approximately 340 samples per hour, 3.4-fold higher throughput than that of the Integra 800 CTS.

**Conclusions:** Cobas c 513 is a precise and accurate automated analyzer for measuring HbA1c. The major advantage of this instrument is its high throughput capable of testing >7500 specimens in 24 hours or 2500 per shift, making it an ideal choice for large laboratories.

## A-201

### The Prevalence and Risk Factors of Diabetic Patients with Normal eGFR and Albuminuria

H. Tsai, C. Lee, S. Lin, F. Wang, D. Ho. *Taipei Veterans General Hospital, Taipei, Taiwan*

**Background**

Diabetic nephropathy (DN) is the leading cause of end-stage-renal-disease (ESRD), of which microalbuminuria is an important early sign. According to the KDOQI Clinical Practice Guidelines and the Clinical Practice Recommendation for Diabetes and Chronic Kidney Disease of National Kidney Foundation, the severity of DN could be evaluated by renal function or urine albumin loss. Renal function is assessed by eGFR and graded into 5 stages: >90, 60~89, 30~59, 15~29 and <15 ml/min/1.73m$^2$. Urine albumin loss is assessed by urine albumin/creatinine ratio (ACR) and graded into 3 levels: normoalbuminuria (<30 mg/g), microalbuminuria (30~300 mg/g) and macroalbuminuria (>300 mg/g). It is generally believed that ACR correlates with renal function. However, in clinical practice, albuminuria is not uncommonly observed in diabetic patients with normal eGFR. Identifying albuminuria in the later situation is important, as it usually indicates progressive deterioration of renal function. The aims of this study were to (1) investigate the association of eGFR and albuminuria, (2) evaluate the associations of albuminuria and routine laboratory data, and (3) identify crucial laboratory data, which could be used to hint the presence of albuminuria.

**Materials and Methods**

The laboratory data that we analyzed were from 10,638 blood samples from diabetic patients of our hospital in 2016. The HbA1c data were available in all these patients who were 18 to 75 years of age. The eGFR of 8,226 of these samples were ≥60 ml/min/1.73m$^2$, while the rest were below this level. The eGFR levels were correlated with ACR, which were stratified as <30 and ≥30 mg/g. The results of 8 biochemical parameters, i.e., HbA1c, triglyceride, cholesterol, HDL, LDL, fasting glucose, 2hr PC glucose, and hemoglobin, which had been related to albuminuria, of the ACR<30 and the ACR ≥30 groups were compared to select those that were significantly different. The odds ratios (OR) were calculated using univariate analysis and were used to select the parameters that were most useful to predict patients who had normal eGFR but had albuminuria.

**Results**

Albuminuria (ACR ≥30 mg/g) was observed in 30% of cases that had normal eGFR (≥60 ml/min/1.73m$^2$), and it was observed in 73.4% of cases with low eGFR (<60). Of the 8 parameters evaluated, the results of 5 of those were significantly different between the ACR <30 and the ACR ≥30 groups. The parameters and the results were listed as follows: HbA1c (7.13±1.37 vs. 7.9 ±1.68; mean±SD), triglyceride (133.7±92.16 vs. 175.17±166.6), HDL (48.1±12.71 vs. 45.0±11.38), fasting glucose (122.95±43.86 vs. 149.63±61.79), and 2hr PC glucose (193.2±73.67 vs. 219.3±80.19). Their *p* values were all <0.0001. Using univariate analysis, the odds ratios (OR) of 3 of the above parameters were more than 2 and their *p* values were <0.0001. They were HbA1c (2.22, 2.02-2.45; OR, 95% CI), triglyceride (2.28, 2.00-2.60) and fasting glucose (2.14, 1.70-2.70).

**Conclusions**

Albuminuria was detected in 30% of diabetic patients with normal eGFR. High levels of HbA1c, triglyceride, and fasting glucose could be used to identify patients who had normal eGFR and albuminuria.

## A-202

### The differential diagnosis and interpretation of discrepant results of thyroid function tests

Y. Yun, H. Kim, A. Yi, H. Moon, M. Hur. *Konkuk Univserity Medical Center and School of Medicine, Seoul, Korea, Republic of*

**Background**

Most thyroid function tests (TFTs) are straightforward to interpret the clinical impression of euthyroidism, hypothyroidism or hyperthyroidism. Some TFTs, however, such as decreased free T4 (FT4) and normal TSH levels could make a difficulty to differentiate among assay interference, thyroxine replacement therapy, TSH-secreting pituitary adenoma and non-thryroidal illness. This study investigated the incidence for TFT patterns grouped by the FT4 and TSH levels in general hospital and to find the possible causes of discordant TFT patterns according to the characteristics of patients by the comparison among the referral departments.

**Methods**

From August 2015 to August 2016, 22,298 TFTs were performed using MODULAR ANALYTICS E170 immunoassay analyzers with Elecsys FT4 II and Elecsys TSH reagents (Roche Diagnostics, Germany) by the department of laboratory medicine, Konkuk University Hospital, Seoul, Korea. We classified TFT results into seven patterns according to the FT4 and TSH levels using the manufacturer's suggested reference ranges and looked into the incidences in each TFT pattern. The proportion of decreased FT4 and normal TSH among the referral departments was investigated.

**Results**

The incidences in seven TFT patterns in 22,298 TFTs were as follows: 62.7% (13,975 with normal FT4 and normal TSH), 11.9% (2,646 with normal FT4 and increased TSH), 9.6% (2,150 with increased FT4 and decreased TSH), 6.3% (1,405 with normal FT4 and decreased TSH), 3.6% (792 with increased FT4 and normal or increased TSH), 3.1% (695 with decreased FT4 and normal or decreased TSH) and 2.9 % (635 with decreased FT4 and increased TSH). The proportion of decreased FT4 and normal or decreased TSH pattern of

3.1% (695 among 22,298 TFTs) was reclassified based on the referral departments: 10.4% (15/144, Neurosurgery, Odds ratio 5.43 by the comparison with the other referral departments, *P* value < 0.0001), 8.5% (33/389, Psychiatry), 7.4% (23/312, Nephrology), 5.0% (54/1074, Emergency medicine), 5.0% (7/141, Neurology), 4.8% (23/476, Orthopedics), 4.5% (29/652, Gastroenterology), 4.2% (9/215, Hematology & oncology), 4.1% (50/1228, Otorhinolaryngology), 2.7% (111/4058, Endocrinology), 1.2% (62/5116, Surgery) and 2.1% (178/8,493, Other referral departments).

**Conclusion**

When TFTs were classified into the seven patterns according to the FT4 and TSH levels, the incidence of discordant TFT patterns such as decreased FT4 and normal or decreased TSH pattern (3.1%) and increased FT4 and normal or increased TSH pattern (3.6%) was to be remarkable. As a result of classifying the TFT results based on the referral departments, the proportion of decreased FT4 and normal or decreased TSH was significantly varied according to the referral departments. This result suggests that the possibility of discordant TFT results is more likely to be attributed to patient factors rather than to assay errors. Further study should be conducted to investigate additional factors needed to discriminate the various patient factors.

## A-203

### A Comparison of Human Chorionic Gonadotrophin Beta-subunit Measurements Using Three Different Assays for the Early Detection of Pregnancy

Y. L. Choo, S. M. Cosio, P. Y. Heng, M. S. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

**Background:** Human chorionic gonadotropin (hCG) is a hormone produced by the placenta shortly after blastocyst implantation. hCG consists of two subunits: the common α(alpha)-subunit which is virtually identical to that of luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone; and the β(beta)-subunit which has unique structure and is distinguishing for hCG. Most laboratory pregnancy tests employ monoclonal antibody specific to the β-subunit of hCG (β-hCG) to reduce cross-reactivity with other hormones mentioned above. This study aims to compare the measurements of β-hCG in human serum using three different assays that are commonly used in the qualitative and quantitative pregnancy tests.

**Methods:** Forty-nine patient serum samples requested for hCG testing in the Khoo Teck Puat Hospital (KTPH) laboratory were randomly selected and tested qualitatively using the QuickVue+ One-Step hCG Combo Test Kit (Quidel Corporation, USA) (hereafter called "QuickVue+ assay"). These samples were also quantitatively measured using the Elecsys HCG+β assay on the MODULAR ANALYTICS E170 (Roche Diagnostics, Switzerland) used in the KTPH laboratory (hereafter called "Elecsys assay') and the Access Total βhCG assay performed on the DxI-80 0 analyzer (Beckman Coulter, Brea, CA) used in the National University Hospital Referral Laboratories Pte. Ltd. (NRL), Singapore (hereafter called "Access assay"). The Elecsys assay uses only mouse monoclonal anti-hCG, whereas a combination of rabbit anti-hCG, mouse monoclonal anti-hCG and goat anti-mouse IgG is used as the capture and tracer antibodies in the Access assay. Aside from the β-hCG, intact hCG and nicked forms of hCG, the Elecsys assay also recognizes β-core fragments that yield no detectable response in the Access assay. The Elecsys and Access assay have been standardized against the 4th IS NIBSC code 75/589 and 5th IS NIBSC code 07/364, respectively.

**Results:** Five negative and forty-four positive results were observed from the QuickVue+ assay. The QuickVue+ assay demonstrated 100% clinical sensitivity and specificity as compared to the Elecsys assay (positive pregnancy cut-off at ≥7IU/L in KTPH), and 100% clinical specificity and 95.65% clinical sensitivity when compared to the Access assays (positive pregnancy cut-off at ≥6.1IU/L in NRL). Method comparison between the two quantitative assays yielded a relationship of y=0.76x-45.79 with $R^2$=0.982. Results measured by the Elecsys assay varied from the Access assay by -9.1% to -45.0%, with an overall significant bias of -25.4% ($p<0.05$).

**Conclusion:** Results from the qualitative pregnancy test (based on single-step lateral flow immunochromatographic assay) correlated well with quantitative hCG tests (based on two-step chemiluminescence immunoenzymatic assay). Our data showed that the Quickvue+ assay could detect positive results in specimens containing as low as 7IU/L hCG, which is lower than the 25IU/L hCG claimed by the manufacturer. Poor correlation was observed between the two quantitative assays for β-hCG measurement; this could be due to the differences in method principles and assay standardization. In conclusion, our study found that these three different assays demonstrated comparable efficiency for early detection of pregnancy. Nevertheless, it is important that the results should always be assessed in conjunction with the patient's medical history and clinical findings for accurate diagnosis.

## A-204

### Assessment of HbA1c Levels in Non-diabetics with Hemoglobin E (Heterozygous E or Homozygous E)

S. Pongudom[1], Y. Chinthammitt[1], I. Klongsanan[1], A. Soh[2]. *[1]Udonthani Hospital, Udonthani, Thailand, [2]Abbott Laboratories (S) Pte. Ltd., Singapore, Singapore*

**Background:** To assess the HbA1c levels in non-diabetic Hb E patients.

**Methods:** Subjects from antenatal care and thalassemia screening programs (n=180) underwent Oral Glucose Tolerant Test (OGTT) and their HbA1c were measured. Subjects with iron deficiency (ferritin <30 µg/dl), blood sugar at 2 hr OGTT > 200 mg/dl, regular blood transfusion and previously diagnosed diabetes were excluded. HbA1c was measured using ion exchange HPLC and an enzymatic assay.

**Results:** The mean HbA1c in heterozygous E (EA) from ion exchange HPLC and Enzymatic Assay were 5.63 (0.55) and 5.29 (0.37) respectively; and the mean HbA1c in homozygous E (EE) from ion exchange HPLC and Enzymatic Assay were 3.37 (0.69) and 4.91 (0.28) respectively. HPLC showed more variation than Enzymatic Assay.

**Conclusion:**

Enzymatic HbA1c assay is an appropriate method for measuring HbA1c in hemoglobin E patients and the results of this study are useful for early diagnosis and monitoring diabetes in Hb E.

## A-205

### Validation of the conversion factor between Activity Assays and direct Immunoassay for Plasma Renin

D. Gomes, M. Pinheiro, R. Gonçalves, S. Argolo, M. Freire. *Laboratory Diagnostic of America (DASA), Duque de Caxias, Brazil*

**Background:** Plasma renin activity(PRA) and the direct renin concentration measured by immunoassay (Ir-PRC) are the methods used for the clinical assessment of primary and secondary Aldosteronism. Favorable and unfavorable factors are found in both assays. PRA is traditionally used, however labor-intensive and requires a great deal of time. While activity assays measure only active renin, direct renin immunoassays measure both active and non-active renin. The automated direct immunoassays stand out for its fast results, however, similarly PRA, conditions such as pregnancy, glucocorticoid excess, estrogen administration overestimation renin. A conversion factor between PRA and Ir-PRC results can be used, but these factor may change according to the method. The objective of our study was to perform an in-house validation of conversion factor 12 between PRA by Elisa-LDN and Liaison direct renin immunoassay (Diasorin) described in literature.

**Methods:**We selected 81 patients, 34 male (age 11-69 years) and 47 female(age 15-85 years). Measurement of renin was performed in both assays. PRA by Elisa-LDN (functional sensitivity 0.14ng/ml.h, range 0.06-4.69 and within-run precision CV 7.2%, inter-assay precision CV 5.67%, reference value 0.2- 3.3 ng/ml.h). In this assay, the plasma sample was aliquoted and the fractions were incubated at 0-4°C and 37°C respectively for 120 minutes, to allow the generation of Angiotensin-I (Ang-I). The PRA were calculated by the Ang-I difference of the sample at 37° and 0-4°. The same samples were analyzed by automated Liaison direct renin immunoassay (Diasorin), (functional sensitivity 1,96 µUI/mL, range 4.4-46.1 and within-run precision CV 3.31%, inter-assay precision CV 8.30% reference value 2.8-39.9 µUI/mL), the results were divided by 12(conversion factor). For statistical analysis were used the Pearson correlation coefficient.

**Results:** 63 results (78%) were in between reference range in both methods, 12 (15%) above the reference value and only 6 (7%) did not correlate. Among the results that not correlate all then had PRA above reference values. The Pearson correlation coefficient was r=0.946 slope 0.8 and intercept 0.6; Among men, r= 0.985 slope 0.7 and intercept 0.6, women r=0.937 slope 0.9 and intercept 0.2

**Conclusion:** The Liaison direct renin immunoassay (Diasorin) has a good correlation with PRA by Elisa-LDN when used conversion factor 12 as already described in the literature.

## A-206

### Effects of Hemoglobin Newyork Traits on Measurements of HbA1c by 11 Methods

D. Wen, W. Wang, S. Xu, M. Suo. *Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China*

**Background**: HbA1c is an important tool for monitoring glucose levels. Hemoglobin variants affect some HbA1c tests . Hb New York is a common β-chain variant in southern China. We aimed to evaluate the interference of Hb New York on 11 HbA1c analytical systems, including IE-HPLC (Biorad VARIANT II, Biorad VARIANT II Turbo, Biorad VARIANT II Turbo2.0, Biorad D10, Mindray H50 ), AC-HPLC(Primus Ultra2 , Premier Hb9210), Immunoturbidimetric (Roche PPI ,Cobas c501), CE(Capillarys 2FP)and Enzymatic (Leadman ) methods . **Methods** 141 samples were included in the study categorized as control (homozygous for HbA; n=120,45 diabetes patients , 75 healthy adults) and Hb New York group(heterozygotes for Hb New York; n=30). Primus Ultra2 was used as comparative system. Deming regression analysis was used and ± 10% bias at 6% and 9% was used as limits to evaluate whether Hb New York had significant interference. **Results** The differences of the 95%CI between the 10 systems and the comparative system in control group were within ±0.70%, bias% were less than 6%, the test results were of no statistically significant difference (P >0.05). In Hb New York group, the differences of 95%CI between the results measured by Biorad VARIANT II,VARIANT II Turbo2.0,D10,Mindray H50

,Premier Hb9210,Roche PPI,Cobas c501,Capillarys 2FP and the comparative system were all within ±0.7%, bias were less than 6%, the test results were of no statistically significant difference (P >0.05). The differences of the 95%CI between the VARIANT II Turbo and Leadman were outside ± 0.7%, bias % were -4.4% ~ -25.3% and -6.2% ~ -31.6% ,the differences were statistically significant (P <0.001). At 6% and 9%, the mean differences of the results were all greater than the clinical acceptable range; **Conclusion** Hb New York interfered with VARIANT II Turbo and Leadman systems. (Figure1).For Hb New York carriers, we suggest using other methods or indicators to monitor glucose levels.

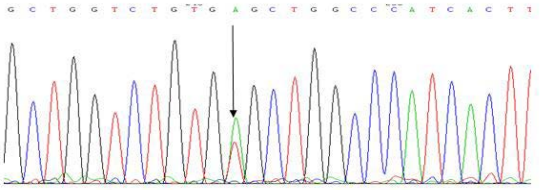


Figure11: The gene sequencing map of Hb New York

## A-207

### A Comparative Effectiveness Analysis of Three Continuous Glucose Monitors: Guardian, G5, and Libre

Y. Young Joo Cha. *Chung-Ang University College of Medicine Seoul, Korea, seoul KOREA, Korea, Republic of*

OBJECTIVE:

Self-monitoring blood glucose (SMBG) with a traditional glucose meter often misses peak post-prandial glucose and hypoglycemia. Currently, continuous glucose monitoring (CGM), which determines diurnal blood glucose patterns on a continuous basis, is being introduced to identify fluctuations and trends of blood glucose levels as soon as possible. This study was aimed to compare the accuracy of three continuous glucose monitoring (CGM) devices in subjects with normal glucose tolerance, type 1 and type 2 diabetes mellitus.

RESEARCH DESIGN AND METHODS:

Nine subjects with normal glucose tolerance (age 23 to 58 years), 9 subjects with type 1 diabetes mellitus (age 27 to 58) and 9 subjects with type 2 diabetes mellitus (age 20 to 67) participated in 96-hour closed-loop blood-glucose control experiments. Capillary blood glucose (BG) obtained 7 times a day were paired in time with corresponding CGM glucose (CGMG) measurements obtained from three CGM devices, the Guardian (Medtronic), G5 (DexCom), and Freestyle Libre (Abbott Diabetes Care) worn simultaneously by each subject. Errors in paired BG-CGMG measurements and data reporting percentages were obtained for each CGM device.

RESULTS:

The accuracy of each device did not change for 5 days. Compared with capillary BG reference readings, the G5 showed the lowest mean absolute relative difference (MARD), with 9.1% overall and 18.1% in the hypoglycemia range. For the Guardian and the Libre, MARD was 16.9%/32.2% and 11.7/14.2%, respectively. Also, the mean and SDs for all BG-ARD pairs associated with BG values within 70-300 mg/dL was lowest in the Libre (6.9±1.5) among 3 devices, indicating higher precision of the Libre. Regarding sensor to sensor variability, the SD for the Guardian was the highest, 14.3%. The Libre and G5 comparable results (6.9% and 8.7%, respectively).

CONCLUSIONS:

This study with three CGM devices for BG values from 35 to 544 mg/dL revealed several differences in performance characteristics that include accuracy, precision and reliability. The G5 and Libre showed comparable accuracy and precision, of which the G5 showed the best accuracy and the Libre showed the best precision.

Acknowledgement:

The research resource was provided by the Clinical evaluation of a small body-attached continuous blood glucose monitoring system with automatic calibration function(NRF-2015M3D5A1065857) through of the MSIP, Korea

## A-208

### Performance of Unicel DXI 800 for 25 (OH) Vitamin D Measurement

E. Üner[1], P. Vatansever[1], T. Çevlik[2], R. Turkal[2], Ö. Şirikçi[1], G. Haklar[1]. *[1]Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey, [2]Biochemistry Laboratory, Marmara University Pendik E&R Hospital,, Istanbul, Turkey*

**Background:** Vitamin D is actually a fat-soluble prohormone steroid that has endocrine, paracrine and autocrine functions. It is not only important in bone metabolism, but also suggested to have etiological roles in cancer, diabetes, neurological and autoimmune diseases. Vitamin D deficiency is a common health problem worldwide. Measurement of serum 25-hydroxy- vitamin D [25 (OH) D]) is necessary to reveal vitamin D status. Due to its hydrophobic character and strong protein binding, measurement of 25(OH) D is technically demanding. We evaluated the analytical performance of Unicel DXI 800 for 25 (OH) D measurement. Pregnancy and high procalcitonin samples were also used to study the effect of vitamin D binding protein (DBP) concentration over DXI 800 assay performance.

**Methods:** Blood samples were collected from healthy volunteers, pregnants (n=30) and cases with high procalcitonin (n=35) into vacutainer tubes with gel seperator (Becton Dickinson, NJ, USA). All analyses were performed at the Marmara University Pendik R&E Hospital Biochemistry Laboratory with Beckman Coulter immunoassay (DXI 800, CA, USA) and Roche immunoassay (Roche Modular autoanalyzer, Mannheim, Germany) and precision, and correlation studies were performed according to 'Approved Guideline' (EP09-A2).

**Results:** For Beckman Coulter immunoassay, within-run imprecisions for 9 and 47 µg/L were 7.5% and 5.6% and and between-run imprecisions for the same concentrations were 17.8% and 6.6%, respectively. Same blood samples were studied with 2 methods. The median (min-max) for Beckman Coulter immunoassay was 22.1 µg/L (4.1-137.4) (n=40 and for Roche immunoassay was 28.8 µg/L (3-130.3) (n=40). All assays were lineer up to 70 µg/L. Lineer regression analysis were performed and there were no significant deviation from linearity. For the effect of different concentrations of DBP, cases with high procalcitonin and pregnant cases were used. Procalcitonin levels range between 18-85 ng/mL and the median (interquartile range) of vit D levels measured by Beckman Coulter immunoassay was 6.4 µg/L (3-7.7) and Roche immunoassay 4.7 µg/L (4.4-8.8). The median (interquartile range) of vit D levels measured by Beckman Coulter immunoassay for pregnants was 6 (4.4-10) and Roche immunoassay 5.8 (3.7-10). There were significant deviations from linearity between two methods in both high procalcitonin and pregnant cases (P<0.001).

**Conclusion:** DBP levels increase by up to 50% in a high-estrogen state, such as pregnancy, and decrease in certain disease states like systemic inflammation. Laboratorians can select the method they need according to their technical utilities and turnaround times. Each method should be verified by the users according to laboratory settings.

## A-209

### Reference values for serum AMH test in Turkish women

D. Topcu, M. S. Gungoren, C. Züngün. *Duzen Laboratories Group, Ankara, Turkey*

**Background:** Anti-Müllerian hormone (AMH) is a glycoprotein with well-known roles in growth and differentiation on reproductive system. Current practices in the evaluation of fertility status of women include analysis of AMH levels, as it is produced by granulosa cells of pre-antral and antral follicles in ovaries. As an ovarian reserve biomarker, serum AMH levels are closely related with several factors such as age and race. Reference values of Roche serum AMH test which were obtained from a worldwide multicenter study are presented as percentiles rather than intervals for narrow age groups. However, we have observed a tendency among AMH test results to be frequently for lower percentiles in reproductive age during our daily practice. This observation implied that Turkish women may have a tendency to have lower serum levels of AMH. The aim of our study was to determine reference values by indirect method from our retrospective data and compare with manufacturer's results of multicenter reference range study. **Methods:** Medical records of female patients from 20 to 50 years of age within the dates of February 2015February 2017 with AMH results were obtained from LIS. Serum AMH analyses were performed with Roche Cobas e601. Outliers from raw data were excluded by Tukey method. 17,571 test results were used for calculation of reference values. Age groups were determined as 20-24, 25-29, 30-34, 35-39, 40-44, 45-50. For each age group, values of 2.5th, 5th, 50th, 95th, 97.5th percentiles were calculated. Statistical analyses were performed with R 3.3.2.**Results:** Reference values calculated were given in Table 1.

All percentile values were 23 to 95% lower than manufacturer's values. The most remarkable difference was observed at 2.5th percentile.

**Conclusion:** Our results show that reference values specific for Turkish female population is required. A nationwide study can be planned for more valid results.

| | Age Groups | AMH (ng/mL) Percentiles | | | | |
|---|---|---|---|---|---|---|
| | | 2.5th | 5th | 50th | 95th | 97.5th |
| Roche | 20-24 | 1.22 | 1.52 | 4.00 | 9.95 | 11.70 |
| | 25-29 | 0.89 | 1.20 | 3.31 | 9.05 | 9.85 |
| | 30-34 | 0.58 | 0.71 | 2.81 | 7.59 | 8.13 |
| | 35-39 | 0.15 | 0.41 | 2.00 | 6.96 | 4.49 |
| | 40-44 | 0.03 | 0.06 | 0.88 | 4.44 | 5.47 |
| | 45-50 | 0.01 | 0.01 | 0.19 | 1.79 | 2.71 |
| Düzen | 20-24 | 0.08 | 0.22 | 2.94 | 7.67 | 8.59 |
| | 25-29 | 0.07 | 0.16 | 1.89 | 6.03 | 6.58 |
| | 30-34 | 0.03 | 0.07 | 1.26 | 4.10 | 4.55 |
| | 35-39 | 0.02 | 0.04 | 0.67 | 2.36 | 2.60 |
| | 40-44 | 0.02 | 0.02 | 0.32 | 1.25 | 1.39 |
| | 45-50 | 0.01 | 0.02 | 0.11 | 0.50 | 0.57 |

## A-210

### Diagnosing Gestational Diabetes Mellitus with the Preferred and Alternate Testing Approaches from the Canadian Diabetes Association

A. Ptolemy[1], M. Godwin[1], H. Li[2], D. Bailey[1], G. Waite[1]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

**Background:** Increased maternal and perinatal morbidity is associated with untreated gestational diabetes mellitus (GDM). In 2013, the Canadian Diabetes Association (CDA) published a preferred and alternate approach for the screening and diagnosis of GDM. The preferred 2-step approach begins with a 50 g glucose challenge test (GCT) in a non-fasting state with plasma glucose (PG) measured 1 hour later. A PG ≥7.8 mmol/L is considered a positive screen and a 75 g oral glucose tolerance test (OGTT) is required for GDM diagnosis. A PG ≥11.1 mmol/L is diagnostic for GDM and does not require a 75 g OGTT for confirmation. GDM is diagnosed if *N*≥1 75 g OGTT result is abnormal (fasting ≥5.3 mmol/L; 1 hour ≥10.6 mmol/L; or 2 hour ≥9.0 mmol/L). The alternate 1-step testing approach deploys a 75 g OGTT only (with no prior 50 g GCT). GDM is diagnosed with this approach if *N*≥1 OGTT result is abnormal (fasting ≥5.1 mmol/L; 1 hour ≥10.0 mmol/L; or 2 hour ≥8.5 mmol/L). **Objectives:** Identify the prevalence of GDM diagnosed by the CDA preferred and alternate approaches to testing within our community-based patient population. Quantify the 75 g OGTT confirmation rate for positive 50 g GCT screens within the CDA preferred testing approach. **Methods:** The PG results from all woman who received 50 g GCT and 75 g OGTT from our regional reference laboratory between Jan 2014 and Dec 2016 were retrospectively reviewed. Patients with both 50 g GCT and 75 g OGTT testing from our laboratory were identified through their date-of-birth and health card number. **Results:** *N*=50866 women received 50 g GCT only. 78.5% (*N*=39935) had a negative 50 g GCT screening result (PG <7.8 mmol/L). 1.3% (*N*=659) had a PG diagnostic for GDM (≥11.1 mmol/L). 20.2% (*N*=10272) of patients had a positive 50 g GCT screen and required 75 g OGTT confirmation testing. 21.6% (*N*=2161) of these women did not receive this testing from our laboratory. 75 g OGTT for patients with a 50 g GCT result between 7.8 and 11.0 mmol/L (*N*=8111) confirmed the diagnosis of GDM in 18.7% (*N*=1518) of these women. *N*=7804 women received 75 g OGTT only. This 1-step approach confirmed the diagnosis of GDM in 24.9% (*N*=1944) of patients. **Conclusion:** The majority of pregnant women received the CDA preferred approach to GDM diagnosis and had a negative 50 g GCT screening result (no 75 g OGTT required). The positivity rate for the 50 g GCT screen within our patient population was ~20% but follow-up 75 g OGTT only confirmed a GDM diagnosis in ~19% of these patients. In total, the CDA preferred 2-step approach confirmed the diagnosis of GDM in ~4% of all patients tested. GDM was diagnosed in ~25% of patients who received the alternate 1-step 75 g OGTT testing. This study provides evidence of the benefits associated with the CDA preferred testing approach. It may also serve as an aid for physicians as they counsel patients with false positive 50 g GCT screening tests.

## A-211

### Evaluation of a plasma renin mass assay as a replacement for plasma renin activity measurement.

D. M. Manthei, C. S. Carter, E. G. East, A. M. Sinay, D. Giacherio. *University of Michigan Hospital, Ann Arbor, MI*

**Background:** Plasma renin is measured in the workup of refractory hypertension to aid in the diagnosis of primary hyperaldosteronism. The Endocrine Society (ES) recommends screening a defined subset of hypertensive patients with the aldosterone / renin ratio (ARR), with follow-up of patients with abnormal results (based on both the ratio **and** increased aldosterone concentration) with more definitive confirmatory testing. Available direct renin mass (DRM) assays present an attractive alternative to PRA for the laboratory because it is performed on an automated, random access analyzer which greatly simplifies workflow compared to the RIA or LC-MSMS based PRA assay. The manufacturer's recommendations for DRM sample handling are different than those for PRA in order to reduce prorenin conversion to immunoreactive renin. **Objective:** To verify pre-analytical sample handling conditions to allow for the measurement of PRA and DRM off of the same sample submitted for routine laboratory analysis. To establish the correlation between PRA and DRM, to evaluate the clinical utility of the ARR with the DRM assay replacing PRA, and to provide clinicians with appropriate interpretive guidelines if the DRM were to replace PRA. **Methods:** Three EDTA plasma samples were drawn from each of 20 healthy volunteer donors. One sample was frozen immediately, one refrigerated for two hours, and one left at room temperature for two hours. All sample were then analyzed by the DRM assay. For assay correlation, 256 samples submitted to the University of Michigan Hospital Special Chemistry laboratory for PRA utilizing a Diasorin RIA kit were also analyzed for DRM on the Diasorin Liason XL. Of these, 188 samples also had aldosterone orders. The ARR was calculated and compared for both the PRA and DRM assays. **Results:** By both paired t test and ANOVA, no statistically significant difference between the sample handling conditions could be demonstrated. P values were 0.903 for the t test and 0.99 for ANOVA. Comparison of the DRM (Y axis) to PRA (X axis) showed a strong linear correlation, with regression equation (Y = 9.05 X + 0.86, r = 0.9620. Comparison of ARR Mass (Y) to ARR Activity (X) showed a slightly poorer but still strong correlation, Y = 0.08 X - 0.05, r = 0.9272. Using a ARR Activity ratio of 20 and an ARR Mass ratio of 2.2 as cutoffs, 127 patients screened negative by both criteria, 41 patients screened positive by both criteria, and 20 patients disagreed with an ARR Activity ratio > 20 but an ARR Mass ratio of < 2.2. However only 4 of these 20 patients had an aldosterone > 15 ng/dL, the suggested requirement in the ES guidelines. **Conclusions:** Immediate post-draw sample handling conditions did not alter DRM as long as samples were centrifuged and frozen with 2 hours. DRM shows a strong correlation with PRA and is a promising potential alternative. Surveys of clinicians at our institution found multiple uses of PRA and ARR. Precise understanding of the relationship between DRM and PRA will be critical to ability to the education of all users for future implementation of DRM.

## A-212

### Evaluation of Analytical Performance of Capillary 2 Flex Piercing against Primus Ultra2

S. Xu, D. Wen, W. Wang, M. Suo. *Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China*

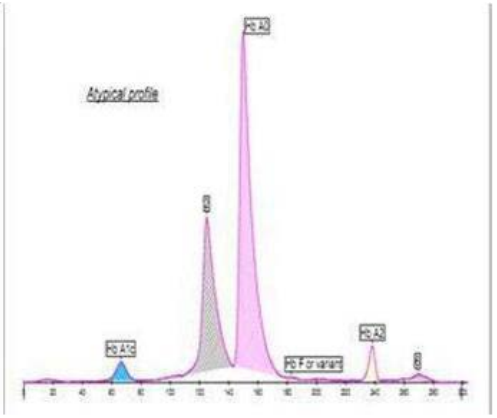
**Background:** HbA1c is an important indicator for the monitoring of glycemic levels in diabetic and prediabetic patients. It could be measured by various methods. Here we report the results of the evaluation of Capillary 2 Flex Piercing, an analyzer using capillary electrophoresis for the separation and quantification of HbA1c against Primus Ultra2,an analyzer using boronate affinity HPLC.

**Methods:** All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity were evaluated according to CLSI protocols EP15-A2, EP9-A2 and EP6-A respectively. Also we have evaluated the influence of HbE and Hb New York regarding HbA1c assays.

**Results:** Intra-tube and between-tubes CVs are respectively lower than 1.8% and 1.26%; The linearity was excellent for HbA1c values ranging from 31 mmol/mol(5.0%) to 138 mmol/mol(14.8%) (r=0.999);The results were well correlated with those obtained by the BA-HPLC method routinely used in the laboratory(Primus Ultra2):HbA1c[CapillaryS2]=0.9926*HbA1c[Primus]-0.0441(r=999); For accuracy :the bias for 39 of the 40 samples were within the range of ±6%;The analytical system were confirmed free from interference of HbE and Hb New York: the deviation of CapillaryS2 to Primus Ultra2 ranges from -0.35 to 0.34 for HbE and from -0.15 to

0.16 for Hb New York; the bias of CapillaryS2 to Primus Ultra2 ranges from -4.8% to 3.0% for HbE and from -2.9% to 3.0% for Hb New York;

**Conclusion:** This evaluation showed that the analytical performances of Capillary 2 Flex Piercing analyzer for HbA1c assay fulfilled quality criteria requested for clinical use for routine practice.




**HbA1c result of a Hb New York carrier with Sebia Capillarys 2 Flex Piercing**

## A-213

### RNase L is Involved in Glucose Homeostasis and Insulin Resistance

D. Liu, N. Alghamdi, R. Wei, A. Zhou. *Cleveland State University, Department of Chemistry, CLEVELAND, OH*

**Background:** Diabetes is characterized by hyperglycemia mainly due to defect in insulin secretion and/or action. Regulation of glucose transport and use by insulin is central to the maintenance of whole-body glucose homeostasis. One of the potential mechanisms associated with insulin sensitivity is the activation of insulin receptor (IR) and subsequently transduces the signal through phosphorylation of insulin receptor substrate (IRS)1 and activation of the PI-3K/Akt pathway. In contrast, activation of the mechanistic target of rapamycin (mTOR) and ribosomal protein S6 kinase (p70S6K) inactivates the signal cascade. RNase L, an IFN-inducible enzyme, plays an important role in IFN functions against viral infection and cell proliferation. However, a direct link between RNase L and insulin sensitivity has yet to be clearly established.

**Methods:** Primary RNase L+/+ and -/- mouse embryonic fibroblasts (MEFs), hepatocytes and adipocytes were used to investigate the role of RNase L in insulin signaling and sensitivity. Cells were treated with insulin at various time points and different concentrations. Activation of the insulin signaling pathway was determined by immunoblot analyses for the protein level and phosphorylation status of these components such as IR/p-IR, IRS1/p-IRS1 and AKT/p-AKT in the presence or absence of chemical inhibitor.

**Results:** We found that RNase L plays an important role in glucose homeostasis through impacting insulin receptor (IR) which is a trans-membrane receptor activated by insulin. The phosphorylation status of IR was significantly reduced in the cells deficient RNase L. As a result, activation of IRS1, the downstream substrate of IR, and the PI3K/AKT pathway was significantly inhibited in RNase L-/- cells. Further investigation of the molecular mechanism underlying the role of RNase L in mediating the activation of IR revealed that RNase L may regulate the cleavage of the precursor of IR via the ubiquitin/ proteasome system. In addition, the level of activated S6 kinase in the mTOR pathway was also markedly elevated.

**Conclusion:** In summary, the role of RNase L in the insulin signaling pathway suggests that RNase L may be a novel target in the design of therapeutic strategies for diabetes. Treatment of this disease may be achieved through regulating the expression and activation of RNase L. In addition, RNase L may be used as a prognostic marker for diabetes as well.

## A-214

### Thyroid Profile, LDL Cholesterol and Oxidized LDL Level in Geriatric Population Visiting a Tertiary Care Hospital

L. Panthi, B. Jha, Y. L. Shakya. *Institute of Medicine (IOM), Maharajgunj Medical Campus, Kathmandu, Nepal*

**Background:** Thyroid dysfunction is one of the most commonly encountered endocrine abnormalities in the elderly. Thyroid dysfunction is a major health problem of Nepal with prevalence of nearly 30% of the population affected in eastern region of Nepal alone. Several changes in thyroid function and thyroid function tests occur with advancing age. This thyroid dysfunction may occur due to alteration in the hypothalamo-pituitary-thyroid axis. It is known that alteration in thyroid hormones produces various metabolic disorder including remarkable effects in lipid metabolism. **Methods:** A cross sectional, comparative study was carried out in the Department of Biochemistry, Tribhuvan University Teaching Hospital, Kathmandu, Nepal between February 2016 and January 2017 to assess and correlate Thyroid Profile, LDL Cholesterol and Oxidized LDL level in healthy geriatric population. One hundred and twelve healthy elderly subjects of both sexes aged > 60 years were taken as study group. They were recruited in the study from General Health Checkup Clinic, TUTH, Kathmandu. In addition, 75 apparently healthy young adult subjects aged 20-30 years were included as control. Serum free thyroxine (FT4), free triiodothyronine (FT3) and thyroid stimulating hormone (TSH) levels were estimated by using ECi analyzer. LDL Cholestreol was measured enzymatically using BT-3000 analyzer and oxidized LDL level in serum was estimated by ELISA. Statistical analysis was done by using ANOVA and Pearson's Correlation Coefficient test as applicable. **Results:** There was significant decrease in FT3 ($p<0.01$), insignificant decrease in FT4 in geriatric population compared to younger population. However, geriatric population showed significant increase in TSH levels ($p<0.05$). Significantly increased oxidized LDL ($p<0.001$), was observed in elderly in comparison to young adults. TSH was strongly positively correlated with LDL Cholesterol ($r=0.789$, $p<0.001$) and oxidized LDL ($r=0.650$, $p<0.001$) in elderly; but it was significantly positively correlated only with LDL Cholesterol in young ($r=0.545$, $p<0.001$). There was weak but significant negative correlation of FT4 with LDL Cholesterol ($r=-0.228$, $p<0.05$) in healthy geriatrics population, despite of no differences in LDL Cholesterol levels between elderly and young. **Conclusion:** This study revealed alterations in thyroid function with advancement of age. There should be a separate reference range for elderly group in our setup to correctly diagnose thyroid disorders in this population. The significantly increased level of oxidized LDL, in elderly as compared to young adults, in geriatric population denotes the presence of increased oxidative stress in this population. The strong positive correlation of TSH with oxidized LDL, weak but significant negative correlation of FT4 with LDL Cholesterol seen only in healthy geriatrics population, despite of no differences in LDL Cholesterol levels between elderly and young, in our study recommends that early therapeutic interventions should be carried out in subclinical hypothyroid patients to prevent the cardiovascular risks related to thyroid dysfunction.

## A-215

### Unexpectedly high adrenocorticotropic hormone values due to its complex formation with immunoglobulin

O. Portakal, S. Sendur, T. Erbas, A. Pinar, M. Bayraktar. *Hacettepe University, Ankara, Turkey*

**Background:** Pituitary-incidentaloma is unsuspected lesion incidentally detected on an imaging-study. Despite lacking of clinical symptoms for a specific pituitary disease, routine functional-evaluation is recommended. This condition can cause a disparity between patient's clinic and laboratory results. Here, we present a pituitary-incidentaloma case with unexpectedly-high adrenocorticotropic-hormone (ACTH) levels, subsequently confirmed as macro-ACTH. **Methods:** A 28-year-old male patient was admitted to the hospital to assess hormonal functions of a pituitary-adenoma which was detected by cranial-MRI performed for differential diagnosis of headache. Past-medical history only revealed a two-year course of anti-epileptic treatment after a head injury. There were not any stigmata on physical examination suggesting pituitary hypo-hyperfunction. Pituitary-MRI revealed a suspected pituitary-microadenoma (6x3.5 mm) at the left-posterior-segment of the gland. Results of pituitary-hormone function were shown in Table-1. Plasma-ACTH was measured by IMMULITE2000 (Siemens-Co, USA), which is a solid-phase two-site immunometric-assay including monoclonal-murine anti-ACTH and polyclonal-rabbit anti-ACTH antibodies. Serum cortisol levels were measured by DXI800 (Beckman Coulter, Co, USA), based on competitive-immunometric assay using paramagnetic particles coating with goat anti rabbit-IgG, and rabbit anti-cortisol antibodies. **Results:** Repeated measurements of

ACTH were high [150, 167, 172 pg/mL (0-46)]. Cortisol was suppressed after 1mg overnight dexamethasone suppression test but ACTH not (Cortisol: 1.9, ACTH: 163). ACTH measurement interference was suspected. Plasma-ACTH was measured by electrochemiluminescent assay (ROCHE Diagnostics, USA), and found 199 pg/mL. Dilution study of patient plasma was non-linear by IMMULITE2000. HBT study (Scantibodies Laboratory, Inc., Santee, CA, USA) showed that there was no heterophilic antibody interferences. A 22.53% recovery was detected by PEG precipitation study. These results show that high ACTH levels are due to its complex formation with immunoglobulin, called macro-ACTH. **Conclusion:** Incompatible high ACTH levels can complicate to detect pituitary adenomas. Clinicians should be aware in terms of prevent unnecessary advanced investigations. Macro-ACTH could reflect pituitary adenoma's altered hormone production or could be a coincidental finding.

Hormone levels of the patient.

| Parameter | Value |
|---|---|
| ACTH (pg/mL) | 176 (0-46) |
| Cortisol (µg/dL) | 11.33 (7-23) |
| TSH (µIU/mL) | 1.4 (0.34-5.6) |
| fT3 (pmol/L) | 7.1 (3.8-6) |
| GH (ng/mL) | 0.49 |
| IGF-1 (ng/mL) | 144 (80-644) |
| FSH (mIU/mL) | 3.8 (1-19) |
| LH (mIU/mL) | 3.9 (2-9) |
| Testosterone (ng/dL) | 512 (181-772) |
| Prolactin (ng/mL) | 7.9 (2-13) |
| fT4 (pmol/L) | 7.9 (2-13) |

## A-216

### Biotin May Lead To High Free Thyroxine Levels in Some Immunoassay Methods

O. Portakal, G. Gulal, N. Gonc, S. Sivri, A. Alikasifoglu, S. Yigit, A. Pinar, A. Ozon, T. Coskun. *Hacettepe University, Ankara, Turkey*

**Background:** Biotin is required for carboxylases including acetyl-CoA carboxylase in cytosol and, pyruvate carboxylase, propyonyl-CoA carboxylase, methyl-crotonyl-CoA carboxylase in mitochondria; all are important for fatty acid synthesis, amino acid metabolism, and gluconeogenesis. Biotin deficiency leads to reduced carboxylase activities, disruption of energy metabolism, and increased organic acids in urine. In brain, biotin deficiency causes lactate accumulation, then seizures and ataxia. Therefore biotin replacement is required.

**Methods:** Here we report two children taking biotin-replacement therapy with high free-throxine (FT4) and free-triiodotyronin (FT3) levels. First patient is a girl, 1y4d, with 61.93 pmol/L (7,86-14,4 pmol/L) of FT4, 14,99 pmol/L (3,8-6,0 pmol/L) of FT3 and 5.04 ug/mL( 0,38-5,33 µg/mL) of TSH levels. Her serum anti-TPO and anti-TG levels were in reference intervals. She was hospitalized with preliminary diagnosis of fatty-acid oxidation defect. She was taking 1 mg/d biotin. The second patient is a boy, 1y4m, with 21.2 pmol/L of FT4 and 1.12 µg/mL of TSH levels. His biotinidase activity was found 1.9 U/L (>3.5 U/L), so was started to use biotin with the diagnosis of partial biotinidase deficiency.

**Results:** All measurements were performed by DXI800 (Beckman Coulter, Co, USA). FT4 levels were measured by another method, ECLIA (ROCHE diagnostic, USA) and found 1.56 ng/dL and 1.48 ng/mL (1.02-1.72 ng/dL), respectively. Beckman-FT4 measurement is a two-step chemiluminescent assay using monoclonal mouse anti-T4 antibody labeled with biotin. Beckman-FT3 assay is a competitive binding immunoenzymatic assay, in which biotinylated-T3 analog is used. In both, at the end of reaction, the substrate and ALP-conjugate are added, and light is produced, that is inversely proportional with analyte-concentrations. Biotin leads to decrease of light, so FT4/FT3 levels are increased. It was confirmed by adding biotin to the sample on DXI800 (Table 1).

**Conclusion:** Clinicians should check whether the patient has received biotin-therapy in high FT4/FT3 results incompatible with the patient's clinic.

## A-217

### Evaluation of the Bio-Rad D-100™ system for the measurement of glycated hemoglobin ($HbA_{1C}$)

N. M. A. White-Al Habeeb[1], H. Dong[2], P. Cheng[2], S. Youkhana[2], S. Lam[2], C. Rolfing[3], R. Little[3], P. Yip[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]University of Missouri School of Medicine, Columbia, MO*

**Background**: $HbA_{1C}$, the main form of glycated hemoglobin, is the gold standard for the monitoring of glycemic control in diabetic patients and has recently been recommended for the diagnosis of diabetes. $HbA_{1C}$ levels also correlate with the development of long-term complications in diabetic patients. It is therefore essential that $HbA_{1C}$ measurements be performed on robust and reliable methods. The aim of this study was to evaluate the D-100™ system (Bio-Rad Laboratories) for the accurate quantification of $HbA_{1C}$.

**Methodology**: Detection of $HbA_{1C}$ in whole blood by the D-100 system is based on ion-exchange quantitative high performance liquid chromatography (HPLC) in a 45 second separation per sample. Precision was assessed for 24 days by measuring Bio-Rad quality control (QC) materials in addition to four patient samples, in duplicate, twice daily. Linearity and accuracy was assessed using proficiency testing (PT) material from the College of American Pathologists (CAP) or Institute for Quality Management in Healthcare (IQMH). Remnant samples after routine analysis were collected and utilized for comparative testing against the Bio-Rad VARIANT™ II Turbo. Interference from known hemoglobin variants (AC, n=55; AD, n=41; AE, n=43; AS, n=37) was assessed by comparing results to those obtained by the Trinity Biotech ultra$^2$ boronate affinity HPLC at a NGSP reference laboratory. An overall test of coincidence of least-squares regression lines was used to test for statistically significant differences compared to AA samples; clinical significance was defined as a relative difference exceeding ±7% versus AA samples at HbA1c levels of 6 and 9 $\%HbA_{1C}$ based on Deming regression..

**Validation**: The Bio-Rad Lyphocheck and Liquicheck QC showed within run and total coefficient of variation (CV) of 0.8-1.0% and 0.9-1.1%, respectively. $HbA_{1C}$ levels in patient samples ranging from 4.8 $\%HbA_{1C}$ to 12.1 $\%HbA_{1C}$ showed total CVs of 0.7–0.8%. Linearity over a measuring range of 5.10-11.17 % $HbA_{1C}$ was acceptable with a slope of 0.947 and intercept of -0.06. PT sample results met CAP and IQMH criteria (allowable error of 6% and 7%, respectively). For the method comparison, samples were selected to maximally cover the measuring range of the assay, 3.5 $\%HbA_{1C}$ to 20.0 $\%HbA_{1C}$. One hundred samples were run in duplicate on the D-100 analyzer and compared to routine measurements on the Bio-Rad Variant II Turbo analyzer. Deming regression analysis showed R=0.9983, slope of 0.944 (0.937-0.952), y-intercept of 0.08 (0.03 – 0.14); the standard error of the estimate was 0.09 $\%HbA_{1C}$. Bland-Altman analysis showed a mean difference of -0.3 $\%HbA_{1C}$ (95% CI: -0.5 – 0.0 $\%HbA_{1C}$). The variant interference evaluation showed no clinically significant interferences for the four variants tested, although there were statistically significant differences for AE and AS ($p<0.05$). In addition, the D-100 Advisor software correctly provided the presumptive identification of the 176 known AS, AC, AD, and AE variants according to defined chromatographic time windows.

**Conclusions**: The Bio-Rad D-100 system is a robust, high-throughput method for the routine determination of $HbA_{1C}$ in clinical laboratories.

## A-218

### Microparticles expressing tissue factor as a marker for antithrombotic metformin effect in polycystic ovarian syndrome

L. M. L. Carvalho[1], C. N. Ferreira[2], A. L. Cândido[3], F. M. Reis[3], I. O. Silva[1], F. F. C. Nunes[1], K. B. Gomes[1]. *[1]Pharmacy Faculty, Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Technical College, Federal University of Minas Gerais, Belo Horizonte, Brazil, [3]Medicine Faculty, Federal University of Minas Gerais, Belo Horizonte, Brazil*

**Background:** Microparticles (MPs) are extracellular vesicles released by the cell membrane during apoptosis or cell activation and are potential mediators of several diseases, since they act as signaling mediators. These structures are increased in women with Polycystic Ovarian Syndrome (PCOS), which is related to thrombotic complications. Several patients with PCOS use metformin because of their hypoglycemic effect, but it is not clear if metformin also improves the hemostatic profile, reducing the thromboembolic risk. This study aimed to evaluate whether the microparticles expressing TF (TFMPs - an important pro-coagulant marker) are altered in PCOS women under metformin treatment. **Methods:** PCOS diagnosis was performed according to the Rotterdam criteria. We quantified the TFMPs in citrate

plasma of 50 patients with PCOS - 13 of these women used metformin (850 mg 2x/day during at least 6 months) and the other 37 did not. The TFMPs were quantified in a BD LSRFortessa® flow cytometer. The presence of phosphatidylserine (marker for MPs) was determined based in the interaction with annexin V, using fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies. Specific monoclonal antibodies CD142-PE to identify MPs expressing tissue factor were also applied. Trucount control tubes were included as a quality control. All data were analyzed by statistics software SPSS (13.0 version). We used the Shapiro-Wilk test to determine the normality. Considering that the distribution was parametric, test t-Student was assess in order to compare the two groups. $p<0.05$ value was considered significant

**Results:** Plasma levels of TFMPs were significantly lower in the group of patients who used metformin when compared to untreated women ($p = 0.003$). **Conclusion:** These results suggest the use of TFMPs as marker to evaluate the antithrombotic effect of metformin. This study included PCOS women, but it could be extended to other diseases associated to insulin resistance and hypercoagulability, which require metformin treatment.

## A-219

### Performance Evaluation of the VITROS® Immunodiagnostic Products Insulin Test* on the VITROS® ECi/ECiQ Immunodiagnostic Systems, VITROS® 3600 Immunodiagnostic System, and VITROS® 5600 Integrated System

H. Groth, L. Colt, L. Ernst, T. Jakubowski, R. Polimeni, K. Teugeman, I. Tuttle. *Ortho Clinical Diagnostics, Rochester, NY*

**Background:** We have developed an enhanced chemiluminescent assay for the measurement of human insulin on the VITROS® ECi/ECiQ Immunodiagnostic System, VITROS® 3600 Immunodiagnostic System, and VITROS® 5600 Integrated System. Performance verification testing was conducted to evaluate precision, Limits of Blank, Detection and Quantitation, comparison to three commercially available insulin assays and to establish a reference interval.

**Methods:** Total Within Lab Precision was evaluated per CLSI EP05-A3 by testing a 5 member panel with concentrations ranging from 5.81 to 206µIU/mL in duplicate 2 times per day for 20 nonconsecutive days. Testing spanned a total of 29 days and included 5 calibration events. Limits of Blank, Detection and Quantitation (LoB, LoD, LoQ) were evaluated per CLSI EP17-A2 by testing 10 replicates of each of 4 LoB fluids and 5 LoD/LoQ fluids 2 times per day for 5 days across 3 calibration events. A total of 130 samples that spanned the assay range were tested in the VITROS Immunodiagnostic Products Insulin Test and an aliquot was sent out for testing on 3 commercially available automated comparator methods. The sample set included random samples, fasting samples, and post meal samples collected from in house volunteer participants. Passing Bablok regression was used to compare the methods. A reference interval for 99 apparently healthy, fasting individuals was established according to CLSI EP28-A3C using a parametric analysis and log-normal transform estimate at the 95% confidence level. All verification testing was conducted using 3 reagent lots across the VITROS ECi, VITROS 3600 and VITROS 5600 systems.

**Results:** The total within lab precision estimates ranged from 2.5% to 7.0% among the 5 panel members across the reagent lot/system combinations. The LoB is 0.033µIU/mL based on 400 determinations of four blank samples. The LoD is 0.077µIU/mL based on 500 determinations of five low-level samples. The LoQ based on 500 determinations with the five LoD pools; and a precision goal of 20% using the functional sensitivity method is 0.077µIU/mL. For the method comparison, Passing Bablok regression analysis yielded a slope of 0.79, intercept of 0.01 and Pearson Correlation Coefficient of 1.00 for comparator method 1; a slope of 1.13, intercept of 0.30 and Pearson Correlation Coefficient of 1.00 for comparator method 2; a slope of 0.86, intercept of -0.28 and Pearson Correlation Coefficient of 1.00 for comparator method 3. The reference interval for the VITROS Insulin Test was 2.30 to 26.0µIU/mL.

**Conclusion:** The performance verification data demonstrate that the VITROS® Immunodiagnostic Products Insulin Test has comparable precision, Limit of Detection/Quantitation, correlation with three commercially available methods, and a fasting reference interval consistent with comparator methods.

*Under development

## A-220

### Thyreoglobulin and anti-thyreoglobulin in the needle washout of neck lymph node biopsies suspected of metastasis of differentiated papillary thyroid cancer

M. C. Gurgel Castelo[1], L. L. A. Cavalcante[2], S. P. Bandeira[2], C. O. W. Sabino[3], R. G. Fontes[4], S. Marui[4], S. A. C. Vencio[5], D. M. V. Gomes[4], G. A. Campana[3]. *[1]Universidade Federal do Ceará, Fortaleza, Brazil, [2]DASA - Diagnósticos da América, Fortaleza, Brazil, [3]DASA - Diagnósticos da América, São Paulo, Brazil, [4]DASA - Diagnósticos da América, Rio de Janeiro, Brazil, [5]DASA - Diagnósticos da América, Goiânia, Brazil*

**Background:** Several studies report that detection of thyroglobulin (Tg) in fine-needle aspiration (FNA) biopsy washout fluid from lymph nodes identifies recurrences/metastases of differentiated papillary thyroid cancer (DPTC) in the neck with higher sensitivity and specificity than fine-needle aspiration cytology (FNAC). However, there are few data on the levels of anti-Tg antibodies in these washouts (TgAb-FNAB), with a restricted number of samples, which compromises the ability to draw further conclusions. **Methods:** To measure Tg-FNAB and TgAb-FNAB in washout samples from patients submitted for FNAB due to the suspected presence of metastases of DPTC in neck lymph nodes. This is a transversal study that enrolled 100 samples for determination of Tg-FNAB and TgAb-FNAB in neck aspirate of lymph nodes suspected of having metastatic disease of DPTC. The study was conducted from January to October 2016. The presence of TgAb-FNAB was analyzed in each sample by two different immunofluorimetric assays (Siemens and Roche). The cutoff value for increased Tg-FNAB was 0.1ng/dL and for increased TgAb-FNAB was 30 IU/mL and 10 IU/mL for Siemens and Roche assays, respectively. **Results:** Among the 100 samples analyzed, 55% were positive for determination of Tg-FNAB. Of these, 34.55% (19/55) were positive TgAb-FNAB using Siemens assay and 62.22% (28/45) using Roche assay. A total of 35.56% (16/45) presented a positive result in both assays and 52,7% in at least one. All samples with negative Tg also had negative TgAb-FNAB by both assays. **Conclusion:** It is still controversial whether the presence of TgAb-FNAB interfere with the assessment of Tg-FNAB. Although previous studies did not find TgAb-FNAB in lymph nodes with positive Tg- FNA, the present study detected TgAb-FNAB in more than half of the analyzed samples. Prospective studies with a larger number of patients are important to identification of a possible causal relationship between levels of TgAb-FNAB and values of Tg-FNAB in patients investigated for presence of metastases of DPTC in neck lymph nodes.

## A-221

### Automated Dispersive Pipette Extraction of Diphenylborinate Complexed Free Catecholamines and Metanephrines in Urine with LC-MS/MS Analysis

W. E. Brewer, K. R. Mastrianni. *DPX Technologies, LLC, COLUMBIA, SC*

**Background:** The catecholamines are bioamines that play an integral role as neurotransmitters in the nervous system. Screening for catecholamines and their O-methylated metabolites is an accepted approach for diagnosis of catecholamine-secreting tumors. Generally, these tumors are benign, but potent effects on the cardiovascular system caused by excess catecholamines can have potentially fatal outcomes. Correct, timely diagnosis is crucial. Unlike plasma, it's recommended both metanephrines and catecholamines be measured in urine. Urine analysis is non-invasive and exhibits sufficiently high levels of target compounds.The proposed method uses diphenylborinic acid (DPBA) as a complexing agent to stabilize and increase lipophilicity for reversed phase retention. Dispersive pipette extraction (DPX) takes place within a pipette tip, which facilitates an easily automated alternative to traditional SPE requiring less sample and solvent volume. The objective was to develop an automated sample preparation method utilizing DPBA with DPX extraction for minimized sample preparation time and high sensitivity analysis of epinephrine, norepinephrine, dopamine, metanephrine, and normetanephrine in urine with LC-MS/MS.**Methods:** A well plate with 300 uL of sample and internal standard was loaded onto a Hamilton Microlab® NIMBUS96® system. Reservoirs of DPBA solution, wash buffer, methanol, and 1M formic acid were also added to the system deck. The system added 600 uL of complexing agent to the urine sample well plate, 500 uL of wash buffer to a second "wash" well plate, 270 uL of 1 M formic acid and 30 uL of methanol to the third "elution" well plate. The system picks up 1 mL DPX RP (reverse phase) tips. After conditioning, the tips aspirated and dispensed the sample solution four times to bind the complexed analytes. After washing with wash buffer, analytes were eluted with 1M formic acid/10% methanol solution. The acidic solution reverses diphenylborinate complexes. Low methanol content aids elution, maximizes selectivity and allows direct injection. The "elution" well plate was placed

into the autosampler. This automated process takes less than 15 min to complete. **Results:** Calibrations from 0.1/0.5-1000 ng/mL resulted in average coefficients of determination ($R^2$) values over 0.998 for all analytes. The limits of detection (LOD) and quantitation (LOQ) were calculated using the average slope and y-intercept standard deviations which resulted in LODs below 0.25 ng/mL and LOQs below 0.7 ng/mL. The method accuracy was determined via quantitation of two levels of quality control samples and each average analyte concentration fell within manufacturer's expected concentration ranges. The average within-run precision was highest at 6% CV for level 1 epinephrine, and between-run precision was highest at 7% CV for level 2 metanephrine. Matrix effects were low with a range of ion suppression from 1-14% except for norepinephrine with ion suppression at 39%. Extraction efficiencies were higher than 96% for all analytes except dopamine, which resulted in 81% efficiency. **Conclusion:** The method reported herein achieves accurate, sensitive analysis of free catecholamines and metanephrines in urine. This method is an excellent alternative to previously published methods, with advantages of ease of implementation, robustness, high sensitivity, and high throughput with 96 samples extracted in less than 15 min.

## A-222

### Low Serum Alkaline Phosphatase Activity in a Teenage Girl

Z. Shajani-Yi, S. J. Casella, M. A. Cervinski. *Dartmouth-Hitchcock Medical Center, Lebanon, NH*

**Background:** Hypophosphatasia (HPP) is a rare and genetic disorder that affects bone mineralization. There are currently six forms of HPP that range in age of onset and severity: perinatal (lethal), perinatal benign, infantile, childhood, adult and odontohypophosphatasia. Perinatal HPP is the most severe and results in death in utero, whereas adult HPP is milder and presents with pain and osteomalacia. HPP is caused by mutations of the *ALPL* gene encoding tissue-non-specific alkaline phosphatase. One of the cardinal features of HPP is a low serum alkaline phosphatase (ALP) activity. Recognition of HPP and differentiation from other causes of ALP activity is required for proper diagnosis and treatment. Here we present a clinical case of a 17-year-old girl who presented with fatigue at her annual medical exam. Results from an external laboratory showed low daytime cortisol concentration. She was referred to endocrinology for chronic fatigue and possible adrenal insufficiency per her father's request. Family history was notable for hypothyroidism and chronic fatigue syndrome. Her medical history was notable for the presence of anti-TPO antibodies below the diagnostic threshold and a normal TSH. Of note she was taking folate, vitamin B12, and multi-vitamins. During her initial workup laboratory results were notable for low ALP activity (24 units/L; reference interval 55-140 unit/L). Low ALP activity can be due to Wilson's disease, hypophosphatasia, pernicious anemia and severe hypothyroidism. Magnesium and zinc deficiencies were once sources of falsely low ALP measurements but current assay formulations incorporate magnesium and zinc to circumvent this issue.

**Methods: :** In order to differentiate the cause of her low serum ALP activity, new samples were collected and ALP activity repeated. In addition ALP-isoenzyme analysis, sequencing of the ATP7B gene, ceruloplasmin, vitamin B6 (P5P) and urine phosphoethanolamine quantification were also performed.

**Results:** The repeat analysis of the patient's serum ALP activity was 23 units/L. ALP-isoenzyme analysis was not possible due to insufficient ALP activity. Initial results showed elevated concentration of vitamin B12 and normal thyroid function, eliminating pernicious anemia and hypothyroidism from the differential. The patient's ceruloplasmin concentration was quantified as 16.5 mg/dL (reference interval 16-45 mg/dL) prompting further evaluation for Wilson's disease via sequencing of the *ATP7B* gene which did not reveal any deleterious mutations. Vitamin B6 and PEA concentrations were 95 µg/L; 5-50 µg/L) and (80 mmol/mg Cr; <88 mmol/mg Cr), respectively, values consistent with hypophosphatasia.

**Conclusion:** Due to the absence of bone abnormalities and impaired growth, HPP was initially considered unlikely; however, the combination of low ALP activity and high vitamin B6 and PEA are consistent with a diagnosis of hypophosphatasia. Her clinical features suggest a mild form of childhood or adult HPP. This case was complicated by ceruloplasmin concentration at the lower limit of the reference interval. The diagnosis was also complicated by a later admission that the patient was receiving cortisol from her father in order to treat her fatigue. The family was advised to stop giving exogenous cortisol and the patient successfully tapered off without any signs of an acute adrenal crisis. The family declined genetic testing of the *ALPL* gene.

## A-223

### Prolactin heterogeneity in inferior petrosal sinus samples

K. N. Nguyen[1], R. H. Langevin[1], I. A. Hashim[2]. *[1]University of Texas Southwestern Medical Center, Dallas, TX, [2]University of Texas Southwestern Medical Center and Parkland Memorial Hospital, Dallas, TX*

Background:

Measuring prolactin levels in inferior petrosal samples may aid in differentiating between pituitary and ectopic ACTH dependent Cushing's. However, circulating peripheral prolactin is known to exhibit molecular heterogeneity but its presence in inferior petrosal (IP) blood is unknown. This project examined the presence of macroprolactin as well as glycosylated forms in inferior petrosal blood draining the pituitary.

Methods:

Twenty one matched samples from peripheral blood, left inferior petrosal, and right inferior petrosal veins were obtained from seven patients being investigated for ACTH-dependent Cushing's.

Prolactin heterogeneity was examined as follows; the presence of macroprolactin variant was investigated using polyethylene glycol (PEG) precipitation (following incubation of 100 ul sample for 20 minutes at room temperature with an equal volume of 25% (v/v) PEG and centrifugation at 1400 xg for 5 minuets), and by immunoadsorption using protein-G suspension (Thermo Scientific, MA, USA). 100 ul sample was incubated with 50 ul Protein-G suspension with agitation for 60 minutes at room temperature before separation on a magnetic rack and elution using 0.1M glycine buffer (pH 2.0) and adjusting pH to 7.4. The presence of glycosylated variants was examined using Concanavalin-A lectin columns (GE Healthcare, USA). 100 ul sample was applied to 1 mL column and bound prolactin was eluted using 0.5M methyl-alpha-D-glucopyranoside. Prolactin levels prior to and following the above treatment protocols were measured using ELISA (Calbiotech, CA, USA) according to manufacturer's instructions.

Results:

Peripheral blood prolactin ranged from 7.9 to 83.5 ng/mL, while left IP sample prolactin levels ranged from 24.1 to 189.0 ng/mL, and right IP sample prolactin levels were from 87.3 to 524.1 ng/mL. None of the samples exhibited macroprolactin, as percentage PEG precipitated prolactin was <40% for all. Similarly, none of the samples showed immunoglobulin bound prolactin evident by all percentage protein-G bound prolactin of <2.6%. However significant glycosylated prolactin variant was present. Percentage of glycosylated prolactin variant ranged from 11.2 to 45.3%.

Conclusion:

The presence of molecular variants in petrosal sinus blood was not previously known. This study showed that although macroprolactin variant was not present in neither petrosal nor peripheral blood from patient being investigated for ACTH dependent Cushing's, a significant proportion of the circulating prolactin was glycosylated. There was no significant difference in the proportion of glycosylated prolactin between peripheral, left or right IP veins. The pathogenesis as well as the impact of prolactin glycosylation on its diagnostic utility needs to be investigated.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Factors Affecting Test Results

## A-224

### Assessment of pipette validation skills and knowledge among selected Clinical Laboratories in Ashanti Region, Ghana

S. A. Sakyi[1], E. O. Anto[2], E. F. Laing[1], F. Basoa[1]. *[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Edith Cowan University, Joondalup, Australia*

**Background:** Pipettes, like all analytical devices can contribute to sources of error during analytical process. Yet, pipettes as sources of error are usually overlooked and factors that impact on their performance are taken for granted. Defective pipette renders a liability to laboratory results. This study seek to validate pipettes and assess knowledge and skills of pipette users among selected laboratories.

**Methods:** Pipette validation and performance were assessed by the gravimetric method. To assess the correct usage of pipettes, each participant delivered 5 µL of sample ten times with a calibrated and functioning 20 µL pipette. Knowledge on pipette validation was assessed by structured questionnaires. Graph Pad Prism was used for data analysis

**Results:** Out of the 30 pipettes validated, 53.3% (16/30) were defective, and 75% (12/16) were silent pipette failures. Laboratories without pipette holders has 1.7 times increase folds of having defective pipettes and those that did not observe periodic pipette cleaning has1.8 increase folds of having defective pipettes. Laboratories that always used pipettes significantly stood 2.7 times increase fold of having defective pipettes. 70% (31/40) of participants obtained formal training on pipette usage, however, 79.5% (32/40) had never validated their pipettes, even though 86.8% (35/40) strongly agreed that pipettes must be validated.

**Conclusion:** Silent defective pipettes existed in most laboratories and remains a liability to volume dependent assays. Furthermore, pipette validation process is not adequately emphasized in most training and quality assurance measures. Pipetting skills and validation procedures should be consciously factored in equipment validation programs.

## A-225

### Correlation Study of Poly Ethylene Glycol PretreatedProlactin Results in Electrochemiluminescence and Chemiluminescence ImmunoassaySystems

S. Pal. *R B Diagnostic Private Limited, Kolkata,West Bengal, India*

**Aims and objectives**

The falsely elevation of Prolactin results due to the presence of macro prolactin in human sample is established. Poly ethylene glycol (PEG) pretreatment has been accepted as reliable, cost effective and user friendly method. The standard dictum is to calculate the recovery and investigate the transferability of the expected values. The comparative study and reference interval measurement in different instruments after PEG pretreatment have also been done but could not be accepted commercially as the range is very small and affinity of macro prolactin is dependent on the epitopes of the antibody resulting variation in binding capacity. The author obtained correlation coefficient after studying results of120 patients with a varying range from 0.238-240ng/ml Prolactin before and after PEG pretreatment. The author tested proficiency testing samples and internal control samples in similar manner. The PEG untreated results were calculated by the correlation coefficient and found out to be comparable with PEG treated results, results of other immunoassay systems and proficiency testing samples.

**Materials and methods**

**Samples**

120 samples for prolactin measurement randomly chosen within the data range 0.238-240ng/ml.

Samples are untreated with PEG.

**Method:**

Prolactin estimation were done in 120 samples by Electrochemiluminescence immunoassay (ECLIA). Then the samples were pretreated using 25% solution of PEG and retested by ECLIA. The proficiency samples and internal quality control samples were tested in the similar way. Results were noted and compared.

**Calculations:**

The patient samples, IQAS &EQAS are being treated with equal volume 25% PEG solution. Hence results should be multiplied by 2.Correlation coefficient was compared between two series of patient samples. After obtaining correlation coefficient 25 samples of varying range were sent in different laboratories and results were compared and regression coefficient calculated. The calculations of untreated samples using correlation coefficient were also done. Regression coefficient of PEG pretreated, and PEG untreated but multiplied by correlation coefficient has also been calculated.

**Results and Discussion**

The correlation coefficient was found out to be very effective. The PEG treated results and untreated samples calculated using correlation coefficient were in accordance and regression coefficient of two series was 0.996. Comparison of EQAS results of different instruments showed results of Cobas e 411 are always high and the correlation coefficient(y=0.722x) worked effectively. 25 patient samples of varying ranges were chosen and sent to 4 laboratories with different immunoassay systems (Advia Centaur, Abbott Architect, Access and Immulite 1000) . The results correlated with PEG treated results. Regression coefficients were within 0.994-0.996.

**Conclusion:**

1. Application of correlation coefficient made the prolactin results comparable with other instruments and methods.

2. Such study was essential as biological reference interval of all four systems are similar creating confusion for patients with elevated values.

3. The consistency in correlation calculation have been observed from 0.238-240ng/ml proving PEG pretreatment a mandatory process for all samples.

## A-226

### Glucose-6-Phosphate Dehydrogenase Activity Deficiency - A Critical Indicator for Appropriate Interpretation of 'Normal' Haemoglobin A1c Result

V. Lo. *Hong Kong Sanatorium & Hospital, Apleichau, Hong Kong*

**Backgrounds:** Clinical decision limit of hemoglobin A1c (HbA1c) level below 6.5% (48 mmol/mol) as good glycemic control, adequate treatment and non-diabetes mellitus (DM) is valid only in patients with normal erythrocyte life span. Any condition reducing erythrocyte survival might associate with falsely low HbA1c level. Glucose-6-Phosphate Dehydrogenase (G6PD) protects red cells against oxidative damage and subjects with G6PD deficiency are prone to intravascular hemolysis when the red cells are exposed to oxidative stress thus shortened the life expectancy. Reports of G6PD deficiency that resulted in lowering of percentage glycosylated haemoglobin and HbA1c level were not only scanty, but also not investigated extensively.

**Methods:** Our laboratory determined HbA1c level by BioRad Variant II Turbo analyzer with a stringent in-house retention time system for correct HbA1c and HbAo peaks identification. Since April 2010 our laboratory adopted an HbA1c reporting algorithm with regard to in-house established reference range 4.9-6.5% (NGSP), 30-48 mmol/mol (IFCC), which was set-up by recruiting 8028 normal subjects of normal fasting blood glucose, 3607 female and 4421 male, age ranged from 4 to 104 years. Cases with unusually low HbA1c result, lower than 4.9% (NGSP), lower than 30 mmol/mol (IFCC), but incompatibly elevated fasting or random blood glucose were further investigated. After verifying transfusion history, eliminating inadequate fasting and haemoglobinopathy, glucose-6-phosphate dehydrogenase (G6PD) activity was determined by Trinity Biotech in Cobas c501, Roche Diagnostic.

**Results:** Among 61643 HbA1c requests from January 2012 to October 2016, we identified 195 G6PD deficiency patients, accounting for 505 (0.8%) requests, who presented with spuriously normal results of HbA1c of lower than 6.5% (lower than 48 mmol/mol) and estimated average glucose (eAG) of lower than 7.8 mmol/L, lower than 104.4 mg/dL but discordant elevation of fasting blood glucose level of higher than 5.6 mmol/L. Thirty nine patients had multiple visits, from twice to sixteen, and showed consistent observations. HbA1c and eAG ranged from 3.3 to 6.5% (NGSP), 13 to 48 mmol/mol (IFCC)), and 2.7 to 7.8 mmol/L, 48.6 to 140.4 mg/dL, respectively. Corresponding fasting blood glucose level ranged from 5.7 to 14.0 mmol/L, 102.6 to 252.0 mg/dL.

**Conclusion:** Patient with G6PD activity deficiency due to shortened erythrocytes life span and rapid red cell turnover might have 'normal' HbA1c level but not indicating

good glycaemic condition due to inefficient and lowered degree of glycation. Laboratory should establish an effective algorithm to avoid reporting of spuriously low or normal HbA1c which was discordant with persistent elevation of fasting blood glucose level. To arouse awareness of requesting doctor to clinical relevance of the HbA1c result, upon verification of discrepancy, laboratory should supplement the HbA1c result with interpretative comments, such as in our laboratory *[The HbA1c level is likely spuriously lowered by the G6PD deficiency status of this patient. Please correlate the result clinically.]*

## A-227

### Ionizing radiation alters membrane integrity in human erythrocytes

S. K. Das. *College of Medicine & JNM Hospital, WBUHS, Kalyani, Nadia, India*

BACKGROUND: Ionizing radiation causes damage to the biomolecules in living cells through oxidative stress by the excessive generation of reactive oxygen species (ROS) from radiolysis of body water. Blood and its components including the cells are exposed to a significant dose of radiation during irradiation. In this study we investigated the biochemical and morphological alteration in red blood cells due to gamma radiation.

METHODS: Fresh blood samples were aseptically collected in the heparinized or EDTA-containing tubes from the healthy male donors (22-30 years). The study was approved by the Institutional Ethics Committee following the ICMR guideline. The samples were irradiated at a dose of 4 Gy in a 60Co γ-radiation unit with a dose rate of 3.05 kGy/ h, incubated for 1 h at 25°C and then transported to the laboratory on ice for experiments.

RESULTS: γ-irradiation for one hour did not appear to change the hematological parameters. However, γ-radiation exposure elevated the levels of thiobarbituric acid-reactive substances (TBARS) in the RBC membrane ghosts, higher levels of $K^+$ ion, and reduced level membrane ATPase activity as compared to the normal blood. The average osmotic fragility ($H_{50}$) and the maximum rate of hemolysis $(dH/dC)_{max}$ increased after the γ-irradiation. The confocal microscopic and atomic force microscopic (AFM) studies confirmed that irradiation induced the transformation of RBC from biconcave cells to echinocytes, increased their surface roughness (170±8 nm in the IR group compared to 81±6 nm in the control group) and decreased the vertical distance (2005±79 nm in the IR group compared to 776±53 nm in the control group).

CONCLUSION: Our results suggested that γ-irradiation-induced oxidative stress and alteration in enzyme activities leading to the structural alteration in the erythrocytes membrane *in vitro*, but did not show any immediate effect on cell count. Membrane integrity of circulating red blood cells (RBCs) is compromised by the deleterious actions of γ-radiation at a dose of 4 Gy in human erythrocytes *in vitro*.

## A-228

### Implementation of Six Sigma metrics for the assessment and process improvement of the quality control program in 22 laboratories from the Secretary of Health in the State of Jalisco, Mexico

M. Lopez-Rodriguez[1], L. M. Brennan-Bourdon[1], G. Cazarez-Navarro[2], B. A. Iñiguez-Carvajal[2], M. G. de Loera[1], A. Silva-Barragán[1], B. Macias-Guzmán[1], D. Y. Gonzalez-Martinez[3], J. Ortega-Martinez[3], S. Builes-Morales[4]. *[1]Laboratorio Estatal de Salud Pública, Zapopan, Mexico, [2]Caisame Estancia Prolongada, Tlajomulco de Zuñiga, Mexico, [3]Distribuidora Quimica y Hospitalaria GAP SA de CV, Zapopan, Mexico, [4]Quality Consulting, Medellín, Colombia*

**Background**: Six Sigma methodology is used to evaluate laboratory performance and improve assay quality by identifying inaccurate and/or imprecise assays in any process in a clinical laboratory. Sigma is a metric that measures the performance of a process as a rate of Defects-Per-Million opportunities. As a pioneer project in Mexico, particularly of the Secretary of Health in the State of Jalisco, our aim was to implement Six Sigma metric analysis as a first phase for the assessment and continuous improvement of the quality control program in laboratories belonging to the Laboratory Network of our state, in order to reduce the number of errors in laboratory results.

**Methods:** From May to December 2016, a total of 22 laboratories from regional and community hospitals as well as health centers were included in our study. In order to evaluate the total allowable errors in the internal quality control program of our laboratory system by sigma metric analysis, we included the performance of clinical chemistry analytes. Sigma metric was calculated for 23 analytes: glucose, total cholesterol, HDL-High Density Lipoprotein, ALP-Alkaline Phosphatase triglyceride, ALB-Albumin, DB-Direct Bilirubin, TB-Total Bilirubin, UA-Uric Acid, ALT-Alanine Aminotransferase, AMY-Amylase, AST-Aspartate Aminotransferase, Lipase, electrolytes: Ca-Calcium, Mg-Magnesium, P-Phosphorus, K-Potassium, Na-Sodium, Cl-Chlorine TP-Total Protein, urea and creatinine, at two control levels. Sigma metrics of each analyte was calculated using the formula [Sigma metric = (TEa-bias)/ CV-Coefficient of Variation]. We used the minimum specifications for total allowable error (TEa), imprecision and bias, based on the 2014 minimum specifications from the Biological Variation Database updated and compiled by Dr. Carmen Ricos and colleagues. The performance values for sigma (σ) were considered at three levels: σ >4: good to excellent, σ from 2-3.99: poor to marginal, and σ <2: unacceptable.

**Results:** A total of 3897 clinical chemistry tests were performed at both control levels (L1: Normal and L2: Abnormal). The σ value with the highest incidence was good to excellent (σ >4) for the analytes: Triglycerides, AST, DB, ALT, Urea, TB, and UA. The sigma value considered as unacceptable (σ<2) was for Na, Ca, Cl, ALB, TP, and Mg. For the rest of the analytes, poor to marginal σ values (2-3.99) were obtained.

**Conclusion** We conclude that analytes with a sigma value <2 require strict monitoring and adjustment of the quality control procedures. Sigma metric analysis provided a cumulative evaluation of the analytical process in clinical chemistry. We established acceptance/rejection criteria in laboratory results, as well as the implementation of standardized processes in clinical chemistry testing within the 22 laboratories of the Laboratory Network in the State of Jalisco. Furthermore, a collaborative and conscious environment was created among laboratory personnel and directors towards the desired goal of quality control.

## A-229

### National efforts to Improve Laboratory Quality and Safety in Clinical and Public Health Laboratories

C. Fitzgerald, N. L. Anderson, N. Cornish, J. Ridderhof, B. Chen, J. Chaitram, S. Howard, D. B. Lake, R. M. Salerno. *CDC, Atlanta, GA*

**Background:** Clinical laboratory testing is performed nationwide in more than 250,000 laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) regulations, ranging from simple tests performed at point-of-care sites to highly complex procedures in reference laboratories. The increasing diversity and importance of laboratory services in patient care and health management warrant national standards, guidance, and training to strengthen laboratory quality and safety. The mission of CDC's Division of Laboratory Systems (DLS) is to strengthen the nation's clinical and public health laboratory system by continually improving quality and safety, data and information science, and workforce competency. **Methods**: DLS accomplishes its mission through multidisciplinary collaborations and engagement with diverse partners and stakeholders, including professional societies, accrediting organizations, proficiency testing programs, healthcare systems, and government agencies at federal and state levels. Examples include managing the Clinical Laboratory Improvement Advisory Committee (CLIAC) in partnership with CMS and FDA; collaborating with ASM, National Guideline Clearing House (NGC), and the American Society for Clinical Laboratory Science (ASCLS) as part of our Laboratory Medicine Best Practices (LMBP) Initiative to address quality improvement practices of interest to the clinical laboratory community; and partnering with the Association of Public Health Laboratories to improve quality, safety, and workforce of public health laboratories nationwide. The division's laboratory training website provides easily accessible learning resources. Through CDC TRAIN, laboratory professionals can register for live and on-demand courses, create learning plans, obtain continuing education credits, and sign up for notifications of new courses. **Results:** Since 2015, DLS has published guidelines on diverse topic areas, including developing an individualized quality control plan, quality practices in next generation sequencing, laboratory professional competencies, and an Informatics Self-Assessment Tool. Ten formal CLIAC recommendations have been submitted to HHS that address interoperability of laboratory information, laboratory safety, integration of laboratory medicine into health care, and non-invasive prenatal testing. Seven LMBP systematic reviews have been published including reviews on effectiveness of practices to reduce blood sample hemolysis in EDs, effectiveness of automated notification and customer service call centers for timely and accurate reporting of critical values and Decision Point Cardiac Troponin (cTn) Threshold Selection, assay selection, serial testing, and Point of Care Testing. In 2016, DLS distributed free tools to 3,798 laboratories that help assure the quality of waived testing and test results, and disseminated an educational booklet to 3,063 laboratories on recommended practices for provider-performed microscopy procedures. DLS' laboratory training website has >300 course offerings with >18,900 registrants in FY16; 92% indicated the training objectives aligned with their training needs. **Conclusion:** DLS and partners have developed

practice guidelines and trainings for a national audience. This work has a substantial positive impact on the laboratory community. In 2017, among other activities, DLS will publicly release many new CDC biosafety courses, and expand its engagement on laboratory biosafety. Through continued collaboration and active engagement with the laboratory community, DLS can collectively strive for exemplary laboratory science and practice across clinical care and population health.

## A-230

### Adjusted calcium: Local application of observations from Big Data

E. Dewar, D. Robertson, I. Rothnie, H. Regan, K. Booth, M. Lum, F. Brandie, W. Simpson, B. Croal. *NHS Grampian, Aberdeen, United Kingdom*

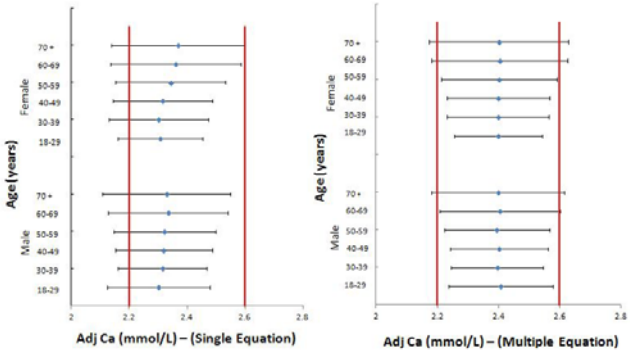
**Background:** Large variation currently exists across pathology services as to the method and choice of equation for adjusted calcium calculations. Recent observations from big data (NHS England) have demonstrated clear age and sex related differences in serum albumin levels which would have significance for choice of adjusted calcium equation in the population sub groups.

**Objective:** To use guidance on adjusted calcium equation generation along with knowledge from big data to construct a multi-equation approach to this problem.

**Methods:** Laboratory data was collected over a four month period (April –August 2015). Primary care results with a measured serum albumin and calcium were pulled using the following exclusion criteria (where available) of age (<18 years), calcium (<2.0 or >2.7 mmol/L), albumin (<20 or >50 g/L), creatinine (>200 µmol/L), urea (>15 mmol/L), ALT (>55 U/L), vitamin D (<25 nmol/L) and PTH (<1.7 or >9 pmol/L) – in line with national UK guidance. Results, divided into gender and 10-year age categories (from 18 to 70 years), were used to derive suitable adjusted calcium equations for each group. A comparison of the distribution of adjusted calcium using this multi-equation approach was made with the existing single adjusted calcium equation technique.

**Results:** The existing single adjusted calcium equation technique demonstrated significant misalignment with the target reference interval of 2.2 - 2.6 mmol/L (8.8-10.4 mg/dL) – with over diagnosis of hypocalcaemia being a particular concern (Figure). The multi-equation approach using 12 distinct sex/age related equations allowed much better alignment with all 95% C.I. distributions fitting within the target reference range.

**Conclusions:** The multi-equation approach for adjusted calcium calculation more closely aligns to targeted reference intervals and minimises inappropriate classification of calcium status. This strategy should replace the current single equation approach in order to better optimise direct and consequential costs to patient care and healthcare finances.




## A-231

### The Effects of Hydrocodone Rescheduling on Laboratory Urine Drug Testing

J. Akin. *University of California San Diego Health, San Diego, CA*

**Background:** Hydrocodone is one of the cornerstone medications of abuse in the current opiate epidemic in the United States. On October 26th, 2014 the DEA rescheduled hydrocodone containing products from schedule III to schedule II in hopes of deterring abuse and aberrant use. This rescheduling event has effectively decreased the number patients prescribed hydrocodone, but little is known about the impact on laboratory urine drug testing. We hypothesize that the overall volume and frequency of urine drug testing will have increased since the rescheduling of hydrocodone containing products. The positivity rate of hydrocodone should be significantly decreased, as well as the mean urine hydrocodone concentration since fewer people will be prescribed the drug. In addition, we speculate that the positivity rate of schedule II alternatives will have increased since the rescheduling event.

**Methods:** Laboratory urine drug results from 18 months prior (pre) and 18 months after (post) the rescheduling event were compared for statistical change. A total of 253,773 laboratory results were extracted from UC San Diego Health. Positive and negative results for thirty-five separate urine drug screen and confirmation tests were compared.

**Results:** There was an increase of 6.8% in the total number of tests, and the mean number of tests per patient increased 7.1% from 1.26 to 1.35. The mean number of tests order per provider increased by 38% from 14.3 to 19.8. The positivity rate for opiates, as a class, was decreased by -0.09% (X2=4.12, p=0.042), as were benzodiazepines at -1.1% (X2=7.62, p=0.006). Codeine increased significantly by 1.3% (X2= 3.99, p=0.046), as well as alpha-hydroxyalprazolam (5.4%, X2=12.1, p=0.00) and temazepam (4%, X2=6.12, p=0.013). Cannabinoids increased slightly by 1.7% (X2=14.1, p=0.000). Hydrocodone (-2.9%, X2=7.85, p=0.005) and hydromorphone (-4.0%, X2=11.8, p=0.001) were the only opiates that saw a decrease. The mean concentration of hydrocodone increased by 249 ng/mL (21.9%, p=0.000, CI 100.2, 407.3).

**Conclusion:** The results suggest a significant difference in the number and frequency of laboratory urine drug testing after the rescheduling event. As hypothesized, the positivity rate of hydrocodone significantly decreased after the hydrocodone rescheduling event. In contrast, the positivity rate of select schedule II alternatives, such as marijuana, select benzodiazepines and codeine increased after rescheduling. A decrease in mean hydrocodone urine concentrations was not observed as hypothesized, as the mean concentration increased after the rescheduling. Although we cannot fully account for all bias in this study, the data presented suggests that the rescheduling of hydrocodone from schedule III to schedule II significantly impacted laboratory urine drug testing results.

## A-232

### Development of a Liquid Ready to Use Multi-Analyte Human Based Linearity Verification Material Covering Five Levels of Analytes Assessed in Clinical Chemistry Tests

W. Wong, A. Shanks, M. L. Rodriguez, L. Adams, J. Campbell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background**

Linearity testing assesses the system's calibration to verify its validity and if a method is linear across the full reportable range of the instrument. Calibration verification involves assaying materials of known concentrations in the same manner as patient samples to substantiate the instrument or test system's calibration throughout the reportable range for patient test results. According to Clinical Laboratory Improvement Amendments, a laboratory should perform and document calibration verification procedures at least once every 6 months, and/or after instrument validation/maintenance, change of reagent lot or after observing an unusual shift in QC performance. This study reports a multi-analyte human-based verification material covering five levels of the analytes most commonly tested in clinical chemistry to challenge the reportable range thus ensuring accurate patient testing.

**Methods**

Human based multi-analyte linearity verification material covering five levels of analytes assessed in clinical chemistry tests (albumin, BUN, calcium, chloride, creatinine, glucose, iron, lactate, lithium, magnesium, phosphorus, potassium, sodium, total protein, triglycerides) was manufactured. Values were assigned on the Roche Cobas C501 system. Open vial stability was determined as the percentage recovery of each level stored at +2$^0$C to +8$^0$C related to a vial of the same material opened at day 7. Freeze thaw stability was determined as a percentage recovery of each level stored at +2$^0$C to +8$^0$C related to a vial of the same material frozen at -20°C and thawed for 3 cycles.

**Results**

Concentration ranges, open vial and freeze thaw stability values are shown in the table below:

| Analyte | Concentration ranges | | Open vial stability at day 7: recovery (%) | | | | | Freeze thaw stability: recovery (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | Level1 | Level5 | Level1 | Level2 | Level3 | Level4 | Level5 | Level1 | Level5 |
| Albumin (g/L) BUN (mmol/L) | 2.8 – 5.2 0.8-1.2 | 46-56 30.5-37.5 | 104.37 100 | 98.59 100.40 | 99.47 100.64 | 99.47 101.09 | 100.38 99.94 | 99.17 100.00 | 99.59 101.13 |
| Calcium (mmol/L) Chloride (mmol/L) | 0.4-0.8 65-75 | 3.8-4.6 121-135 | ≤0.125± mmol/L from target value for all levels 100.65 99.94 99.77 100.33 100.04 | | | | | ≤0.125± mmol/L from target value 99.93 100.40 | |
| Creatinine (µmol/L) Glucose (mmol/L) | 20-30 0.18-0.42 | 1820-2080 32-39 | 100.00 96.55 | 100.23 100.02 | 101.03 98.44 | 100.46 99.26 | 99.27 98.88 | 98.48 96.15 | 99.57 99.94 |
| Iron (µmol/L) Lactate (mmol/L) | 1.4-3.6 0.35-0.75 | 169-177 13.2-14.8 | 97.65 101.35 | 101.84 99.50 | 101.52 99.13 | 100.35 100.24 | 98.99 99.25 | 99.60 99.77 | 100.68 100.64 |
| Lithium (mmol/L) Magnesium (mmol/L) | 0.1-0.2 0.25-0.55 | 2.4-2.8 1.5-1.9 | 99.62 101.16 | 97.37 100.00 | 99.45 99.48 | 98.84 100.61 | 100.98 100.09 | 101.98 104.09 | 100.16 101.22 |
| Phosphorus (mmol/L) Potassium (mmol/L) | 0.25-0.55 1.9-2.7 | 5.3-5.9 8.8-9.2 | 95.58 98.08 98.19 99.45 98.19 ≤0.25± mmol/L from target value for all levels | | | | | 101.44 101.26 ≤0.25± mmol/L from target value | |
| Sodium (mmol/L) Total protein (g/L) | 86-94 5-11 | 161-175 100-115 | ≤2± mmol/L from target value for all levels 99.26 99.68 99.43 100.30 100.29 | | | | | ≤2± mmol/L from target value 103.45 100.19 | |
| Triglycerides (mmol/L) | 0.25-0.55 | 8.9-9.5 | 99.21 | 99.71 | 99.97 | 99.67 | 99.71 | 101.54 | 100.22 |

**Conclusion**

The developed linearity verification material covers the reportable range of the analyser. The material is stable for 7 days (at +2$^0$C-+8$^0$C) and presents a freeze thaw stability of up to 3 cycles. This material has the potential to be used with other systems to ensure accurate sample assessment in clinical chemistry tests.

## A-233

### Effect of Bilirubin Interference on Hemoglobin Quantitation using the NanoDrop ND-1000 and the Roche Cobas Indices

S. Miller, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

**Background**: Evaluation of hemoglobin and bilirubin concentrations in serum specimens is an important pre-analytical step to identify potential sources of error in clinical laboratory measurements. For ELISA based immunoassays, our laboratory performs determination of hemoglobin and bilirubin concentrations using the NanoDrop ND-1000 spectrophotometer. Neuron Specific Enolase (NSE) and 14-3-3 are two immunoassays where hemoglobin concentrations as low as 20 mg/dL could lead to inaccurate results. For these assays, we have observed that in some samples bilirubin interferes with the NanoDrop measurement of hemoglobin, resulting in falsely decreased hemoglobin concentrations. The objective of this study was to evaluate the effect of bilirubin on hemoglobin quantitation using the NanoDrop spectrophotometer. The Roche Cobas serum indices were evaluated as an alternate method for hemoglobin quantitation in samples with an elevated bilirubin**.**

**Methods:** Correlation between the NanoDrop spectrophotometer and the Roche Cobas indices measured on a c501 instrument was determined using serum samples (n=95) with hemoglobin concentrations between 1.3-273 mg/dL. For bilirubin interference studies, serum pools with various concentrations of hemoglobin (3.5-200 mg/dL) were spiked with increasing concentration of bilirubin (0-15 mg/dL). Samples were measured on the NanoDrop spectrophotometer and the Roche Cobas c501serum indices. A change of 20% between the nonspiked and bilirubin spiked sample was considered a significant change.

**Results:** For hemoglobin concentrations >20mg/dL, the Nanodrop and Roche Cobas methods showed an excellent agreement with a Spearman correlation coefficient of 0.971, slope of 1.02 and intercept of -5.41 by Passing-Bablok regression fit. When the hemoglobin concentration was <20 mg/dL, the Spearman correlation coefficient was 0.699, slope of 0.95 and intercept of -1.49. Hemoglobin quantitation using the NanoDrop spectrophotometer was susceptible to bilirubin interference. At 20 mg/dL hemoglobin, a decrease of 30%, 42% and 55% from the unspiked sample was observed at bilirubin concentrations of 2.5, 3.75 and 5 mg/dL, respectively. A similar bilirubin dose dependent interference effect was observed for hemoglobin concentrations of 3.5 and 10 mg/dL. At 60 and 200 mg/dL hemoglobin, bilirubin concentrations up to 5 and 15 mg/dL, respectively, did not affect hemoglobin quantitation in the NanoDrop. The Roche Cobas hemolysis index was unaffected by up to 15 mg/dL of bilirubin at hemoglobin concentration of 20, 60 and 200 mg/dL.

**Conclusions:** Quantitation of low concentrations of hemoglobin (<20mg/dL) using the NanoDrop spectrometer is significantly affected by bilirubin concentrations as low as 0.5 mg/dL resulting in falsely low hemoglobin concentrations. The use of the Roche Cobas H-index is a good alternative method in these situations since this methodology is not susceptible to bilirubin interference.

## A-234

### Analyzer Maintenance Affects the Observable Sigma Metrics of Clinical Chemistry Analytes

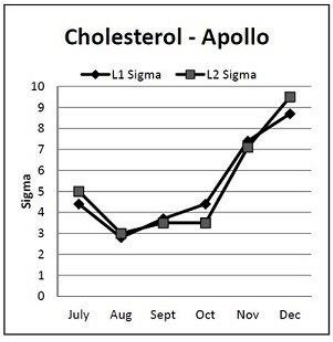
J. A. Montgomery, W. C. Miller, J. F. Licata. *Asante Rogue Regional Medical Center, Medford, OR*

**Background:** To calculate sigma metrics on paired Beckman Coulter AU5812 analyzers and evaluate the efficacy of measures to improve 3 sigma and 4 sigma analytes.

**Methods:** 22 CLIA-regulated analytes were examined across both platforms with sigma values calculated at two levels of concentration. CLIA total error allowable (TEa) limits and Unassayed Chemistry Control QAP (Bio-Rad--over four months) were used to calculate the initial sigma as: Sigma = (TEa%-Bias%)/CV%. Performance metrics followed a "Plan-Do-Study-Act" model.

**Results:** One AU5812 (nicknamed Zeus, maintained by day shift) had 35 of 44 analyte/levels at 5 sigma or better. The alternate AU5812 (nicknamed Apollo, maintained by night shift) had 36 of 44 analyte/levels at 5 sigma or better. Studying the performance and differences between the two instruments for 3 sigma and 4 sigma analytes, it was noted that for cholesterol (both levels), Zeus had a lower CV% (i.e., 2.0, 1.8) resulting in higher sigma values (i.e., 4.3, 5.1) than were observed on Apollo (i.e., CV% 2.7, 2.8; sigma 3.5, 3.5). Additionally, Zeus Level 2 QC for calcium had less variation and a higher sigma (i.e., CV% 1.4; sigma 5.5) than Apollo (i.e., CV% 1.7; sigma 4.5). Following the "Plan-Do-Study-Act "model, these observations inspired a "Plan" to increase the frequency and monitoring of Apollo's sample probes, syringe replacement and instrument maintenance. The plan was implemented ("Do") and a "Study" of the next two months' QAP data showed significant improvement in Apollo's CV% and sigma values for both cholesterol and calcium, raising the sigma to above 6 for two consecutive months. Following the study, the "Act" is to continue monitoring the system.

**Conclusion:** An assay's sigma is greatly influenced by CV% and can be improved with increased maintenance and observation. Changes in instrument performance over time indicate sigma values are not static and should be monitored periodically.




## A-235

### Reporting real gene coverage resolution for clinical diagnostic tests

R. L. M. Guedes[1], M. A. Pereira[1], F. S. V. Malta[1], M. C. M. Freire[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil*

**Background:** Next-generation sequencing (NGS) technology allowed users to analyze thousand to million of DNA sequences in a short time, revolutionizing the field of genomics and medical diagnosis. Build a custom panel targeting specific genes or genomic regions of interest is more cost-effective and useful for studies of disease- or phenotype-related genes when compared to broader approaches (e.g. whole-genome and whole-exome sequencing). It is expected that all genes/regions are completely

covered and it is known that the panel coverage is highly dependent on a wide range of factors such as the sequencing platform, sequence complexity, nucleotide composition (GC content), location of variants, uniqueness of regions and primers specificity, efficiency and interference. **Objective:** Report a simple tool to analyze the actual coverage achieved across the targeted regions in each patient panel report. **Methods:** The tool was developed in PERL scripting language. Samtools was used to count sequencing coverage at base-level resolution for a BAM alignment output file. A BED format file used to design the panel was parsed to extract every gene coordinates and sizes. Human genome annotation GTF files was used to extract all gene isoforms coordinates. **Results:** Two output files are generated: a text file reporting the percentage of bases above the established coverage threshold for each gene and a total percentage for the whole panel; and a BED file reporting the coordinates of all bases below the coverage threshold. The report can be performed for the complete gene or any specified isoform. The tool tooks only 3'40'' minutes to process ~1 million reads from a panel with 40 gene isoforms with ~130k bases using a single processor in a common desktop computer. **Conclusions**: The coverage threshold is an essential parameter to detect variants in any NGS sequencing experiment. Furthermore, if full coverage is not achieved, it is recommended to provide the actual values obtained across the targeted regions for each patient report. We developed a tool to calculate the percentage of bases above a minimum established coverage threshold for each gene/region and for the whole panel. This tool is compatible with all sequencing platforms and can be used to guide primer design for further Sanger sequencing or simply used to incorporate as essential information in the final individual report.

## A-236

### Comparability of two different urine total protein methods in patients with monoclonal proteinuria

C. D. Giesen, J. C. Lieske. *Mayo Clinic, Rochester, MN*

**Background:** Quantitation of urine total protein has long been problematic. No gold standard method exists as many protein species are present in varying amounts in the urine under normal physiological conditions and disease states. All available assays reliably detect albumin but have well-documented limitations for detecting monoclonal and other non-albumin proteins. The present study compared two commercial methods for quantifying urinary total protein in a cohort of patients with and without kidney disease and with and without known monoclonal M-spikes.

**Methods:** Waste urine samples were selected for analysis from among those submitted to the Mayo Clinic Renal Testing Laboratory for random urinalysis (RUA, n=99) and to the Mayo Clinic Protein Immunology Laboratory for monoclonal protein testing (n=61). Urine total protein was measured using pyrogallol red (QuanTtest Red, Redondo Beach CA) and benzethonium chloride (Roche Diagnostics Total Protein Gen. 2, Indianapolis IN) on a Roche Cobas 6000 c501. The pyrogallol red assay was modified by the addition of 30 mg/L sodium dodecyl sulfate (Sigma-Aldrich, St. Louis MO) previously observed to increase detection of monoclonal proteins. The modified pyrogallol red assay, currently used in our laboratory, served as the reference assay for this analysis

**Results:** Among the RUA samples without known monoclonal species there was excellent correlation between the two methodologies by Passing & Bablok analysis (n=99; y=0.99x+0.79; range 0.9-229.9 mg/dL). Overall bias by Bland-Altman analysis was also acceptable (10.8% [3 mg/dL]) but was somewhat higher in samples with a low protein concentration of <10 mg/dL (22.2%) than those in the more clinically relevant ranges (10-30 mg/dL [4.5%]; >30 mg/dL [3.9%]). Among the samples with known M-spikes, the Roche assay yielded higher results than the modified Quantimetrix assay (Passing & Bablok regression y=1.30x+0.32; range 3.5-623.9 mg/dL; Bland-Altman % Bias = 35.1%).

**Conclusion:** Both urine total protein assays gave comparable results in random urine samples obtained from clinic patients with and without kidney disease, a population in which albumin is usually the major protein present in urine. However, quantitation of monoclonal proteins was quite variable between these 2 assays, with the Roche benzethonium chloride assay yielding values approximately 30% greater than the modified pyrogallol red method. These results have implication regarding the sensitivity of total protein assays for detecting and quantifying urine monoclonal proteins. Individual laboratories should take this into account together with their local patient population when choosing a urine total protein assay.

## A-237

### Equivalence of urine albumin to creatinine ratio measurements in 12h overnight urine and first morning urine

L. J. Tolentino[1], C. B. Maluf[1], R. C. Fiqueiredo[2], R. C. P. dos Reis[1], P. G. Vidigal[1]. *[1]School of medicine. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]School of medicine. Universidade Federal de São João Del'Rei, São João Del'Rei, Brazil*

**Background**: Sustained albuminuria is a criteria for diagnosis of chronic kidney disease. Its early identification with adequate management reduces cardiovascular and renal risk, especially in hypertensives and diabetics. Albuminuria measured with timed urine is the gold standard, but its collection is laborious and susceptible to errors. Urine albumin to creatinine ratio (UACR) in random sample is an alternative to the gold standard. The aim of this study was to evaluate the equivalence between albuminuria in 12h urine (overnight) compared to first void urine considering the urinary stasis interval and the collection time. **Methods:** 123 participants collected urine for 12h, beginning in the evening. The urine overnight was stored in bottle 1, and kept in the refrigerator. First morning urine, identified as the urine performed after waking up, was collected in bottle 2. The hour of all urine collections were registered. Samples were analyzed using the Vitros 5600 Ortho Clinical Diagnostics Integrated System (Raritan, New Jersey), at the same analytical run. Equivalence between two samples was evaluated using Lin's, Pearson's and Bland Altman's methods. **Results:** Strong correlation and accordance between tests in samples with a 2 to 4 h stasis interval was found, being stronger if collected between 06:30 and 08:15 a.m (TAB.1). **Conclusion:** Although UACR is recommended for detection of albuminuria, lack of standardization of sample characteristics is a barrier to its reliability. Our results indicate that the best sample is obtained between 06:30 and 08:15 a.m. after a retention interval of 2 to 4 hours which may contribute for better standardization of albuminuria dosages.TABLE 1Equivalence of UACR measurements in 12h overnight and first morning urine

| | N | CORRELATION COEFFICIENT | | BLAND ALTMAN | | |
|---|---|---|---|---|---|---|
| | | Lin | r's Pearson | Mean difference | Standard deviation | Limits of agreement |
| UACR 12 h vs UACRm | 123 | 0,558 | 0,646 | -4,927 | 29,57 | -62,88 |
| UACR 12 h vs UACR 2-4h | 41 | 0.958 | 0.974 | 2.67 | 11.76 | -20.38 |
| UACR 12h vs UACR 6:30 | 26 | 0.994 | 0.997 | 0.48 | 5.16 | -9.631 |

UACR: Urine albumin to creatinine ratio; UACR 12 h: in 12h overnight urine samples; UACRm: in morning urine samples; UACR 2-4h: in samples with stasis time of 2 to 4 h; UACR 6:30: collected between 06:30 and 08:15 am.

## A-238

### Establishing and adjusting the calibration interval based on reagent stability

E. Cho, K. Park, J. Kim, E. Shin, H. Hong, W. Lee, S. Chun, W. Min. *Asan Medical Center, Seoul, Korea, Republic of*

**Background:** The calibration criteria in the guidelines follow the manufacturer's recommended interval, but there are many differences in the volume of test between laboratories, applying the manufacturer's calibration interval uniformly is unreasonable. We tried to verify the calibration interval recommended by the manufacturer by investigating the in-use stability of the reagents in this study. We compared the point of time when the percent change of calibration factors exceeds the acceptance criteria with the manufacturer's recommended calibration interval. **Methods:** Using the equipment of Roche cobas 8000 (Roche Diagnostics, Switzerland), 22 general chemistry items (albumin, ALP, ALT, amylase, AST, BUN, Ca, cholesterol, CK, creatinine, direct bilirubin [DB], glucose, iron, LD, lipase, Mg, γ-GT, phosphorus [P], total bilirubin [TB], triglyceride [TG], total protein [TP], uric acid [UA]) were examined. The allowable range of the calibration factor variation was set as the optimal, desirable, minimum imprecision goal based on the biological variability and the coefficients of variation (CV) of the cumulative internal quality control results. For instruments using calibration only reagents, two lot numbers of reagents were used to evaluate each for 7 days. Only one reagent cassette was used in each test items and reagent was used only for calibration. The average of the calibration factor and the 95% confidence interval (CI) were calculated 12 times a day for each lot number of reagents. In addition, the calibration factors from the

day when the reagent cassette was installed on the instrument to the maximum 7 days were analyzed using the instrument performing high-volume and small-volume tests in the actual laboratory. The time at which the 95% CI of the mean percent change deviates from the acceptance criteria was obtained. **Results:** Among the instrument that use calibration-only reagents, instrument performing high-volume and small-volume tests, there were no differences in the 15 items such as ALT, amylase, BUN, Ca, cholesterol, CK, creatinine, glucose, iron, LD, lipase, Mg, P, TB and UA at the time of exceeding the allowable limit. However, in albumin, ALP, AST, DB, γ-GT, TP and TG, it is observed that the earlier time point exceeding the allowable limit. When applying the optimal goal or the CV, the time point when the limit is exceeded obtained from the instrument using a calibration-only reagent, compared with the manufacturer's recommendation. The albumin, ALP, DB, LD, Mg and TP were required to shorten the recommended interval of the manufacturer, but Ca needs to increase the recommended interval. The cholesterol, creatinine, iron, and TG were found to be consistent with the manufacturer 's recommendation. The 11 items such as ALT, amylase, AST, BUN, CK, glucose, lipase, P, γ-GT, TB and UA did not exceed the limit for 7 days. **Conclusion:** When the desirable or minimum imprecision goal are applied as the acceptance criteria, the time point exceeding the allowable limit can be changed. Therefore, it is necessary to determine the calibration interval according to the appropriate acceptance criteria and the volume of test rather than the uniform calibration interval suggested by the manufacturer in the laboratory.

## A-239

### Evaluation of hemolysis interference for high sensitivity cardiac troponin I immunoassay on ABBOTT Architect i2000 system

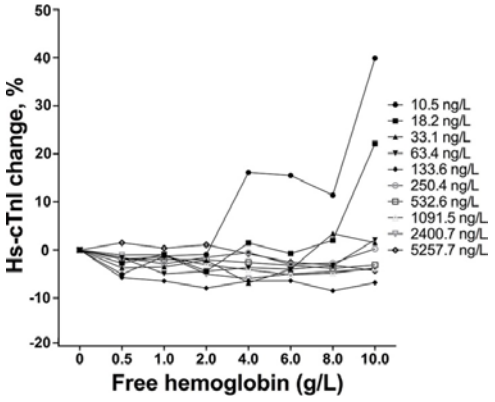
Y. Huang, L. Jiang. *Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu, China*

**Background:** Hemolysis is the most common endogenous interference that effects clinical laboratory test results. Our study aims to investigate hemolysis interference on ABBOTT immunoassay of high sensitivity cardiac troponin I (hs-cTnI), one of the most important markers for acute myocardial infarction diagnosis.

**Methods:** None-hemolyzed serum with high concentrations of hs-cTnI was pooled and spiked with hemolysate. Basal and seven hemolysis groups were constituted with free hemoglobin levels at 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 g/L, respectively. Each group contains 10 samples with serial hs-cTnI concentration at 10.5 ng/L, 18.2 ng/L, 33.1 ng/L, 63.4 ng/L, 133.6 ng/L, 250.4 ng/L, 532.6 ng/L, 1091.5 ng/L, 2400.7 ng/L and 5257.7 ng/L. Hs-cTnI concentrations for all 80 samples were analyzed in triplicate on i2000 analyzer (ABBOTT, USA) to evaluate the hemolysis interferences.

**Results:** ABBOTT hs-cTnI immunoassay had minimal interference with hemolysis. When applied a previously proposed serial cTnI change criteria to our spiked samples, no hs-cTnI results were effected by more than 20%, except for 10.5 ng/L (40.0%) and 18.2 ng/L (22.0%) at the free hemoglobin concentration of 10.0 g/L, which is rarely observed clinically. According to another suggested cTnI change criterion, results changed by 28 ng/L (All give lower levels) at the hemoglobin concentration above 2.0 g/L. The cTnI concentrations in these samples were at 1091.5 ng/L, 2400.7 ng/L and 5257.7 ng/L, respectively, which were far above cutoff value for clinical diagnosis.

**Conclusion:** Our results demonstrated that hemolysis has minimal effect on the immunoassay of hs-cTnI concentration on ABBOTT i2000 system, except for at very high cTnI levels or at extremely high hemoglobin levels. Thus, hemolysis is not a confounding factor of the hs-cTnI assay on ABBOTT i2000 system for clinical interpretation at most of clinical situations.




## A-240

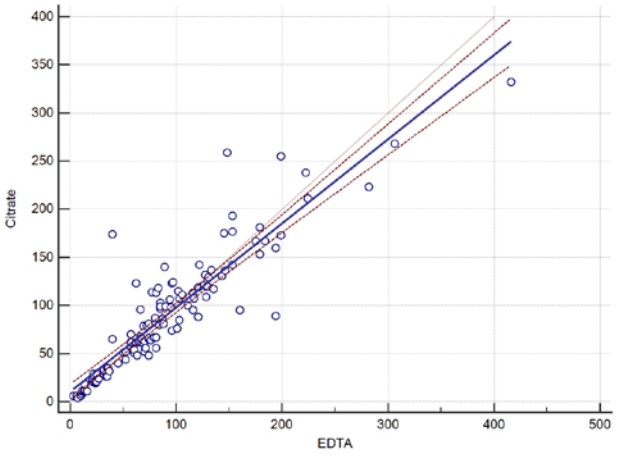
### Effect of Sodium Citrate on Automated Platelet Count

M. O. Nakashima. *Cleveland Clinic, Cleveland, OH*

**Background:** Platelet clumping due to EDTA-induced antibodies can falsely decrease automated platelet counts. Mechanical disaggregation, such as vortexing, may be strictly prohibited by hematology analyzer instructions for use. Using sodium citrate as an anticoagulant has been suggested as an alternative to EDTA to reduce likelihood of clumping. We did a retrospective analysis to see how results from platelet counts ordered on citrated samples compared to those obtained from EDTA-anticoagulated samples.

**Methods:** Orders for platelet counts in EDTA and citrate which were simultaneously placed over a period of four months were retrieved from the laboratory information system. Platelet counts were obtained on Sysmex XE-5000 analyzers (Kobe, Japan). A 1.1x correction was applied to the citrated count to correct for dilution by the anticoagulant. Regression analysis was performed (MedCalc v 14.8.1, Ostend, Belgium).

**Results:** There were 164 concurrent orders for EDTA and citrate platelet counts. Platelet clumps were reported in the EDTA samples only for 11 orders (6.7%), the citrate sample only in 27 orders (16.4%), and in both citrate and EDTA samples in 7 (4.3%) . Linear regression yielded an equation of $y = 0.874x + 10.6399$ (95% CI for slope 0.8012-0.9467) with $R^2 = 0.8289$ (figure; thick solid line indicates regression, dashed lines are 95% CI, thin line is y = x). When analyzing only thrombocytopenic samples (defined as EDTA platelet count <150 x $10^9$/L), $y = 10.259x + 0.9201$ (95% CI for slope 0.9092-1.1425) with $R^2 = 0.7528$.




**Conclusion:** Use of citrate anticoagulant does not prevent platelet clumps, and citrate samples were more likely to contain platelet clumps than EDTA samples. Although there was a relatively linear correlation between the results, there was bias in the citrate result. In thrombocytopenic samples, bias was smaller, however there was higher variability. These counts should be interpreted with caution especially in thrombocytopenic patients.

## A-241

### Automated urinary NGAL assay for early acute kidney injury detection

N. Voskoboev, T. S. Larson, J. C. Lieske. *Mayo Clinic, Rochester, MN*

**Background:** Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) is one potential biomarker for early detection of acute kidney injury (AKI), since it increases before serum creatinine or even cystatin C. However, previous NGAL assays were not available on platforms that would allow rapid and cost efficient turnaround for clinical use. Here we validated an NGAL assay developed for a chemistry autoanalyzer

**Methods:** An enzymatic NGAL assay (BioPorto Diagnostics A/S, Denmark) was studied using a Cobas c501 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Validation was performed using residual urine samples from patients with and without documented kidney damage. All samples were centrifuged to remove white blood cells which are known to artificially increase NGAL values. A reference value study was completed in healthy volunteers without known kidney disease (54 females, age

24-85 years old; 42 males 23-85 years old). Accuracy was assessed via spike recovery. Mixing recovery was performed using high and low concentration samples in 1:1 ratios. Mean (range) % recovery was calculated as (measured/expected x100%) for each experiment with recoveries of 100+/-10% considered acceptable. Serial dilution was performed in water.

**Results:** This automated urinary NGAL assay was analytically robust between 40 ng/mL and 3000 ng/mL. Intrassay precision was acceptable (5%) at 112 ng/mL, and improved to 2% at 2084 ng/mL. Average recovery with serial dilutions using water, calculated as (measured/expected x100%), was 94%. Recovery upon mixing high and low samples 1:1 (n=3 pairs) was 101%. The upper 95% reference value in the healthy male and female donors was 109ng/mL. Samples were stable after centrifugation for up to 7 days ambient, 4°C, -20°C, or -70°C, with toluene or sodium bicarbonate preservatives, and with up to 3 freeze-thaw cycles. The analytic turnaround time is 12 minutes.

**Conclusion:** An automated NGAL assay can be used on a chemistry analyzer to rapidly and accurately quantitate NGAL in urine. This platform should facilitate widespread clinical validation studies and implementation into practice if clinical utility is demonstrated.

## A-242

### Evaluation of BD Vacutainer® Barricor™ Plasma Blood Collection Tubes Under Various Transport Conditions

K. McDougall, B. Gormley. *BD, Franklin Lakes, NJ*

**Objective**: Two studies were performed to assess the integrity of the barrier in BD Vacutainer® Barricor™ Plasma Blood Collection Tubes (BD Barricor) under various transport conditions. One study evaluated filled tubes transported via a pneumatic transport system (PTS) pre- and post-centrifugation to determine if there was any effect on the mechanical separator in BD Barricor Tubes; the second study evaluated filled tubes during simulated ground transportation conditions post centrifugation for maintenance of the separator barrier.

**Background**: Although pneumatic tube systems (PTS) provide a rapid means of tube transport to the laboratory, the samples withstand forces of pressure, such as changes in air pressure, movement or shaking of blood in the test tube, vibrations, and sudden accelerations and decelerations. Studies tested the effect of PTS on the mechanical separator in BD Barricor Tubes. Barrier integrity was also assessed under simulated ground transportation conditions that were designed to emulate conditions that centrifuged blood specimens collected in BD Barricor Tubes would be subjected to during transport from remote clinics and physicians' offices to a testing laboratory.

**Methods**: Three hundred 3.0 mL, 13x75 mm and 300 4.5 mL, 13x100 mm BD Barricor Tubes were filled with sheep's blood prior to centrifugation, inspected for correct positioning of the separator and transported in the PTS. Six hundred varied draw BD Barricor Tubes were filled with sheep's blood and centrifuged at 4000g for 3 minutes. Tubes with no or trace hemolysis were subjected to the PTS and re-inspected for plasma color change, an indicator of barrier leakage and loss of barrier integrity. To simulate ground transportation conditions, bagged human blood in 300 BD Barricor Tubes each of 3.0 mL, 13x75 mm and 4.5 mL, 5.5 mL, 13x 100 mm, was centrifuged at 4000g for 3 minutes and inspected at four time intervals.

**Results**: Pre-centrifugation, post PTS transport showed no separator movement from the original position. There was no loss of barrier integrity in 300 3.0 mL and 299 4.5 mL BD Barricor Tubes and post PTS transport for varied draw tubes. The 95% confidence limit for the failure rate was less than 1.0% for all tube configurations. This demonstrated compatibility for PTS transport, as all tubes maintained barrier integrity through simulated transportation. No deterioration was observed in barrier performance, which met the acceptance criterion of 95% confidence of 95% reliability to maintain barrier integrity at each time interval.

**Conclusions**: The mechanical separator in BD Barricor Tubes maintained its position at the top of the uncentrifuged tubes through pneumatic tube transport. The separator formed a robust barrier between cells and plasma in centrifuged tubes, which remained integral throughout the transport conditions.

## A-243

### Differential interferences of a kinetic Jaffe creatinine method by three ketones

D. Li[1], C. Cheng[2], S. Wong[1], M. Pudek[1]. *[1]Vancouver General Hospital, Vancouver, BC, Canada, [2]University of British Columbia, Vancouver, BC, Canada*

**Objective:**

It is well-known that ketones interfere with creatinine measurement by Jaffe methods. However, which ketone(s) are responsible for this interference have not been previously elucidated. Here we report the effects of beta-hydroxybutyrate, acetoacetate, and acetone on creatinine analysis by a Jaffe method and an enzymatic method. We also propose the use of a creatinine gap in the differential diagnosis of an excess osmolal gap.

**Methods:**

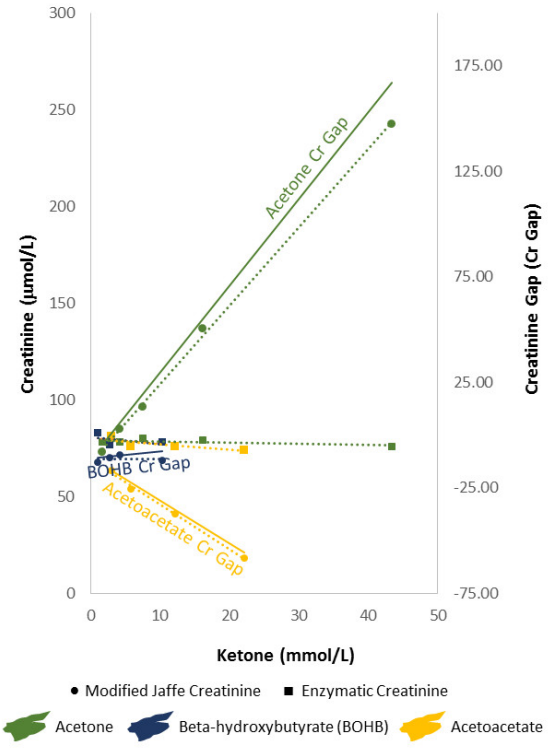
DL-beta-hydroxybutyric acid sodium salt and lithium acetoacetate were purchased from Sigma-Aldrich, acetone from Thermo Fisher Scientific. Stock solutions of beta-hydroxybutyrate, acetoacetate, and acetone were prepared in distilled water. Plasma samples with creatinine concentration of ~80 umol/L were pooled. The pooled plasma was spiked with distilled water (control) or with the prepared stock ketone solutions to create specimens with varying ketone concentrations. Creatinine levels in these spiked samples were measured by both a kinetic Jaffe method (Siemens Vista) and an enzymatic method (ABL800). The creatinine gap was calculated as the Vista creatinine concentration - the ABL800 creatinine concentration. Beta-hydroxybutyrate was measured on the Vista, acetone by gas chromatography; acetoacetate level was estimated by the sample's lithium concentration, as measured on the Vista platform.

**Results:**

Ketones do not interfere with the enzymatic creatinine method. In contrast, ketone-specific interferences are observed with the kinetic Jaffe creatinine method: acetone causes concentration-dependent falsely high creatinine levels, acetoacetate causes concentration-dependent falsely low creatinine levels, and beta-hydroxybutyrate does not interfere with the creatinine measurement (see figure).

**Conclusion:**

Ketones interfere with creatinine analysis by the kinetic Jaffe method. This interference is ketone-specific. Osmolal gap, anion gap, and lactate gap are often used to expedite diagnosis and treatment of toxic alcohol ingestions. As acetone is the metabolite of isopropanol, calculation of the creatinine gap, as determined by creatinine measurements between Vista and ABL800 analyzers, can be used as a surrogate marker for isopropanol ingestion.



## A-244

### Negative interference by Calcium Dobesilate in Five Trinder Reaction Assays

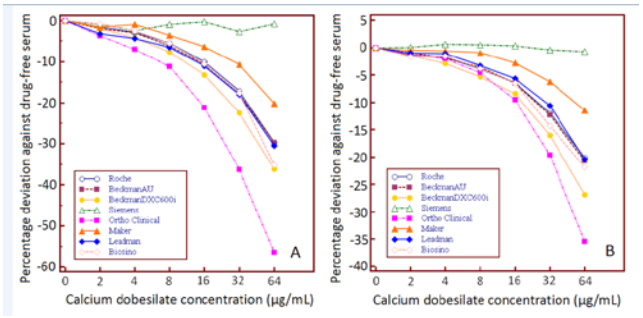
X. Guo, L. Qiu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

**Background:** Previously, we reported the strong negative interference of calcium dobesilate, a vasoprotective agent, in creatinine assays involving the Trinder reaction. It is hypothesized that a similar effect occurs in the detection of uric acid (UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The interference of calcium dobesilate in the five serum analytes was investigated.

**Methods:** Calcium dobesilate standard was added into two sets of the blank serum of each analyte at final concentrations of 0, 2, 4, 8, 16, 32, and 64 μg/mL. Each analyte was measured using eight different assay systems. The percentage deviation of each analyte value was calculated between each drug concentration and the drug-free samples, and the effects were compared among eight different assay systems for each analyte.

**Results:** Considering the clinically acceptable deviation of ±4.5% for UA, the exogenous addition of calcium dobesilate clearly exhibited dose-dependent negative interference with the determination of UA in all seven Trinder reaction-based assays (Figure 1). In the presence of 16mg/mL calcium dobesilate, all seven Trinder reaction-based UA assays exhibited deviations ranging from -6.3% to -21.2% in the low UA serum group (Figure 1A). As a control assay, the Siemens system using the Uricase-UV method did not show any interference (Figure 1). The clinically acceptable error levels for TC, TG, HDL-C, and LDL-C were defined as±4.0%, ±5.0%, ±5.2%, and ±6.8%, respectively. At 16 μg/mL calcium dobesilate, six TG assay systems, three HDL-C assay systems and one TC assay system exhibited negative drug interference. The deviations for all eight LDL-C assay systems were less than 6.8% at calcium dobesilate concentrations ≤64 μg/mL.

**Conclusion:** Calcium dobesilate negatively interferes with the detection of UA, TG, TC, and HDL-C in assay systems based on the Trinder reaction. The effect was most significant in UA and TG detection but imperceptible for LDL-C detection.




## A-245

### Calcium Dobesilate can Negatively Interfere with the Detection of Glycated Albumin

X. Guo, L. Qiu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

**Background:** Glycated albumin (GA) is considered as a potential intermediate-term glycation index to fill the gap between self-monitoring of blood glucose and haemoglobin A1c testing in diabetes management ADDIN NE.Ref.{6B0F0033-2506-4A2A-AF78-F095247FA411}. The enzymatic method has been the primary method for assessing GA in medical laboratories owing to its high sensitivity and specificity, and ability for automated analysis. However, recently, we noted that calcium dobesilate, which is a vasoprotective agent widely used for the treatment of diabetic retinopathy, can negatively interfere with GA detection using the enzymatic method.

**Methods:** We quantified this analytical interference using both in vitro and in vivo methods. A calcium dobesilate standard was added to 3 serum samples (GA levels: 39.4%, 23.3%, and 17.6%, respectively) to prepare dose-response series according to the CLSI EP7-A2 guidelines. For in vivo interference experiments, baseline serum samples were collected from 8 healthy participants. Calcium dobesilate (500 mg) was then administered orally 3 times daily for 3 days to achieve a steady state according to pharmacokinetic information. Fasting blood samples were collected at trough drug levels on the morning of the 4th day (0 hours), and at 2 hours after administering another 500-mg dose of calcium dobesilate. GA levels were measured with the Asahi Kasei GA assay (Asahi Kasei, Inc., Japan) using a Beckman AU5800 analyser (Beckman Coulter, Inc., Brea, CA), according to the manufacturer's recommended procedure. Calcium dobesilate (mg/mL) concentrations were measured using a high-performance liquid chromatography method.

**Results:** The exogenous addition of calcium dobesilate clearly exhibited dose-dependent negative interference with GA determination. In the presence of 16 mg/mL calcium dobesilate, the percentage deviations from drug-free serum were -9.9%, -11.6%, and -10.2% for the high-, medium-, and low-GA interference samples, respectively. The degree of interference reached about 30% at a calcium dobesilate concentration of 64 μg/mL. In the in vivo interference experiments, after 3 days of calcium dobesilate administration, the mean calcium dobesilate concentrations at 0 and 2 hours were 7.33 (range, 4.63-9.55; interquartile range [IQR], 6.25-8.18) and 18.52 (range, 7.04-20.2; IQR, 15.90-19.67) mg/mL, respectively, resulting in a decrease in GA values of -4.8% and -12.8%, respectively, relative to that in the baseline control samples.

Calcium dobesilate is believed to remain mainly in its original form after excretion through the kidney and intestinal tract. Currently, the recommended clinical dose of calcium dobesilate is 500 mg TID, and its steady-state plasma concentration is estimated to be above 15 μg/mL. We previously reported that calcium dobesilate interfered in creatinine assays using the Trinder reaction method. We also noted that the calcium dobesilate concentration in patients administered this drug reached 63.35 μg/mL, and interference is expected to be more substantial at such high concentrations.

**Conclusion:** In conclusion, calcium dobesilate was confirmed to negatively interfere with GA determination in both in vitro and in vivo experiments, which may result in false glycaemic status evaluations in the management of diabetic patients. Extra care should be taken in the evaluation of GA levels in patients receiving calcium dobesilate.

## A-246

### Evaluation of Sekisui LDL-C Reagent against Wako LDL-C Reagent on Beckman Coulter AU5800 and its Impact on Patient and External Quality Assurance Results

S. Phun, Z. Yang, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore*

**Background:** National University Hospital(NUH) has participated and achieved satisfactory results in College of American Pathologists(CAP) surveys for LDL-Cholesterol(LDL-C) for many years. In April 2014, we changed to Beckman Coulter AU5800 and observed higher recovery than our peers for all our CAP General Chemistry and Accuracy-Based surveys. Our investigations revealed that Sekisui Medical Co. Ltd LDL-C reagent was supplied to US customers and Wako Pure Chemical Industries Ltd LDL-C reagent to customers outside US. Both LDL-C reagents passed the Centre for Disease Control and Prevention(CDC) Lipid Standardisation Program and both calibrators were traceable to CDC Reference method. However, the differences in method performance between the two reagents were not evaluated in detail.

**Methods:** The study was performed over a period of 5 days with daily calibrations and valid QC runs. Three methods were evaluated- Sekisui(Reference method), Wako(Test Method 1) and Wako with adjusted LDL calibration factor(Test Method 2). A total of 200 fresh patient samples with Triglyceride values less than 2.25mmol/L and LDL-C values across analytical measurement range were tested. The correlation results were evaluated for percent bias from Reference Method across clinically significant decision levels.

**Results:**Both Test Methods 1 and 2 had an over-recovery ranging from 10.9-20.3% and 2.4-10.9% respectively. Test Method 2 achieved closer recovery compared to Reference Method using calibrator adjustment alone. The table below shows the percent bias of the Test Methods.

| Percent Bias from Reference Method Based on Best Fit Line | | |
|---|---|---|
| LDL-C Clinical Decision Levels | Test Method 1 | Test Method 2 |
| 2.6 mmol/L | 20.3% | 10.9% |
| 3.4mmol/L | 15.6% | 6.6% |
| 4.1 mmol/L | 13.0% | 4.3% |
| 4.9 mmol/L | 10.9% | 2.4% |

**Conclusion:** This study highlights the problems faced by international customers when diagnostic companies provide different reagents to different parts of the

world, leading to the laboratories' inability to obtain appropriate performance (and sometimes failure) when assessed against peers in CAP surveys.

## A-247

### Multivariate Models for Combinations of Hemolysis, Icterus, and Lipemia Interference

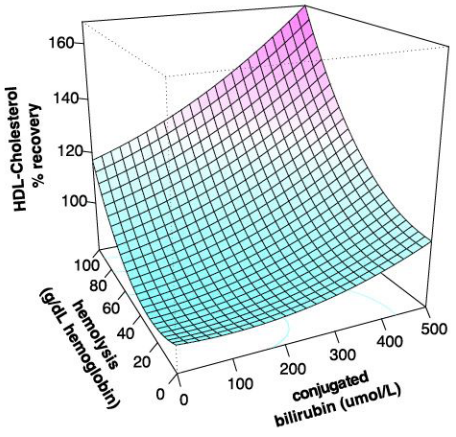
C. McCudden, N. Mitchell, T. Ranko, A. Burgess. *The Ottawa Hospital, Ottawa, ON, Canada*

**Background:** Hemolysis, icterus, and lipemia interferences can affect the accuracy of patient results. Both manufacturers and laboratories spend considerable financial and personnel resources performing interference studies, but there remains limited data on the effect of combinations of interferences. The objective of this study was to use multivariate experimental design to efficiently determine the effect of combinations of hemolysis, icterus, and lipemia interferences on common biochemistry test results.

**Methods:** To model the combined effects of hemolysis, icterus, and lipemia on creatinine, HDL-cholesterol, AST, and ALT, we used a face-centered central composite design (CCD). CCD is an efficient experimental design method using limited experimental data to model individual factors on a response; in this study, factors included hemolysis, icterus (conjugated and unconjugated), lipemia, and analyte concentration and the response was %recovery. Interferences were added in combination to represent low, medium, and high values for each. The design yielded a mathematical model for each analyte and interference combination with only 30 samples. Analytes were tested on the Siemens Vista 1500, where %recovery was calculated as: (expected - measured)/expected. Results were analyzed using response surface modeling to identify linear and exponential effects and well as interactions (e.g. hemolysis:lipemia in combination).

**Results:** At low concentrations, AST showed markedly increased recovery due to hemolysis. Also at low analyte concentrations, ALT was subject to positive bias due to lipemia interference. There was significant positive bias on HDL-cholesterol recovery in the combined presence of hemolysis and conjugated bilirubin. Enzymatic creatinine showed positive bias with icterus and hemolysis in combination.

**Conclusion:** Creatinine and HDL-cholesterol showed an until now unrecognized synergistic positive bias from hemolysis and icterus. ALT and AST analysis confirmed existing package insert information supporting that there is no multivariate effect from interference combinations. Multivariate experimental design is an efficient way to obtain complete data for multiple interferences in combination.




## A-248

### Study of hemolysis of samples by hospital services.

D. González Benito, R. Escobar Conesa, V. García Moreira, A. Llorente Torres, E. Fernández Rodríguez. *Hospital Universitario de Cabueñes, Gijón, Spain*

Background: The main goal of clinical laboratories is to obtain results with precision and accuracy. The hemolysis is produced by a wrong sampling and/or an incorrect sample shipment. It also involves the most frequent analytical interference and it is the cause of 60 percent of the rejected samples in the laboratory. As consequence, it produces an alteration of the results, and therefore, a wrong diagnosis or treatment plan may be reached. Our goal is to conduct a detailed study of the hemolysis in the received blood samples for biochemical analysis in our emergency laboratory from different clinical departments of the hospital and to assess the results.

Methods: During 1 year (From February 2015 to January 2016) a total of 54035 samples were studied from different clinical departments of the hospital (emergency room, internal medicine, operating room, neonatology, intensive care unit, gynecology and surgery and traumatology).

The degree of hemolysis was measured by absorbance at Dimension EXL 200 via standardized index HIL (hemolysis, icterus and lipemia) and it was classified into 3 types: non-hemolyzed (NH) <25 mg/dl of hemoglobin, slightly hemolyzed (SH) 25-200 mg/dl of hemoglobin, and heavily hemolyzed (HH) >200 mg/dl of hemoglobin or visible hemolysis.

Results: We observed that the percentage of hemolysis is very different depending on the department that the samples are handled. The highest hemolysis degree is the in neonatology (19.78% SH and 2.25% HH) and in emergency services (21.67% SH and 3.41% HH). On the other hand, we observed a minor ratio of hemolyzed samples from operating room (3.11% SH and 0.22% HH) and from surgery and traumatology (5.03% SH and 0.44% HH).

Conclusions: We believe that the main cause of these differences is the sample extraction. In neonatology, the main cause of the difficulty in the sample extraction is the type of patients, while in emergencies, the difference may be caused by some of these reasons: the extraction by direct venipuncture, the possible lower training and the excessive changes in the staff, the higher quantity of work and the type of patient, among other reasons. On the other hand, we observe less hemolyzed samples from surgery room and from traumatology, probably for opposite reasons (as a clearer protocol of sampling, made with more time and accuracy), comparing with the emergency department.

## A-249

### Glucose preanalytical variation: Influence of time-to-centrifugation and environment temperature

L. Bautista Balbás, R. Gómez Rioja, M. Duque Alcorta, C. Eisman Valdés, L. Alba Valdivia, A. Buño Soto. *Hospital La Paz, Madrid, Spain*

**Background:** Glucose determination in samples collected in external centers is very susceptible to preanalytical errors; particularly due to glucose consumption. In this observational study we want to describe these variations in our particular conditions (Northern area of Madrid, Spain).

**Materials and methods:** We retrospectively gathered the serum glucose results of outpatients (primary care and specialized care) from 2015-2016; all these were drawn outside our hospital and delivered by road in portable non-electric coolers.

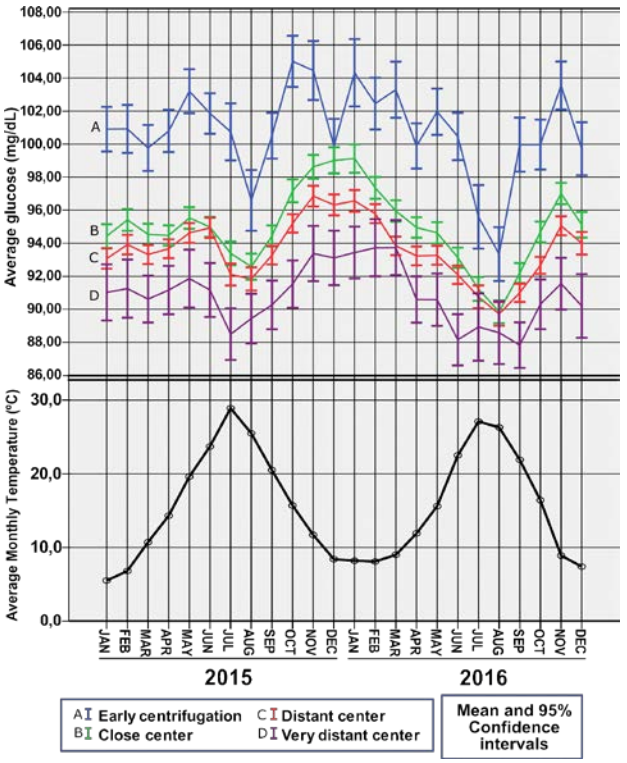
We calculated the time-to-centrifugation (TTC) according to our daily preanalytical unit logbook and we estimated the approximate TTC for each centre. We obtained the Average monthly temperatures in our area from Spanish Meteorological Agency (AEMET). We classified the samples in early centrifugation (before road transport, in less than 30 minutes), close centers (30-90 minutes), distant centers (90-120 minutes) and very distant centers (more than 120 minutes). Glucose was measured in Advia 2400 (Siemens Healthineers) (hexokinase).

**Results:** We gathered 361,268 results: 39228 in Early centrifugation, 134311 in Close centers, 164245 in Distant centers and 23485 in Very distant centers. Slight differences were observed in the mean age between the groups (52-56), and in sex (37-40% males).

In a general linear model age, sex, the average TTC of the centre and Average monthly temperature (ºC) in our area were significantly associated with the glucose value (although, as we expected, these factors only explained a small percentage of the patient results variability: $R^2$=0.109).

**Conclusions:** Observed glucose variations of up to 10 mg/dL between groups of samples are coherent with described glycolysis rates of 2-7% per hour. This could be prevented by using tubes with glycolysis inhibitors or centrifuging samples in collection centers.

We can conclude from these observational results that in part due to preanalytical factors serum glucose results could vary up to 10% in samples from external centers.




## A-250

### Assessment of Lipemia Interference using Human Triglyceride-Rich Lipoproteins

J. P. Nagy[1], R. Nguyen[2], J. H. Contois[1]. *[1]True Health Diagnostics, Richmond, VA, [2]Sun Diagnostics, New Gloucester, ME*

**Background:** IntraLipid® continues to be used as a lipemia interferent despite data that suggests it fails to replicate the lipemia seen with human lipoproteins. Therefore, we evaluated interference by triglycerides on common chemistry tests and compared results to manufacturer's claims based on IntraLipid. **Methods:** Base Pool was prepared using serum pools with low triglycerides. High Pool was prepared by spiking concentrated triglyceride-rich lipoprotein (TRL) into the Base Pool (Assurance™ Interference Test Kit, Sun Diagnostics, New Gloucester, ME). Base Pool and High Pool were intermixed to create five levels of triglycerides (107, 397, 677, 964, and 1227 mg/dL). Multiple analytes were measured on the Beckman AU5800. **Results:** Minimal interference was seen with alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase (AMY), bicarbonate (CO2), Calcium (CA), creatine kinase (CK), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), lipase (LIP), magnesium (MG), phosphorus (PHOS), total protein (TP), apolipoprotein AI (ApoAI), uric acid (UA), adiponectin (ADN), cystatin C (CYSC), high sensitivity C-reactive protein (hsCRP), ferritin (FER), 1,5-anhydroglucitol (1,5-AG), potassium (K), or chloride (CL), consistent with manufacturer claims using IntraLipid. We saw less lipemia interference with TRL compared to manufacturer's claim using IntraLipid for total bilirubin (TBIL), direct bilirubin (DBIL), iron (FE), urea nitrogen (BUN), and creatinine (CRE). We saw no TRL interference with K or CL, although higher triglyceride concentration likely support manufacturer's claim of potential decrease due to volume displacement. Sodium (NA), however, did display decreasing values with increased triglyceride concentrations. Albumin (ALB), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations increased with increasing triglyceride interferent concentration, in contrast with manufacturers' claims of minimal lipemia interference using IntraLipid. **Conclusion:** The use of TRL versus IntraLipid as an interferent offers a more accurate depiction of the potential for triglyceride interference during laboratory testing.

## A-251

### Recognition of Rare Hemoglobin Variants during Hemoglobin A1c Analysis by Capillary Electrophoresis

S. W. Strickland[1], S. T. Campbell[1], R. R. Little[2], D. E. Bruns[1], L. A. L. Bazydlo[1]. *[1]University of Virginia, Charlottesville, VA, [2]University of Missouri Diabetes Diagnostics Laboratory, Columbia, MO*

**Background:** Hemoglobin A1c (HbA1c) is the result of a non-enzymatic glycation of hemoglobin A and is measured by multiple analytical techniques, including immunoassay, boronate affinity chromatography, HPLC, and capillary electrophoresis. Few studies have investigated the effects of rare variants on HbA1c analysis by high resolution capillary electrophoresis (CE).

**Methods:** During routine HbA1c analysis by CE in our laboratory, potential hemoglobin variants were identified by examination of CE electropherograms. Variants were confirmed by sequencing and A1c was subsequently measured in selected samples by 3 additional HbA1c measurement procedures (2 ion-exchange HPLC and 1 boronate HPLC).

**Results:** During a 13-month period, 35 patients were identified as having an "atypical profile" with the CE method. Of those, 11 samples were confirmed to have hemoglobin variants and 6 samples appeared to harbor a hemoglobin variant, but additional blood samples for sequencing were unavailable. Four variants (Hb Roanne, La Desirade, Nouakchott, and A2 prime) had not been reported previously to influence the quantification of HbA1c by the CE method. Three of these variants (Hb Roanne, La Desirade, and Fannin-Lubbock) were further tested by 3 additional methods for measurement of HbA1c, and the results were compared to the CE results. HbA1c as measured by the CE method differed by 0.8%, 0.7%, and 2.2% for Hb Roanne, La Desirade, and Fannin-Lubbock, respectively, from the mean of the other 3 methods. Hb Roanne and Fannin-Lubbock were resolved from HbA only by the high resolution CE method and not by either HPLC method (ion-exchange or boronate affinity), while Hb La Desirade displayed aberrant peaks only in the CE and the G8 Tosoh HPLC methods.

**Conclusions:** Failure to identify these silent variants could lead to misleading HbA1c results and negatively impact patient outcomes. The high resolution of the CE method allows for better identification of hemoglobin variants in patients requiring routine HbA1c analysis. For patients with a hemoglobin variant that prevents measurement by HPLC or CE, another method is needed or alternative assays, such as measurement of fructosamine or glycated albumin, may also be required for appropriately monitoring glycemic control.

## A-252

### Effect of Seasonal Temperature on Specimens Stored Outside in Courier Lock Boxes

J. R. Wiencek, J. H. Nichols. *Vanderbilt University Medical Center, Nashville, TN*

**Background:** Lock boxes for patient specimens are commonly used by outpatient clinics to store processed specimens awaiting pick-up for courier transport to a laboratory. Temperature conditions and delays in courier pick-up are critical factors in the preanalytic quality of these specimens and have not received rigorous investigation.

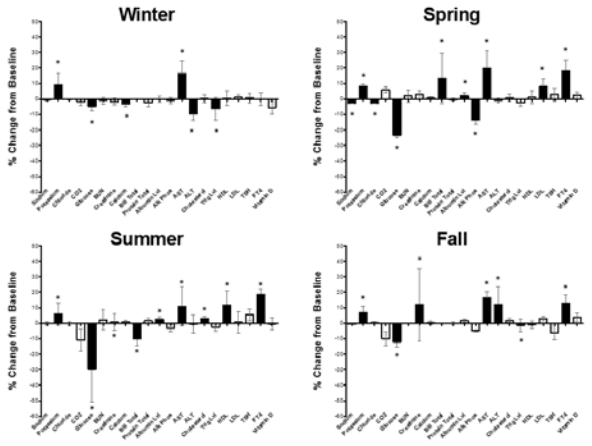
**Objective:** To determine the effects of sample exposure to ambient temperature changes and delayed shipping conditions (over a weekend) on several commonly measured analytes in the clinical laboratory at different times of the year.

**Methods:** Paired lithium heparin BD PST Vacutainer® tubes were drawn from healthy volunteers (n=4) during four seasons (winter, spring, summer, fall) in the Southern US. Specimens were processed immediately after collection. Paired specimens were analyzed either immediately by Abbott Architect analyzers or after 72 hours of holding at ambient temperature in an outside courier lock-box. Temperature was continuously monitored every 15min with an INKBIRD Temperature Data Logger. Each sample was analyzed for a comprehensive metabolic panel, lipid profile, thyroid panel and Vitamin D. Acceptable tolerance limits for analytes were determined by significant change limit (SCL) analysis. For each analyte, SCLs were calculated from 12-months of cumulative performance data (quality control coefficient of variation) multiplied by ±2.8.

**Results:** The average temperatures were: winter -3.1°C (Range: -9.9-22.3°C), spring 16.1°C (Range: 7.0-25.2°C), summer 25.0°C (Range: 21.4-29.8°C) and fall 10.6°C (Range: 2.2-22.8°C). Large deviations were observed for glucose, $K^+$, and AST when compared to initial measurements across all seasons. Colder temperatures seen in the

fall and winter experiments showed 7/21 and 6/21 of the analytes were outside of the SCL tolerance limits (black shaded bars in seasonal graphs $p<0.05$ from $t_0$). More analytes were affected by warmer seasons with 10/21 in spring and 9/21 in summer outside SCL tolerance limits.

**Conclusion**: Delayed sample delivery and fluctuating seasonal temperatures in the Southern US are important sources of preanalytical error.




## A-253

### Surface Characterization and Free Thyroid Hormone Measurements of Chemically Modified Polyethylene Terephthalate Films and Blood Collection Tubes (BCT) to Eliminate Potential BCT Surfactant Interference on Some Immunoassay Tests

E. Jalali Dil[1], S. Kim[2], A. Saffar[1], A. Sattayapiwat[3], V. Esguerra[3], A. Ajji[1], R. Zare[3], R. A. Bowen[3]. *[1]Ecole Polytechnique de Montreal, Montreal, QC, Canada, [2]University of California, San Francisco, San Francisco, CA, [3]Stanford University, Palo Alto, CA*

**Background:** Blood collection tubes (BCTs) are not inert specimen carriers, as shown by some studies reporting significant differences in serum hormone concentrations collected in polyethylene terephthalate (PET) BCTs due to surfactants. We recently developed chemically modified BCTs (chemoPETs) that have an interior surface similar to glass BCT and lacks surfactant(s). However, the chemoPET surfaces were not fully characterized and free triiodothyronine ($FT_3$) and free thyroxine ($FT_4$) concentrations were not studied. Free thyroid hormones in blood is important because they are metabolically active and useful for the diagnosis of thyroid diseases. This study's first objective is to characterize and compare unmodified PET and chemoPET film surfaces to determine if the latter is significantly hydrophilic. The second objective is to determine if the biases in $FT_3$ and $FT_4$ concentrations from chemoPET and other serum BCTs compared to glass tubes are statistically and/or clinically significant. **Methods:** PET and chemoPET film surfaces were characterized by X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and scanning electron microscopy (SEM). The PET film contact angles and surface energy were determined with liquid probes. For serum specimens, one glass (control BCT) and five plastic (Vacuette™, SST™, RST™, plain red-top, and chemoPET) BCT types were used. The chemoPET film and tubes were made from a base-catalyzed transesterification reaction. Blood samples were drawn in a randomized order from 25 volunteers (10 males, 15 females; age range: 25-70 years), mixed, and allowed to clot for 1 hour. Following centrifugation, serum specimens were transferred to plastic tubes and stored at -70 ºC until analysis. The concentrations of $FT_3$ and $FT_4$ in the BCTs were analyzed in singleton on an Immulite 1000 instrument. Repeated measures ANOVA with Bonferroni correction were used to determine significant differences in $FT_3$ and $FT_4$ concentrations. To assess whether the BCT-related biases (deviation from glass tubes) were clinically significant for $FT_3$ and $FT_4$ concentrations, each BCT bias was compared to the desirable allowable bias, derived from biological variation. **Results:** XPS analysis results indicate that the carbon/oxygen ratio on the PET film surfaces decreased from 2.9 for unmodified PET to 2.1 for chemoPET surfaces. Tof-SIMS analysis shows a higher peak intensity of oxygen and hydroxyl groups on the chemoPET compared to unmodified PET film surface. The surface topography by SEM demonstrated that the chemoPET film surface was smooth without any holes. Water contact angle measurements show a decrease from 70° for unmodified PET to 44° for chemoPET film surfaces and the surface energy and polarity increased from 37.9 to 51.9 ($mJ/m^2$) and 25% to 71%, respectively. These results clearly indicate that the chemoPET surface is more hydrophilic than the unmodified PET. No statistically significant differences in $FT_3$ ($p$=0.01) and $FT_4$ ($p$=0.77) concentrations were observed when chemoPET and other BCTs were compared to glass BCTs. However, compared to glass tubes, clinically significant differences were found in $FT_3$ (6.71%; desirable bias: 4.80%) and $FT_4$ (3.36%; desirable bias: 3.30%) concentrations in SST and RST tubes, respectively. **Conclusion:** ChemoPET tubes may be used to eliminate BCTs that contain surfactants known to interfere with some immunoassays.

## A-254

### Risk Factors Associated with Indeterminate QuantiFERON-TB Gold In-Tube Assay Results in Diagnosis of Latent Tuberculosis Infection

Y. J. Choi[1], S. J. Kee[1], E. J. Won[1], Y. J. Kwon[1], H. S. Lee[1], H. J. Choi[1], J. H. Shin[1], B. H. Park[2], S. P. Suh[1]. *[1]Chonnam National University Hospital, Gwangju, Korea, Republic of, [2]Mokpo National University, Muan, Korea, Republic of*

**Background:** QuantiFERON-TB Gold-In Tube test (QFT-GIT) is a recommended method for diagnosing latent TB in general population. However, indeterminate results of GFT-GIT are is a well-documented limitation. The aims of this study were to identify factors associated with indeterminate results and to investigate the impact of using automated enzyme-linked immunosorbent assay (ELISA) processor on QFT-GIT results.

**Methods:** We performed univariate and multivariate logistic regression analysis to QFT-GIT results in Chonnam National University Hospital, from March 2009 to February 2013. We also compared precision between manual methods and automated methods on QFT-GIT assay.

**Results:** Among the total of 4,925 QFT-GIT results, 4,501 (90.9%) were determinate results and 451 (9.1%) were indeterminate results. In univariate analysis, age, season, turnaround time (TAT), ELISA processing methods, lymphocyte count, neutrophil/lymphocyte ratio, platelet count, hemoglobin, albumin, and C-reactive protein (CRP) were associated with indeterminate results. In multivariate analysis with all factors above, younger age ($P$ <0.001), winter ($P$ <0.01), prolonged TAT ($P$ <0.01), automated method ($P$ <0.001), higher neutrophil/lymphocyte ratio ($P$ <0.001), higher platelet count ($P$ <0.05), lower hemoglobin ($P$ <0.01), lower albumin ($P$ <0.05), higher CRP ($P$ <0.05) were significantly independent predictors of indeterminate results. Automated methods presented higher precision in lower concentration samples.

**Conclusion:** Using automated ELISA processor on QFT-GIT assay was factors that reduce indeterminate results and could be controlled in clinical laboratory. We expect that automated methods may contribute accurate clinical decision by reduction of indeterminate results.

## A-255

### Comparison of Electrophoretic Systems to Detect Occult IgA Monoclonal Immunoglobulins

D. Yin[1], K. Cost[2], R. Elin[1]. *[1]University of Louisville, LOUISVILLE, KY, [2]Norton Hospital, LOUISVILLE, KY*

**Background:** Monoclonal immunoglobulins (MI) are diagnostic and prognostic biomarkers for several clonal diseases including multiple myeloma – these antibodies (IgG, IgA, IgM, IgD, and IgE) are secreted by mature plasma cells. Approximately 3-4% of patients with multiple myeloma do not produce or secrete monoclonals proteins. MIs are screened using serum protein electrophoresis (SPE), where proteins are separated in an agarose gel based on their electrophoretic mobility and charge distribution of the protein to produce a pattern of major fractions: albumin, alpha-1, alpha-2, beta, and polyclonal gamma globulins. MIs are present in the gamma globulin region, but can also be found in the beta region or alpha-2, which can make quantitation and interpretation inaccurate. Elevated beta globulin, of which the major fraction is transferrin, may be an indication for iron deficiency anemia, 3rd trimester pregnancy, or the use of oral contraceptives. Capillary electrophoresis (CE) can resolve the beta-globulin as two peaks; $beta_1$ (transferrin) and $beta_2$ (complement proteins [C3]) regions. We present three cases that illustrate the resolving power of these two methods to detect the presence of an IgA MI band hidden within the beta region.

**Method:** SPE and CE were performed using the Helena SPIFE 3000 and Sebia Capillarys2 systems respectively. Protein fractions were quantitated by densitometry

(Helena) with total serum protein concentration as the reference or direct UV absorbance (Sebia) at 200 nm. The presence of a MI was confirmed by immunofixation electrophoresis (IFE). The Beckman DxC and Vitros 5,1 FS were used to quantitate immunoglobulins. Reference ranges: $beta_1$ (0.30 – 0.60 g/dL), $beta_2$ (0.20 – 0.50 g/dL).

**Results:** Patient A showed a normal electrophoretic pattern and protein concentration from the SPE, but an elevated $beta_2$ (0.87 g/dL) versus normal $beta_1$ (0.45 g/dL) using the CE method. Immunoglobulin quantitation methods showed an elevated IgA (Beckman: 677; Vitros: 634 mg/dL), and normal IgG and IgM - IFE confirmed an IgA lambda. Patient B showed an elevated beta globulin but otherwise a normal electrophoretic pattern from the SPE. The CE method showed an elevated $beta_2$ (1.0 g/dL) versus a normal $beta_1$ (0.42 g/dL). Immunoglobulin quantitation methods showed an elevated IgA (Beckman: 913; Vitros: 851 mg/dL), normal IgG and IgM - IFE confirmed an IgA lambda. Patient C showed an elevated monoclonal protein adjacent to the beta globulin peak on the SPE. The CE method showed an elevated $beta_2$ (1.6 g/dL) versus a normal $beta_1$ (0.38 g/dL).. Immunoglobulin quantitation methods showed an elevated IgA (Beckman: 913; Vitros: 851 mg/dL), normal IgG and IgM - IFE confirmed an IgA lambda. These results demonstrate that a capillary method is superior to agarose gel electrophoresis due to its ability to separate the beta region into $beta_1$ and $beta_2$, thus allowing better detection of hidden IgA MIs. Furthermore, we observe a pattern whereby the concentration difference between $beta_1$ and $beta_2$ become reversed ("flipped") in the presence of a hidden IgA MI. Further studies with a larger sample size may be warranted to establish a useful rule for IFE reflexing when the $beta_2$ fraction is greater than the $beta_1$.

## A-256

### Analysis of serum indices measurement results as partial evaluation of preanalytical phase

M. S. Gungoren, D. I. Topcu, C. Zungun. *Duzen Laboratories Group, Ankara, Turkey*

**Background:** Serum indices are calculations of spectrophotometric measurements that represent levels of icterus (I), hemolysis (H), or lipemia (L) in serum samples. Main purpose of serum indices is to evaluate sample integrity and avoid analytical errors due to inappropriate sample. Additionally, measuring the presence and the extent of hemolysis, icterus and lipemia of a serum sample can give us some idea about patient's health and nutrition conditions and quality of phlebotomy. As these are several preanalytical factors, we decided to evaluate preanalytical errors related with blood sample collection and inadequate collection of patient information by analysing results of serum indices together with demographical features of patients.**Methods:** Patient records with serum indices results of last 12 months were imported from Laboratory Information System. Lower limits for H, I and L indices were determined as 15, 1 and 15, respectively. Total of 38 tests were selected for evaluation (23 for H, 6 for I and 9 for L). Serum indices were measured with Roche SI2 Reagent using Roche Cobas c501 (Roche Diagnostics, Mannheim, Germany). Statistical analyses were performed with R 3.3.2 (R Working Group, Vienna, Austria).**Results:** Total number of samples with selected 38 tests was 34394. Firstly, ratio of out-of-range results were found. 20.3% of all H index results, 12.0 % of all I index results and 11.0 % of all L index results were above lower limits. Positive results were evaluated by categorizing patients as 8 age groups (<1, 1-3, 4-11, 12-17, 18-64, 65-74, 75-84, >85). Rates of icterus (19.3 %) and lipemia (57.8 %) were higher among babies under 12 months of age. Additionally, rates of hemolysis were remarkably high among samples from patients aged 0-12 months (43.8 %) and >85 years (20.8 %).**Conclusion:** Improving quality of sampling by training phlebotomists especially for working with babies and elderly may reduce rate of hemolysis, whereas adequate questioning of feeding status of babies may improve rate of lipemia.

## A-257

### Reduce the impact of hook effect on hCG immunoassay by proactive backtracking confirmation

P. Gu, Y. Wen, C. Lin, H. Ning. *Chang Gung Memorial Hospital, Taoyuan, Taiwan*

**Aim:** Immunoassay is a general methodology for hCG measurement in clinical laboratories. Hook effect occurs when hCG concentration is extremely high, resulting in an falsely low result. In our study, we develop a proactive backtracking confirmation strategy to reduce the error rate caused by hook effect.

**Methods:** SIEMENS Immulite2000 XPi was used to quantitate hCG in our laboratory. The hCG calibration range is 0.4~5,000mIU/mL. For samples with hCG concentration greater than 5,000mIU/mL, the instrument was set up to switch to auto-dilution program in order to quantitate hCG correctly. The vendor claimed that there is no high-dose hook effect at concentrations up to 600,000 mIU/mL. There is no appropriate way to identify the extremely high-concentration samples if the instrument did not switch to auto-dilution mode. The laboratory could report the falsely low results. Therefore, to decrease the error rate, we developed a backtracking confirmation rule: When an over 5,000mIU/mL hCG result was shown, laboratory technologists should proactively search 7-day history results of this patient. If hCG 7-day history results were below 5,000mIU/mL, technologists retested the previous sample in a dilution mode. An over 5,000mIU/mL retested result implied that due to hook effect, the previous result was underestimated. We then corrected the result in laboratory information system (LIS) and notified clinicians. To verify the practicality of the backtracking confirmation rule, we applied it to history hCG results retrieving from LIS during the period from January through November 2016. This retrospective study was confirmed by resident doctors.

**Results:** Among 5,045 prescribed hCG tests from January to November in 2016, 19 cases met the inclusion criteria of the backtracking confirmation rule. In these cases, one patient was diagnosed as having hydatidiform mole, and hCG results of this patient before and after surgery were 2,247 mIU/mL and 101,105 mIU/mL respectively. Hook effect may explain this phenomenon. We further modified the inclusion criteria, extended the history data search setting from 7-day to 14-day. No cases revealing possible evidences of hook effect were observed. According to medical records, all cases were pregnant women or in vitro fertilization. By applying the backtracking confirmation rule, we screened out one case in December 2016. The patient was diagnosed as having hydatidiform mole. These hCG results before and after the surgery were 2,279 mIU/mL and 81,779mIU/mL respectively. The 400-fold diluted of the first sample was retested, the final result was 1,610,533 mIU/mL. The hCG results were revised and the clinician was informed timely.

**Conclusion:** A proactive backtracking confirmation rule was applied to hCG measurement. Our laboratory can timely recall and revise incorrect results caused by hook effect. Through proactive notification from laboratory technologists to clinicians, patient safety will be improved.

## A-258

### Increasing the reliability of variant reports

F. S. V. Malta[1], R. L. M. Guedes[1], G. C. Lopes[2], M. A. Pereira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil*

**Background:** Variant Callers (VC) are programs designed to detect sequence variants in genomic datasets. They have several parameters that define conditions that must be fulfilled for confirming a candidate as variant. The parameters set used in VC can be adjusted according to each strategy, for example, less stringent parameters may decrease false negatives, but increase false positives. The challenge is to define a parameter set to obtain the lowest error rate. In our laboratory routine we noticed that some parameters have more influence to the number of false negatives. These parameters (e.g., total minimum depth or minimum depth per strand) assume that a minimum number of reads must be available in the dataset, but the minimum depth parameter is not achieved, the VC will not consider these regions for the variant calling, regardless of the presence of an eventual variant. This event cannot be considered an algorithm interpretation error. In fact, those positions were not even assessed because they did not satisfy the established threshold. Here we describe a simple workflow that can be applicable by any health service laboratory that wants to increase the accuracy of variant reports. **Methods:** The dataset used was obtained from the sequencing of nine genes of NA12878 human reference sample using the Ion PGM platform. The variant calling process was performed using Torrent Variant Caller v5.0 (TVC). Our workflow basically comprised in the use of modified "low stringency" default parameters of TVC with more stringent adjustments (mapping quality, minimum total coverage and minimum coverage per strand) combined with the use of a bioinformatics tool (developed in PERL). Our tool was designed to recover regions that did not fill the minimum depth adjusted parameter in the VC. And then these regions were evaluated by manual curation and/or Sanger sequencing. The result obtained by this workflow was compared with the results obtained using the "low stringency" default parameters in the same variant caller. **Results:** Using the default parameter on TVC, we obtained 11 concordance, 2 false negatives and 4 false positives while in the analysis using our workflow, we obtained 12 concordance, 1 false negative and 1 false positive. **Discussion:** Considering those results, our workflow had better performance than the "low stringency" default. The workflow was able to reduce the number of false positives and negatives, allowing the user to recover the information about the discarded regions. This workflow will allow users to use more rigorous parameters to ensure better quality, without the risk of ignoring

target regions and variants that could be better evaluated by another methodology. **Conclusion:** We consider that our analysis workflow is an alternative to ensure more reliable results. Furthermore, other parameters can have the same behavior like the minimum depth and therefore can also be treated in the same way. Therefore we believe that improvements in the tool still can be made and enhance this workflow.

## A-259

### National Survey of Adult and Pediatric Reference Intervals in Clinical Laboratories across Canada: A Report of the CSCC Working Group on Reference Interval Harmonization

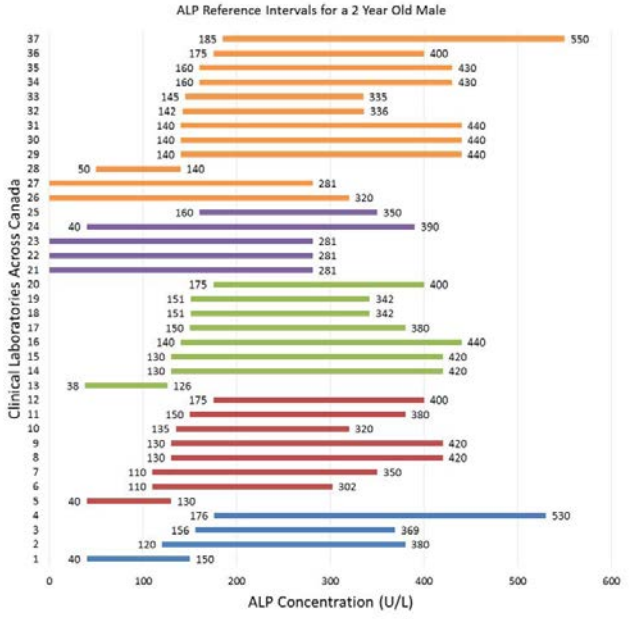
V. Higgins[1], D. Seccombe[2], C. Collier[3], C. Balion[4], G. Cembrowski[5], A. Venner[6], J. Shaw[7], K. Adeli[1]. *[1]SickKids Hospital, University of Toronto, Toronto, ON, Canada, [2]CEQAL, Vancouver, BC, Canada, [3]Kingston General Hospital and Queen's University, Kingston, ON, Canada, [4]McMaster University, Hamilton, ON, Canada, [5]University of Alberta, Calgary, AB, Canada, [6]University of Alberta, Red Deer Regional Hospital, Calgary, AB, Canada, [7]University of Ottawa, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada*

**Background:** Reference intervals are health-associated benchmarks used to interpret laboratory test results in clinical practice. There is evidence suggesting that there is wide variation in reference intervals, even between laboratories using the same analytical equipment and the same reagents. This poses a major problem for test result interpretation and patient safety, contributing to the lack of consistency in test result interpretation and decreased transferability of test results between labs. Here, we present data from a recent national survey conducted by the Canadian Society of Clinical Chemistry (CSCC) Reference Interval Harmonization (hRI) Working Group that examines the variation in pediatric and adult reference intervals, as well as a reference sample measurement in Canadian laboratories.

**Methods:** Reference intervals currently used by 37 Canadian laboratories were collected through a national survey for seven biochemical markers. Additionally, 40 clinical laboratories measured six analytes in a reference sample as a baseline assessment. The CVs and percent biases for reported reference intervals and test sample measurements were calculated and compared.

**Results:** Reference intervals for alanine aminotransferase, alkaline phosphatase (**Fig. 1**), and creatinine were most variable. As expected, reference interval variation was more substantial in the pediatric population and varied between laboratories using instruments/reagents from the same manufacturer. Test sample results differed between laboratories, particularly for alanine aminotransferase and free thyroxine. Reference interval variation was greater than test result variation for most analytes and was often not related to the observed bias of the assay.

**Conclusion:** The survey data highlights the critical lack of standardization in laboratory reference intervals across Canada, particularly for the pediatric population. The CSCC hRI Working Group aims to address this critical issue by establishing and recommending harmonized reference intervals across Canada, based on evidence-based reference data.




## A-260

### Assessment of Clinical Significance of Differences Between K2 and K3 EDTA Tubes for Routine Hematological Analyses and Glycated Hemoglobin Measurements

C. Züngün, D. I. Topcu, M. S. Gungoren. *Duzen Laboratories Group, ANKARA, Turkey*

**Background:** $K_2$ and $K_3$EDTA are both most frequently preferred additives for hematological analysis and glycated hemoglobin (A1c) measurements. The aim of this study is to compare the routine hematology test results obtained by the $K_2$ and $K_3$EDTA tubes and to investigate the analytical significance of differences between two additives for four hours stability. A1c results were also compared between $K_2$- and $K_3$EDTA tubes. **Methods:** Blood samples of 50 patients for hematological analyses and of 47 patients for A1c analyses were collected into both BD Vacutainer $K_2$ and $K_3$EDTA containing tubes considering the sufficient blood volume. For routine hematological analyses $K_2$ and $K_3$EDTA tubes were analyzed at 0th minute and four hours after the initial analysis by using Sysmex XN-1000. White blood cell, red blood cell, platelet counts and hematocrit, mean corpuscular volume and mean corpuscular hemoglobin indices obtained from both tubes which are related to cell shrinkage or dilution effect were compared to ascertain the differences between $K_2$ and $K_3$EDTA. The samples were evaluated for 6 complete blood count parameters. For short term stability testing, results of four hours preserved samples were compared against initial results. A1c analyses were performed within one-hour after sampling by Roche Cobas c501. The calculated differences were compared with total allowable error values based on biological variation to assess clinical significance. Statistical analyses were performed with R 3.3.2 (R Working Group, Vienna, Austria). **Results:** At 0th minute, the percentage of differences between $K_2$ and $K_3$EDTA tubes were WBC:0.02%, RBC:1.85%, PLT:4.39%, Hct:4.10%, MCV:2.24% and MCH:0.17%. The percentage of differences between K2 and K3-EDTA tubes at 4th hour were WBC :0.75%, RBC:0.71%, PLT:0.66%, Hct:2.96%, MCV:2.30% and MCH:0.19%. The results of four hour stability studies for K2-EDTA WBC:0.24%, RBC:1.17%, PLT:4.63%, Hct:0.04%, MCV:1.26% and MCH:0.13% and for K3-EDTA were WBC (0.97%), RBC (0.02%), PLT (0.93%), Hct (1.18%), MCV (1.19%) and MCH (0.22%). For all of 6 parameters the percentage of differences between $K_2$ and $K_3$EDTA tubes at both 0th minute and 4 hours were found below the total allowable error limits. Mean of HbA1c results of K2-EDTA samples was 38.32±10.16 mmol/mol, whereas mean of HbA1c results of K3-EDTA samples was 38.47±10.55 mmol/mol. HbA1c results of $K_2$ and $K_3$EDTA samples were positively correlated (r=0.99). The mean difference between K2 and K3 samples was calculated as 0.4%. Analytical coefficients of variation (CV's) for A1c measurements were calculated with measurement results of manufacturer's internal quality control samples. **Conclusions:** Our results demonstrated that BD Vacutainer $K_2$ and $K_3$EDTA tubes performed equivalently for routine hemataological parameters. Both tubes are found convenient to be used interchangeably for routine hematological analysis with Sysmex XN-1000. For the stability test, all of the results were found comparable in the aspect of clinical signficance of differences when compared against initial analysis. As mean difference between $K_2$ and $K_3$EDTA samples was much lower than analytical CV's, it can be concluded that there is no significant difference between BD Vacutainer $K_2$ and $K_3$EDTA in A1c results for analysis with Roche Cobas c501.

## A-261

### Correction of Lactate Dehydrogenase and Potassium Values of Hemolyzed Specimens

Y. Wang[1], S. Clapps[1], B. Horne[1], M. Ladson[1], E. Champion-Lyons[1], Y. Zhu[2]. *[1]Medical University of South Carolina, Charleston, SC, [2]Penn State University Hershey Medical Center, Hershey, PA*

**Background:** Test cancellation on hemolyzed samples is routinely performed to avoid reporting falsely elevated lactate dehydrogenase (LDH) and potassium (K) concentrations. However, this common practice is associated with an increase in turnaround time and a delay of patient treatment. This study aims to use mathematic models to predict the true values of these two analytes in hemolyzed specimens in order to reduce associated test cancellations.

**Methods:** Non-hemolyzed patient plasma containing LDH (126 U/L -1792 U/L) or K (2.6 mmol/L-5.9 mmol/L) was spiked with free Hb (0-666 mg/dL) prepared from fresh blood, with a corresponding hemolysis index ranging from 0 to 4 as determined by the Abbott Architect analyzer. Multivariate regression analysis based on concurrent measurement of LDH or K and Hb was used to establish their correction equations. Prediction validation was performed using 100 paired hemolyzed and non-hemolyzed patient samples to assess their predictive performance. Either the predicted

or measured value was used to determine the sample rejection by comparing the difference between the paired samples to the total allowable error as defined by CLIA.

**Results:** Average increase in LDH and potassium were 1.332(±0.052) U/L and 0.0031(±0.0002) mmol/L per mg/dL spiked Hb, respectively. Correction equations for the true LDH or K value (Fig. 1A, B) were established via multivariate regression, with $R^2$ of 0.993 and 0.996, respectively. The predicted LDH and K values of hemolyzed sample corresponded well with their non-hemolyzed counterparts (Fig. 1C, D). Moreover, the correction significantly reduced the difference in LDH or K values between the paired samples (P<.0.01), and consequently led to 83% and 78% reduction of sample rejection for LDH and K, respectively (Fig. 1E, F).

**Conclusion:** Our data demonstrate that mathematic corrections of LDH and K values might be a beneficial alternative to report reliable values of hemolyzed specimens and reduce unnecessary test cancellations.

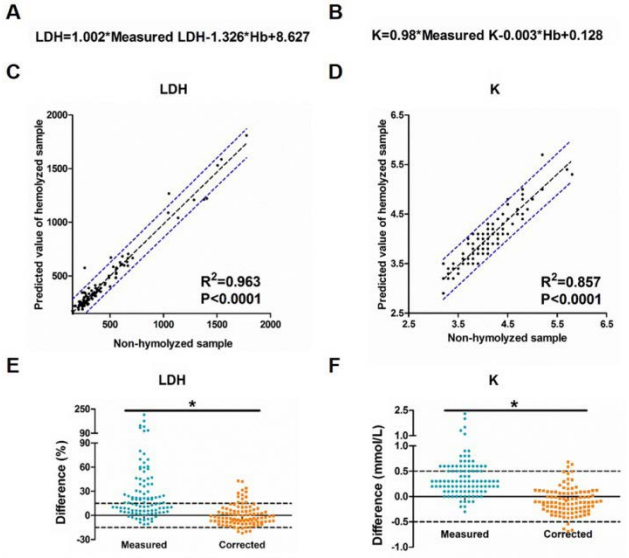



**Figure 1. Correction of LDH and K in hemolyzed specimen.** Correction equation for LDH (A) and K (B). Linear correlation between predicted values for for LDH (C) and K (D) of hemolyzed samples and its non-Hemolyzed counterparts. Dash lines indicate 95% prediction interval. Difference in LDH (E) and K (F) between the paired samples. Dash lines indicate total allowable errors.

## A-262

### Effect of non-fasting and non-morning samples on results of serum TSH and fT4 tests

C. Zungun, M. S. Gungoren, D. I. Topcu. *Duzen Laboratories Group, Ankara, Turkey*

**Background:** Sample collection requirements known to date for thyroid function tests are sampling in the morning and after an overnight (8-12 hours) fasting. However, laboratories encounter inappropriate demands from some patients who cannot meet these requirements from time to time. Although morning sample after an overnight fasting has been accepted as an acknowledged information, the number of studies found in scientific literature is limited with controversial findings. With this study, it has been aimed to compare results of fasting morning samples with non-fasting and non-morning samples for serum TSH and fT4 tests.**Methods:** This study was conducted with left-over samples of 50 individuals who was admitted to Duzen Laboratories Group for serum fasting and postprandial (120th min.) glucose tests. Samples for fasting glucose test were drawn in the morning (08.00-10.00 a.m.) whereas samples for postprandial glucose tests were drawn after 12.00 a.m. Individuals being at the age of 18 to 70, who were not on medications and had no chronic systemic disease were included. Samples were scanned from Laboratory Information System every day at around 16.00 and any eligible sample was analysed at the same day with Roche Cobas e601. Descriptive statistics and paired sample t-test were performed with R 3.3.2 (R Working Group, Vienna, Austria). **Results:** 100 samples from 50 patients were analysed. Average interval between fasting and postprandial samples were found to be 192±73 minutes. Means of fasting and non-fasting TSH results were 2.41±1.75 and 1.87±1.14 µIU/mL, respectively. There was significant difference between fasting and non-fasting samples (p<0,001). Means of fasting and non-fasting fT4 results were 16.56±2.84 and 16.51±3.28, respectively. There was no statistically significant difference between fasting and non-fasting samples (p=0.73).**Conclusion:** These results prove that non-fasting and non-morning samples cause variations in TSH levels. On the other hand, changes in conditions do not effect fT4 levels.

## A-263

### Between-generation Hemoglobin $A_{1c}$ discrepancies for the Roche Tina-quant assay

Y. Yang, E. K. Y. Leung, S. E. Groboske, K. T. J. Yeo. *University of Chicago, Chicago, IL*

**Background:** The N-terminal glycated hemoglobin A ($HbA_{1c}$) is an important biomarker for diabetes mellitus. During an in-house verification process, two generations of the Roche $HbA_{1c}$ assays exhibited significant discrepancies at lower values.

**Objective:** The study was aimed to compare the performance characteristics of the two generations of $HbA_{1c}$ assay and investigate the significant differences of $HbA_{1c}$% measurement by the two assays observed in patient samples containing low concentrations of $HbA_{1c}$.

**Method:** The $HbA_{1c}$% in the assay is calculated based on separate measurements of total Hb and $HbA_{1C}$, by absorption spectrophotometry and turbidimetric immunoassay respectively. The 3rd generations of assays were both evaluated based on analytical measuring range, precision, sensitivity, and method comparison. In addition, samples displaying significant discrepancies between the two generations of assays were further evaluated by comparison to an ion-exchange chromatography method traceable to the DCCT reference method and certified NGSP samples.

**Results:** Overall, the analytical characteristics of the new generation (3rd) $HbA_{1c}$ test were comparable with the existing older generation (2nd) assay. In a patient comparison study (n=55) performed between the two generations, the Passing-Bablok regression analysis for HbA1c% showed a slope of 1.14 and an intercept of -1.12%. The average bias between the two methods were -0.21%. More detailed analysis was performed on samples with $HbA_{1c}$% values closer to the prediabetes and diabetes diagnostic cutoff values of 5.7% and 6.5%, based on the American Diabetes Association (ADA) guidelines. In those samples (n=33), we observed a more significant difference between the two methods. Specifically, the Passing-Bablok regression analysis showed a slope of 1.29, an intercept of -2.00%, and an overall mean bias of -0.34%. Of which, 6 patient samples showed differences greater than -0.5%. Samples with biggest discrepancies were measured using a method traceable to the DCCT reference method and the reported values were between the results obtained from the 2nd and 3rd generation assay. This indicated a negative bias for the 2nd generation assay, and a positive bias for the 3rd generation. Because $HbA_{1c}$% is reported based on the measured $HbA_{1c}$ and total Hb concentration, we also compared the measured $HbA_{1c}$ and total Hb results obtained from both methods. It was identified that the significant discrepancies between the two methods were observed in samples with low amounts of $HbA_{1c}$ (<0.45 g/dL, near the LOQ of the assays). For the certified NGSP materials, the two methods reported comparative results across the measuring range (4.7% - 22.4%). Nevertheless, NGSP samples with lower $HbA_{1c}$ (<0.45 g/dL) were reported with more significant discrepancies (0.8%) between the two methods.

**Conclusion:** In patient samples with low $HbA_{1c}$ (<0.45 g/dL), significant systematic bias (1.0%) in $HbA_{1c}$% were observed between two generations of assays. As 5.7% and 6.5% are utilized as the diagnostic cutoff values for prediabetes and diabetes according ADA guidelines, such biases between the two methods can lead to different clinical interpretations for diagnosis and management of diabetes when a laboratory switches from one generation to another generation of assay.

## A-264

### Midodrine, an Antihypotensive Medication, May Produce Falsely Elevated Free Plasma Metanephrine Levels due to Analytical Interference

A. J. McShane, S. Wang. *Cleveland Clinic, Cleveland, OH*

**Background**: Free plasma metanephrines (Met) and normetanephrines (Normet) are biomarkers that aid in the diagnosis of catecholamine-secreting tumors. If left untreated, these tumors may cause severe to life-threatening repercussions. Our laboratory provides Met and Normet testing on a liquid chromatography tandem mass spectrometry (LC-MS/MS) platform. The sample preparation involves the addition of deuterated internal standards to the plasma specimen followed by weak cation exchange solid phase extraction. Recently, upon routine chromatography review a small shoulder was noticed on a Met peak in a patient specimen. Furthermore, there

was a slight retention difference from the internal standard (0.03 minutes), which is atypical for this assay. The patient's medication list was reviewed and one possible interferent was found, midodrine, an antihypotensive agent. This medication is a prodrug that biotransforms via amide hydrolysis to desglymidodrine. This active metabolite is an isobar of Met with a similar structure. Herein, is the investigation of desglymidodrine interference with the Met assay. **Methods**: An EDTA plasma pool was spiked with 25 ng/mL desglymidodrine and serially diluted to 0.78 ng/mL with blank EDTA plasma. Along with the blank each serial dilution was extracted and analyzed in triplicates. Next, desglymidodrine and a Met standard were separately infused into the mass spectrometer to obtain fragment ion spectra. A unique fragment for desglymidodrine was identified and the transition added to the panel. Samples from additional patients, midodrine-presribed and otherwise, were then analyzed. **Results**: The desglymidodrine spiking study demonstrated a linear rise ($R^2$=0.972) in Met results with increasing desglymidodrine levels. The blank plasma pool (0 ng/mL desglymidodrine) was found to have an average endogenous Met value of 14 pg/mL, but the highest spiked desglymidodrine level (25 ng/mL) averaged a Met level of 358 pg/mL. During the fragmentation studies, a 180/117 transition for desglymidodrine was found to be unique with respect to Met. No unique transitions were identified for Met with respect to desglymidodrine. The desglymidodrine transition was added to the Met assay, and monitored in midodrine-prescribed patients and midodrine-free patients. The midrodrine-prescibed patients demonstrated peaks, near the Met internal standard, in the desglymidodrine (180/117) and Met (180/148) chromatograms. For the midodrine-free patients, the desglymidodrine chromatograms did not have peaks near the internal standard. Our routine chromatogram review, for the met assay, was amended to include monitoring the unique desglymidodrine transition. If similar peaks are present in both the desglymidodrine and Met chromatograms, and they co-elute with the Met internal standard, prompt communication with the ordering physician is warranted. **Conclusions**: Patients prescribed midodrine may have falsely-elevated free plasma Met results in LC-MS/MS assays. This is due to an active metabolite, desglymidodrine, sharing the same transition as Met with a similar retention time. The same interference was not found in our laboratory's urinary Met assay. Laboratories should be aware of this potential analytical interference to avoid reporting erroneously-high Met levels.

## A-265

### Comparison of automated immunoassays across different platforms to evaluate combinability for clinical trials

E. Wagner[1], S. Narla[1], C. Thomason[1], N. Patel[2], S. Patel[2], L. Pritchard[1], P. Oefinger[1]. *[1]Covance Central Laboratory Services, Indianapolis, IN, [2]Covance Central Laboratory Services, Cranford, NJ*

**Background**

In global clinical trials, pharmaceutical sponsors rely on central laboratory systems to deliver harmonized results regardless of testing location. This is typically achieved by deploying the same instrument platforms across central laboratory sites; however, scenarios arise where different platforms are utilized across a laboratory network. Here, we performed correlation studies to investigate the combinability of immunoassays performed on different analyzers.

**Methods**

A minimum of 30 human serum or plasma samples spanning the analytical measurement range were tested in parallel over a three-day period for beta-hCG, testosterone, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), total and free thyroxine (TT4 and FT4), total triiodothyronine (TT3), prostate-specific antigen (PSA), B-type natriuretic peptide (BNP), folate, ferritin, C-peptide, insulin, immunoglobulin E (IgE), troponin I (TnI), and estradiol at the Indianapolis, IN and Cranford, NJ central laboratory locations on Siemens (Malvern, PA), Beckman Coulter (Brea, CA), and Roche (Indianapolis, IN) analyzers. Estradiol, TnI, and IgE served as positive controls for like platforms (Siemens Centaur). All assays were calibrated twice over the three day period. Quality control samples were assayed on each day prior to testing. Data reduction was performed using the Alternate Method Comparison Module on Data Innovations EP Evaluator® Version 9.4.0 software (South Burlington, VT). Methods were considered combinable if they met standard operating procedure acceptance thresholds for slope, y-intercept, and correlation coefficient calculated from a Deming linear regression.

**Results**

Correlation outcomes are listed in the table below. Positive control assays (IgE, TnI, and estradiol) tested on the Centaur CP or XP platforms demonstrated acceptable statistical correlation.

| Assay | Cranford Method | Indianapolis Method | Correlation Outcome | Average Bias (%) |
|---|---|---|---|---|
| beta-hCG | Centaur CP | cobas e601 | Fail | -21 |
| Testosterone | Centaur CP | Access DXI | Fail | -14 |
| FSH | Centaur CP | Access DXI | Pass | +1 |
| TSH | Centaur CP | Access DXI | Pass | -1 |
| TT4 | Centaur CP | Access DXI | Pass | +6 |
| FT4 | Centaur CP | Access DXI | Fail | -6 |
| TT3 | Centaur CP | Access DXI | Fail | -22 |
| PSA | Centaur CP | Access DXI | Fail | +11 |
| BNP | Centaur CP | Access DXI | Fail | +16 |
| Folate | Centaur CP | Access DXI | Fail | +10 |
| Ferritin | Centaur CP | Access DXI | Fail | -29 |
| Insulin | Immulite 2000 | Access DXI | Fail | +55 |
| C-peptide | Immulite 2000 | Centaur XP | Pass | -7 |

**Conclusions**

This study highlights the gap in standardization across automated immunoassays offered by different vendors. The lack of correlation between some vendors could not be attributed to experimental design, as assays evaluated on two generations of the Siemens Centaur platform demonstrated acceptable statistical correlation.

## A-266

### Hematuria Without Microalbuminuria

A. M. Ferguson, V. Chadha, A. Wiebold, U. Garg. *Children's Mercy Hospitals and Clinics, Kansas Ctiy, MO*

**Background:** Microalbuminuria is a condition in which a small amount of albumin is excreted in the urine in the absence of overt nephropathy. It is an early marker of kidney injury in diabetes, hypertension, and glomerular disease. Microalbuminuria is defined as persistent albumin excretion between 30-300 mg/day, 20–200 mcg/min, or 30-300 mcg/mg creatinine. Laboratory analysis of microalbuminuria is clinically utilized to detect early kidney injury, when treatment is more effective in slowing down the progression of the disease. Since urine dipstick and protein precipitation methods lack sensitivity, immunoassays are used for the measurement of trace amount of albumin in urine. It is commonly believed that in the presence of hematuria, testing for microalbuminuria is not valid. Based on this belief and manufacturers' recommendations, many laboratories cancel microalbumin testing if the urine dipstick is positive for blood. During routine clinical testing, we have encountered several urine samples that were dipstick positive for blood but tested negative for microalbumin when the clinician requested the testing be completed. **Methods:** Although this does not mimic true patient samples, to study this aspect more objectively, several urine samples with a typical creatinine concentration were spiked with 5, 10, 20 and 50 uL of whole blood (total volume 10 mL). Using a dipstick, these aliquots were tested for blood and protein. One of these samples is described here. **Results:** All of the aliquots except the 5 uL aliquot were visually positive for blood. All aliquots were dipstick positive for blood (3+), and aliquots with 10, 20 and 50 uL were positive for proteins (trace, trace and 1+, respectively.) After centrifugation, these aliquots were tested for microalbumin. All of the aliquots were negative for microalbuminuria (< 30 mcg/mg creatinine) except the 50 uL aliquot. **Conclusion:** These findings suggest that samples with hematuria may be negative for microalbumin, and therefore, laboratories should not automatically cancel microalbumin testing if a sample is dipstick positive for blood. A better strategy may be to test the sample for protein using a dipstick and cancel the test for microalbumin only if the sample is positive for greater than trace protein. Further studies using non-spiked patient samples are underway.

## A-267

### Assessment of Body Fluid Testing Requests and Validation of Methods on the VITROS® 5600

P. Lai[1], L. Trowski[2], S. Sohi[3], L. Wang[2], B. Jung[2], S. Wong[3], V. E. Barakauskas[2]. *[1]University of British Columbia, Vancouver, BC, Canada, [2]BC Children's and Women's Health Centre, Vancouver, BC, Canada, [3]Vancouver General Hospital, Vancouver, BC, Canada*

**Background:** Chemistry testing of body fluids can be useful in clinical and post mortem investigations. However, these sample types are often not validated by assay manufacturers. The objective of this study is to catalogue fluid testing requests

received by the laboratory at BC Children's and Women's Hospital (BCCW) to guide in-house validation studies.

**Methods:** *Part A*: Fluid testing practices at BCCW were evaluated by retrospective review of test orders and results in the laboratory information system (LIS) spanning a 21-month period (January 2015 through September 2016). The data query was limited to fluid-specific test codes as well as miscellaneous test requests (requests without dedicated LIS codes) and included sample (fluid) type and test result.

*Part B:* The most frequently encountered body fluids (excluding CSF) and analytes were identified as targets for initial matrix validation studies. Residual clinical samples were saved from discard and stored at -30ºC for up to 8 months for use in linearity and accuracy experiments on an Ortho-Clinical Diagnostic VITROS 5600. Due to limited sample volume, linearity studies used an admixture design. Linearity in one clinically relevant fluid type was assessed per analyte (as determined in Part A). To expand the linear range tested, for some analytes a high concentration fluid was created by spiking with urine, plasma or dextrose solution, or by mixing two different fluid types. Accuracy was assessed by spike-recovery using calibrator materials. The volume of spiking material was limited to 10% of total sample volume and percentage recovery was calculated relative to the expected concentration. Multiple samples of 2-4 fluid types were included in recovery studies for each analyte. Recovery from fluids was also compared to recovery from plasma specimens. Data analysis was performed using Excel (2010).

**Results:** A total of 4525 body fluid tests were reported over the query period. CSF and vitreous fluids accounted for 59.4% and 17.1% tests, respectively. Drain, abdominal, pleural, stomal and dialysate fluids accounted for the majority of remaining orders. Most frequently requested analytes (defined as >10 orders in 21 months) included: glucose, sodium, creatinine, urea, potassium, protein, chloride, triglyceride, lactate dehydrogenase (LDH), phosphate, albumin, amylase, bilirubin, cholesterol and lactate.

Linearity studies were performed for amylase, lipase, chloride, albumin, cholesterol, triglyceride, sodium, potassium, LDH, protein, urea, creatinine and glucose. Good linearity over the range spanned by available samples was seen for most analytes ($R^2$>0.995), minor non-linearity was seen for sodium, potassium and LDH ($R^2$>0.98), although the latter experiments consisted of admixtures of different fluid types. Maximum deviation from a linear regression line was < 7% for all analytes.

The majority of spiking experiments showed recovery within 10% of the expected concentration. Larger deviations were seen in some samples for triglyceride, LDH, lipase and creatinine (up to 30%).

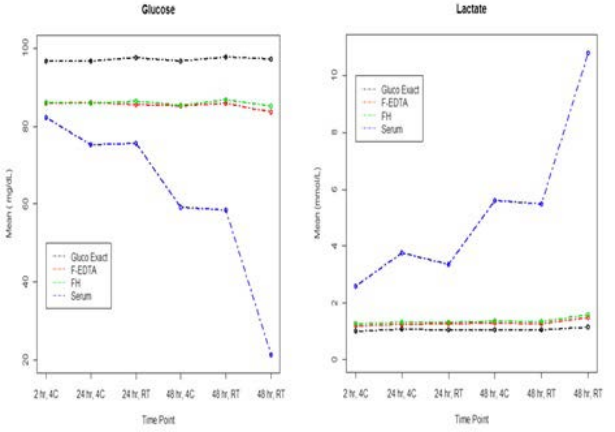
**Conclusions:** A significant number of body fluid test requests are received by the laboratory at BCCW. Linearity and accuracy studies suggest acceptable performance of many assays in multiple fluid types. Further evaluation of factors affecting electrolytes, LDH, triglyceride and creatinine measurement in select fluids is warranted.

## A-268

### Stability of Glucose and Lactate in Samples with and without Glycolytic Inhibitors: Fluoride and Citrate

J. Ulloor[1], M. Orth[2], L. Lennartz[3], K. Sikder[1], M. Beischer[1], J. Reid[4]. *[1]Abbott Laboratories, Irving, TX, [2]Marienhospital, Stuttgart, Germany, [3]Abbott Laboratories, Wiesbaden, Germany, [4]Abbott Laboratories, Longford, Ireland*

**Background:** Glycolysis decreases Glucose and increases Lactate concentrations in blood collected at room temperature (RT) in the absence of stabilizers. Although cold temperatures (4°C) inhibit glycolysis, processing blood samples at this temperature is difficult to achieve in routine clinical practice. This study evaluated the effect of different tube types and storage temperatures on Glucose and Lactate measurements. **Methods:** Fasting blood samples were collected from 34 healthy volunteers into 3 sets each comprising of Serum, Fluoride/Heparin (FH), Fluoride/EDTA (FE), Fluoride/EDTA/Citrate (GlucoEXACT) Sarstedt S-Monovette tubes. Set 1: Samples stored at 2-8°C throughout the study period and measured at 2, 24 and 48 hours post collection. Set 2: Samples stored at 2-8°C before centrifugation but after centrifugation stored at RT for the rest of the study period and measured at 24 and 48 hours. Set 3: Samples stored at RT throughout the study period, centrifuged and measured 48 hours post collection. All Glucose and Lactate measurements were performed on Abbott ARCHITECT c8000 systems using Glucose (LN 3L82) and Lactic Acid (LN 9P18) reagents. **Results:**Mean measured Glucose concentration is ~12% higher and Lactate is ~20% lower in GlucoEXACT tubes when compared to FH tubes at 2 hours (Figure 1). Samples in tubes with glycolytic inhibitors demonstrated comparable recovery for up to 48 hours compared to the corresponding tube type at 2 hour. In contrast, samples stored without glycolytic inhibitors showed ~74% lower and ~315% higher recovery at 48 hours after collection for Glucose and Lactate, respectively. **Conclusion:** The study demonstrates that in routine clinical practice, compared to Fluoride additive tubes, use of Citrate additive tubes may result in overestimation of Glucose and underestimation of Lactate. Use of Citrate additive tubes may cause higher number of Glucose determinations above the decision limit for diabetes. This may necessitate decision limits and reference intervals to be redefined for effective patient management.




## A-269

### Lipemic Interference of Ceruloplasmin Assays - An Evaluation of Lipid Removal Methods

J. J. H. Hunsaker[1], S. L. La'ulu[1], S. P. Wyness[1], J. R. Genzen[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT*

**Background**: Ceruloplasmin (CERU) is a ferroxidase and a major copper-containing protein in the circulation. CERU is typically measured on automated chemistry and/or immunoanalyzers using nephelometric or turbidimetric methods. Interference due to lipemia with some CERU assays has previously been described. It is unknown, however, whether CERU assays from different manufactures have different susceptibility to lipemic interference, or if methods to remove interferent (e.g. dilution, ultracentrifugation, or lipemia-clearing reagent) are effective when applied to specimens across different platforms. The present studies were conducted to characterize lipemic interference of CERU assays and to evaluate procedures designed to address lipemia while minimizing analytical interference with lipemic and non-lipemic patient specimens.

**Methods**: Residual human serum specimens were obtained from frozen storage (-20°C) and de-identified according to an IRB-approved protocol. Three FDA-cleared CERU assays were evaluated on instruments from their corresponding manufacturers: ARCHITECT *ci*8200 (Abbott; Abbott Park, IL); AU5800 (Beckman Coulter; Brea, CA); and Cobas Integra 400 Plus (Roche Diagnostics; Indianapolis, IN). Lipemic index was measured on each instrument concurrently with CERU. Precision studies (low and high; 2x/day in duplicate over 5 days) were conducted to assess assay imprecision. As diluents referenced in package inserts differed by assay, a diluent evaluation study comparing distilled water ($dH_2O$), 0.85% saline, and 9% saline was conducted to evaluate linearity and % recovery of CERU with serial dilutions of human AB sera (Mediatech, Inc., Manassa, VA) enriched with human CERU (SigmaAldrich, St. Louis, MO). Lipemic interference was evaluated by performing serial dilutions of human sera enriched with human CERU after spiking with triglyceride-rich lipoproteins (Sun Diagnostics, New Gloucester, ME). Methods for minimizing lipemic interference - 1:5 dilution, ultracentrifugation (AirFuge; Beckman Coulter), and LipoClear (StatSpin; Norwood, MA) - were then evaluated using human serum specimens with varying degrees of lipemia.

**Results**: CERU assays across platforms demonstrated acceptable imprecision (%CV; low, high): *ci*8200, 1.2%, 1.1%; AU5800, 3.3%, 1.5%; Integra 400 Plus, 6.1%, 2.0%. Diluent evaluation showed linearity and acceptable % recovery (85-115%) for most diluent/analyzer combinations; although a trend toward slight over-recovery (+17%) was observed on the *ci*8200 at greater than 1:4 dilution for each diluent. The CERU assay on the *ci*8200 showed greater susceptibility to interference by spiked triglyceride-rich lipoproteins than the AU5800 or Integra 400 Plus. Ultracentrifugation was more effective than LipoClear or dilution at removing lipemic interference without impacting baseline CERU results observed in non-lipemic specimens.

**Conclusions**: The present study provides comparative data for three methods of addressing lipemic interference in three different CERU assays. These findings demonstrate that the ultracentrifugation method of lipemia elimination was the least likely to interfere with underlying CERU concentration in specimens regardless of lipemia.

## A-270

### Building consensus in the Wild West: The Alberta reference interval harmonization project

J. L. Gifford[1], S. M. H. Sadrzadeh[1], T. Higgins[2], Y. Butorin[3], C. Paluck[3], A. A. Venner[1]. *[1]Calgary Laboratory Services & University of Calgary, Calgary, AB, Canada, [2]DynaLIFEDX & University of Alberta, Edmonton, AB, Canada, [3]Alberta Health Services, Red Deer, AB, Canada*

**Background:** Reference intervals (RI) are fundamental laboratory decision-making tool necessary for healthcare providers to appropriately interpret patient test results. RI should ideally represent the range of values that are present in a healthy population of a corresponding laboratory. Results that fall outside of the RI may be interpreted as abnormal and indicate the need for additional medical follow-up. Unfortunately, many labs depend on outdated, inappropriately determined or incomplete RI when reporting test results. To address this issue, there is growing interest in harmonizing RI across large geographic areas. Clinical laboratories in the province of Alberta include high volume community laboratories, rapid response laboratories located in secondary and tertiary care urban hospitals, and rural hospital laboratories. Many patients visit more than one laboratory during their clinical follow-up for testing of the same analyte, providing opportunity for result interpretation confusion and error. As such, standardizing RI within and/or across analytical platforms will reduce medical errors and provide improved patient care. The objective of this project is to develop harmonized references intervals, including appropriate age and sex partitions, for twelve frequently ordered clinical chemistry tests in the province of Alberta, Canada.

**Methods:** A posteriori indirect sampling approach was used to develop harmonized RI for glucose, creatinine, sodium, potassium, chloride, CO2, total bilirubin, calcium, total protein, albumin, alkaline phosphatase, and phosphorous. For each analyte, a survey was initially used to determine the current RI, sex and age partitions, as well as the analytical testing platform employed in every clinical laboratory in Alberta. Subsequently, de-identified patient results were obtained from the lab information systems. Due to the high volumes of tests performed in the province (>50 million/year), adult RI were developed solely from test values measured in community patients. For pediatric partitions, additional results from hospitalized patients or out-patients were required. Bhattacharya analysis was used to determine the statistically appropriate RI. Bhattacharya analysis requires large number datasets ($N$>3000) and excludes outliers by sampling the central distribution. This data was then used to help build clinically relevant RI by assessing clinical significance and through group consensus.

**Results:** Platforms measuring the twelve analytes are from Roche Diagnostics, Beckman Coulter, Ortho Clinical Diagnostics, and Siemens. The survey of current RI reinforced the need for our study as several out of date/incomplete RI were identified. After appropriate partitioning of the data according to age, sex and patient location, Bhattacharya analysis was performed using data sets of $N$=3,400-25,000 results, depending on the analyte. Consensus is being reached for all analytes; implementation is ongoing following final clinical laboratory approval and laboratory information system resource allocation.

**Conclusion:** Large data sets, a posteriori indirect sampling, Bhattacharya statistical analysis, and clinical judgment have enabled frequently ordered chemistry tests to be harmonized across multiple laboratories and analytical platforms. This not only benefits the small rural hospitals that often have minimal clinical support, but will also provide significant improvement to the care of patients within Alberta.

## A-271

### Evaluation of monoclonal peaks migrating in beta zone for the analysis of transferrin isoforms

A. Alyakut, A. Pınar, F. Akbıyık. *Hacettepe Faculty of Medicine, Ankara, Turkey*

**Background:**The congenital disorders of glycosylation were originally called carbohydrate-deficient glycoprotein syndromes, affecting primarily N-glycans. In CDG, serum glycoproteins have altered glycosylation. Transferrin contains 2 complex type N-glycans with terminal sialic acid residues. Therefore the current screening test for CDG based on analysis of serum transferrin isoforms. Transferrin isoforms can be identified by using isoelectric focusing electrophoresis (IEF), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). In this study we analysed the effect of beta migrating monoclonal components on the analysis of Tf isoforms. Our aim was to compare HPLC and two different CE methods' performances in presence of monoclonal band interferences

**Methods:**Total 27 serum samples with monoclonal gammopathy migrating in beta zone were evaluated according to their transferrin isoform pattern. HPLC system was performed on a gradient HPLC (Shimadzu Europe, Germany), using column and reagents provided in kit (EUREKA-CDT test in serum by UV/VIS-FAST). First CE system analyses were carried out with Sebia 2 Capillarys™ (Capillarys™, Sebia, France ) and second CE system analyses were carried out with Helena Biosciences' V8® E-class analyser (Helena Biosciences Europe, UK). The study was approved by the Hacettepe University Clinical Research Ethics Committee

**Results:**Transferrin isoform patterns were compared with this systems for the monoclonal band interferences. We observed that CE systems were effected by interferences, but HPLC system were minimally effected (Table)

| | | 1.CE System | | 2. CE System | | HPLC System | |
|---|---|---|---|---|---|---|---|
| | | Interference | | | | | |
| | **Total** | + | - | + | - | + | - |
| **IgG-K** | 3 | - | 3 | 1 | 2 | 1 | 2 |
| **IgG-L** | 3 | - | 3 | 3 | - | 2 | 1 |
| **IgA-K** | 11 | 8 | 3 | 7 | 2* | - | 11 |
| **IgA-L** | 7 | 6 | 1 | 5 | 1** | 1 | 5 |
| **IgM-K** | 2 | 2 | - | 2 | 1 | 1 | 2 |
| **IgD-L** | 1 | 1 | - | 1 | - | - | 1 |
| **Total** | 27 | 17 | 10 | 19 | 3 | 5 | 22 |

* 2 and ** 1 samples cannot be analysed due to insufficient serum

**Conclusion:**In this study we evaluated serum samples with monoclonal gammopathy migrating in beta zone and showed significant interferences on the CE systems. We demonstrated that analysis with HPLC system were more reliable for CDG diagnosis in terms of monoclonal gammopathy migrating in beta zone.

## A-272

### Testosterone Content in Hyaluronidase Powder: Evaluation of Commercially-Available Sources for the Pretreatment of Viscous Body Fluid Specimens

S. L. La'ulu[1], D. R. Turner[2], E. Zupan[2], J. R. Genzen[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Department of Pathology, University of Utah, Salt Lake City, UT*

**Background**: Hyaluronidase (HYAL) is frequently used for the treatment of viscous body fluid specimens prior to chemical and cellular analysis. By catalyzing the hydrolysis of hyaluronic acid, addition of HYAL helps to liquefy viscous specimens and facilitate accurate aspiration by automated analyzers. Most commercially-available HYAL used for this purpose is sourced from mammalian testes, although potential contamination by testes-derived hormones in commercial HYAL has not been investigated. The present study was therefore designed to characterize the presence and/or relative quantity of testosterone (T) in commercially available HYAL with the goal of identifying a source that is relatively free of T while still remaining effective in reducing body fluid viscosity.

**Methods**: Five HYAL powders were evaluated (3 bovine, 2 ovine): HYAL-1) bovine type I-S (Sigma Aldrich; St. Louis, MO); HYAL-2) bovine (VWR / MP Biomedicals; Santa Ana, CA); HYAL-3) ovine type V (Sigma Aldrich); HYAL-4) ovine (VWR / MP Biomedicals); and HYAL-5) ovine type II (Sigma Aldrich). Each HYAL source was reconstituted in Universal Diluent (Roche Diagnostics; Indianapolis, IN) to yield a 20 mg/mL solution. T was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS; in-house assay). HYAL with low T content was then used for serum and body fluid spiking studies to exclude potential assay interference. These experiments used human AB sera (Mediatech; Manassa, VA) and residual, clinical body fluids (biliary/hepatic, cerebrospinal, drain, pancreatic, peritoneal, pericardial, pleural, synovial, and vitreous) that were de-identified according to an IRB-approved protocol. Assays investigated included amylase, blood urea nitrogen, cancer antigen 19-9, carcinoembryonic antigen, chloride, creatinine, glucose, lactate dehydrogenase, lipase, potassium, rheumatoid factor, sodium, and uric acid on Roche cobas 8000 instrumentation. Interference studies for body fluids were limited to assays available on our test-menu for the particular fluid type. HYAL with low T content was further investigated for its ability to liquefy gels (0.5-2% in distilled $H_2O$) prepared from

hyaluronic acid powder (Alfa Aesar; Ward Hill, MA) and viscous human body fluid specimens.

**Results**: T amounts in the corresponding commercial powders were as follows (ng T per mg HYAL powder): HYAL-1) 0.4272 ± 0.0108; HYAL-2) 0.3565 ± 0.0076; HYAL-3) 0.0004 ± 0.0001; HYAL-4) 0.0590 ± 0.0016; HYAL-5) 0.0018 ± 0.0002. HYAL-3 was therefore used in spiking studies of human serum. All serum assays investigated showed % differences within acceptable limits after HYAL-3 treatment (acceptability threshold ±15%). Interference was also not observed using HYAL-3 for assay/body fluid combinations evaluated. HYAL-3 was able to effectively liquefy hyaluronic acid gels and body fluid specimens with similar performance to our previous reagent (HYAL-1).

**Conclusions**: The present study identified a commercial HYAL source that was free from T contamination and without significant interference for the laboratory assays investigated.

## A-273

### Evaluation of a New HbA1c Analyzer Mindray H50 against Bio-Rad Variant II Turbo2.0 and Interference Assessment of HbE and HbF

D. Wen, W. Wang, S. Xu, M. Li. *Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China*

**Background:** Hemoglobin A1c (HbA1c) is integral for monitoring long-term glycemic control i, and to diagnostic diabetes. It is imperative that methods used to measure HbA1c meet established performance goals and readily differentiate

hemoglobin variants that may affect HbA1c quantitation. This study was aimed to evaluate the analytical performance of a new IE-HPLC analyser to measure HbA1c (Mindray H50) . and evaluate Mindray H50 in comparison to boronate affinity HPLC method (Primus Ultra2) in patients with normal and abnormal Hb( HbE and HbF)

**Methods:** All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity and interference were evaluated according to CLSI protocols EP5-A2, EP9-A3, EP6-A and EP7-A2 respectively. Measurements of HbA1c by the three methods were made in blood from 141 patients with normal Hb(HbA )and 41 patients with abnormal Hb(Hb E, n=30, 11 HbF,n=11).Primus Ultra2 was used as comparative system. Comparative analysis and bias evaluation were conducted on the results from two detection systems, Appropriateness of data for linear regression analysis was checked regards CLSI EP9-A3 document, then performed both linear regression and difference plot analyses.

**Results:** The within-run imprecision values(CV%) were less than 0.6% and the total imprecision values(CV%) were less than 1.3%**.** Bias using reference samples from NGSP ranged from -1.68 to 2.11%. The linearity of was excellent in the range between 3.9% and 16.9%.Comparison of against Primus Ultra2 demonstrated significant correlation (r = 0.994; slope= 1.030; intercept =0.017). The differences of the 95% confidence interval (95%CI) between the test systems and the comparative system in normal HbA samples and HbE samples,were within ±0.70% HbA1c, bias% were less than 6%, (P >0.05).The results showed that the Mindray H50

was not affected by CM,Hb,F-Bil,C-Bil,Acetal,HbE and HbF(<7.1%).

**Conclusion:** The Mindray H50 shows excellent analytical performance,demonstrated high analytical performance similar to previous systems such as Primus Ultra2 and Bio-Rad Variant II turbo2.0widely used HPLC systems. were not affected by HbE and HbF(<7.1%). and is therefore suitable for its utilization in modern clinical laboratories.

## A-274

### Elimination of False Positive ELISA Signals in RF Positive Patient Specimens

M. Halat, E. Gravens. *Omega Biologicals, Inc., Bozeman, MT*

**Background:** Heterophilic antibody interference remains a prevalent and persistent source of potential error in immunoassays. Spuriously high signals may lead to further diagnostic tests or procedures that are unnecessary, costly, and potentially detrimental to the patient. Rheumatoid factor (RF) is a well known interfering substance present in the majority of rheumatoid arthritis (RA) patients. This study was designed to identify false positive signals in commercial ELISA test kits observed with RF-positive serum and plasma specimens. Two biomarkers, Human Cardiac Troponin I (cTnI) and Human Mucin 16 (CA125), were selected for their clinical importance. Human Cardiac Troponin I is a critical biomarker for assessing myocardial disease, and Human Mucin 16 is the most widely used biomarker for detection of ovarian cancer.

**Methods:** Ten RF-positive serum specimens (4 male, age 35-88 and 6 female, age 29-75, RF titer 20-160 IU/mL) and nine RF-positive plasma specimens (9 female, age 43-76, RF titer 40-900 IU/mL) were tested in commercial ELISA kits, Human Cardiac Troponin I and Human Mucin 16, per the manufacturer's protocol. Kit assay diluent was also prepared according to the manufacturer's instructions. HeteroBlock®, a commercially available blocking reagent, was added directly to the assay diluent without any additional steps such as filtering or heating. Patient specimens were diluted per the manufacturer's recommendations (2- to 2.5-fold) just prior to testing with and without HeteroBlock present in the assay diluent.

**Results:** For the Human Cardiac Troponin I ELISA test kit, elevated signals were observed for seven of the ten RF-positive serum specimens; the seven elevated signals were eliminated in the specimens prepared with assay diluent containing HeteroBlock. Nine of the nine RF-positive plasma specimens produced elevated signals; the nine elevated signals were eliminated in the specimens prepared with assay diluent containing HeteroBlock.

For the Human Mucin 16 ELISA test kit, elevated signals were observed for six of the ten RF-positive serum specimens; specifically, four of the six specimens produced a result greater than the clinically significant level of 35 U/mL. None of the ten serum specimens prepared with assay diluent containing HeteroBlock generated a signal above the limit of detection. All nine RF-positive plasma specimens gave elevated CA125 results; seven of those nine results were greater than the clinically significant level of 35 U/mL. The elevated signals were reduced below the clinically significant level of 35 U/mL when the plasma specimens were prepared with assay diluent containing HeteroBlock.

**Conclusion:** This study reinforces the need for vigilance regarding the potential for false positive results caused by heterophilic antibody interference. In this study, the addition of HeteroBlock to the assay diluents for Human Cardiac Troponin I and Human Mucin 16 commercial ELISA kits demonstrated a simple and effective means of blocking heterophilic antibody interference.

## A-275

### A Central Laboratory Interlaboratory Comparison Program to Assess the Comparability of Data of Five Chemistry Tests from Four Regional Laboratories Involved in Global Clinical Trials over a Six Month Period

C. R. Daniels, V. R. Rajagopal, T. Turner. *Medpace Reference Laboratories, Cincinnati, OH*

**Objective:** The objective of this study was to develop and support a process for assessing the comparability of data used in global clinical trials from four individual laboratories, wholly owned by the same central laboratory entity, and that the same five chemistry tests on the same samples are under statistical control and acceptable limits of variation.

**Relevance:** Data from central laboratories have been key for assessing safety, tolerability, and efficacy of new drugs in clinical trials. With the increasing complexity and global scale of many clinical trials, it is important to maintain harmonization among the regional laboratories as part of a central laboratory participating in the same study. This is usually accomplished by maintaining standardized operating procedures (SOPs) and identical assay platforms, calibrators, and quality control material across all laboratories. However, continuous monitoring of same samples run at all laboratory locations may not be common practice.

**Methodology:** The interlaboratory comparison program was established in 2006 and involves locations in the US, Europe, Singapore, and China. The program involves multiple platforms to assess over 40 analytes tested globally, encompassing several therapeutic areas including Oncology, Cardiometabolic, Infectious disease and others. Pooled serum/plasma/urine samples were aliquoted, frozen at -70ºC, and distributed quarterly to each laboratory for analysis. Here we present the dataset for five chemistry tests using the Beckman Coulter AU series chemistry analyzers. Samples were analyzed weekly on the same day at each laboratory for six months (N= 260). College of American Pathologist evaluation limits, Westgard database, and Royal College of Pathologist of Australasia were sources used to establish bias criteria. The percent bias was calculated for each result using the US laboratory as the reference laboratory. The percent bias for each week and month was also calculated. A Bland-Altman plot was created between each laboratory and the reference laboratory for the six month period, and a student T-test run using a relative bias limit for each test and a significance level of 5%.

**Results:** Overall, during the six month period all five chemistry tests had a mean bias within the acceptable bias limit for the individual test compared to the reference laboratory. When comparing the mean weekly bias for the five chemistry tests from all laboratories, any week where the bias was outside acceptable limits, investigation and

corrective action was warranted. One week during the six month period two chemistry tests from the same laboratory had a mean weekly bias outside the acceptable limit. After investigation it was determined that a pre-analytical issue with thawing/mixing of frozen samples was the cause of the bias.

**Conclusion:** An interlaboratory program where frequent monitoring of identical samples run at all laboratories involved in clinical trials can provide valuable information into the harmonization of data reported by the central laboratory, and help mitigate pre-analytic, analytic, and post-analytic issues that may arise when assessing data used in the development of new therapeutics.

## A-276

### Stability of 5-Hydroxyindole-3-acetic Acid and Vanillylmandelic Acid in Urine Specimens

J. A. Jenkins, S. Wang. *Cleveland Clinic, Cleveland, OH*

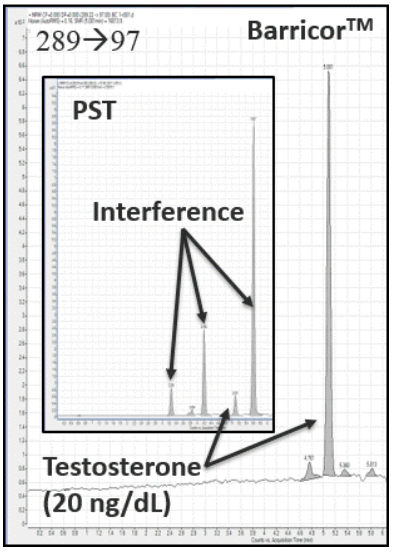
**Background:** Measurement of 5-hydroxyindole-3-acetic acid (5HIAA) and vanillylmandelic acid (VMA) in urine is useful for diagnosis and treatment of neuroblastic and carcinoid tumors. However, there is insufficient information in the literature regarding the stability of these two analytes at typical clinical lab storage conditions. The aim of this study was to determine short and long term stability of 5HIAA and VMA in native and pH-adjusted specimens at storage conditions of room temperature (RT), 4°C, and -20°C. **Method:** Leftover patient urine specimens with clinical orders for either 5HIAA or/and VMA were selected based on their original testing results. There were three groups for 5HIAA: 3 patient samples in the low range (<2.0 ug/mL), 5 in mid-range (2.0-8.0 ug/mL), and 2 in high range (> 8.0 ug/mL). There were two groups for VMA: 4 patient samples in the low range (<2.0 ug/mL) and 6 in mid-range (2.0-8.0 ug/mL). Half of each patient specimen was acidified with 12 mol/L HCl to pH 2-3. The native urine (n=10) and the acidified urine samples (n=10) were aliquoted and stored in triplicate at RT, 4°C, and -20°C for the time periods (0, 7, 14, 28, 90 and 180 days). The aliquots were analyzed by an established HPLC-MS/MS method when the specific time point was reached. All the results at different time points of different storage conditions were compared with the results of original native urine specimen at time 0. The analyte was considered stable for the specific time point at the specific storage condition if the triplicate mean was within 80% of the original result at time 0. **Results:** 5HIAA was not stable for 7 days at RT in either acidified or native urine. At RT, VMA was stable for up to 7 days in both acidified and native urine. 5HIAA was not stable at 4°C for 14 days in either acidified or native urine. VMA was stable at 4°C for up to 28 days in both acidified and native urine samples. Both 5HIAA and VMA were stable at -20°C for 90 days except acidified specimens for 5HIAA, which was not stable even for 14 days. **Conclusion:** 5HIAA stability was not conclusive for storage at RT or 4°C. 5HIAA was stable at -20°C in native urine for 90 days, while was not stable in the acidified urine. VMA was found stable for 7 days, 28 days, and 180 days at RT, 4°C, and -20°C, respectively in either acidified or native urine samples. Acidification of urine samples was shown to have little impact on VMA stability, while caused decreased stability for 5HIAA.

## A-277

### The new BD Barricor™ tube does not introduce interferences in the measurement of testosterone by liquid chromatography tandem mass spectrometry.

C. Mazur, J. Hayden. *Weill Cornell Medical College, New York City, NY*

The objective of our study was to see if the new Barricor™ tube (BC) could be used for analysis of testosterone by liquid chromatography tandem mass spectrometry (LCMS). Gel-based separator tubes introduce an isobaric substance that interferes with LCMS analysis of testosterone; this requires labs to extend their chromatography (limiting throughput) or draw an alternative tube (causing an additional blood draw). Since the BC uses an elastomer instead of a thixotropic polyester gel, it might offer a separator tube that yields samples amenable to testosterone analysis by LCMS. To investigate, samples were obtained in lithium heparin, plasma separator tubes (PST) and the BC. Consistent with literature reports, the PST showed intense, isobaric interferences which eluted near testosterone (289->97). These interferences dwarfed the true testosterone signal at lower levels, which is especially problematic as it is at these levels that analysis by LCMS is most important. In contrast, the BC and lithium heparin tube were free of this interference. Furthermore, relative to the lithium heparin tube the baseline noise in the BC was substantially reduced. These results show that the BC can be used for testosterone analysis by LCMS. This will allow labs to request and process a single tube when there are orders for automated chemistry testing and LCMS testing of testosterone.




## A-278

### Blood collection practices in the Emergency Department: Association with sample hemolysis rate

I.A. Hashim[1], A. Jones[1], M. Ruscheninsky[2], R. H. Langevin[2], G. Donahue[3], J. Cuthbert[2]. *[1]University of Texas Southwestern Medical Center and Parkland Memorial Hospital, Dallas, TX, [2]University of Texas Southwestern Medical Center, Dallas, TX, [3]Parkland Memorial Hospital, Dallas, TX*

Background:

Hemolysis is the major reason for rendering samples unsuitable for biochemical analysis. Although hemolysed hemolyzed samples are received into the laboratory from all locations within the hospital, the majority originates at the emergency Emergency departmentDepartment (ED). This project iIn collaboration with Center for Disease Control (CDC) we examined blood collection practices in effort to evaluate the effectiveness of evidence based LMBP WG recommended practices to reduce hemolysis rate in the Emergency DepartmentED.

Methods:

During the first level of this projectInitially, we collected baseline data on current blood sample collection practices in use at a large teaching county hospital (Parkland Memorial Hospital) accompanied with the baseline hemolysis rate. PHemolysis preventing measurement of potassium levels wasis used to calculate hemolysis rates.as an indicator for both denominator (total number of samples) as well as the number of rejected samples due to hemolysis because of its exquisite sensitivity associated with hemolysis. For the practices, the bBaseline data were collected from 120 patients admitted to the emergency departmentED. using tTwo approaches were used,; direct observations (n = 59) of the practices in use to draw blood specimen and retrograderetrospective interview sessions (n = 61) with the sample collection staff forassociated with specimens identified hemolyzed specimens by the laboratory as hemolyzed. A Survey Monkey toolsurvey was developed with specific observation parameters was developed; the technique of sample collection (straight needle or existing intravenousl/V line), needle size, use of saline lock, use of syringe, and syringe size, and use of J-loop, and direct use of a vacutainer collection device. Collection practice variables as well as sample quality outcomes were analyzed.

Results:

The institutional Hhemolysis rate of was 6.7%. Collection practices were varied with many different options, 25 different combinations were observed. Among hemolyzed samples 79 % were collected using existing intravenous lines with 9 different combination of practices. Among the non-hemolysed hemolyzed collections, only 6% used the existing intravenous lines to collect blood specimens.

Conclusion:

There was no standardized blood collection practice in the emergency departmentED with various options for needle size, and collection techniques. Although there was no particular practice responsible for hemolysis, the use of intravenous lines was commonly associated with hemolysed hemolyzed samples.

As next stepsNext, we will be implementing recommended practices (straight needle) to draw blood as well as other identified practices identified to reduce the hemolysis rate.

## A-279

### Validation of Lipemia, Icterus, and Hemolysis Interference for Common Chemistry Analytes using the Beckman AU 5800

J. Noguez, O. Duckworth-Liebermann, J. Meyers, T. Barker, C. Schmotzer. *University Hospitals Cleveland Medical Center, Cleveland, OH*

Background: Interfering substances can be a significant source of error in clinical laboratory measurements and may lead to inappropriate follow-up testing, incorrect diagnoses, or adversely affect treatment decisions. Hemolysis, icterus, and lipemia account for the most common interferences in clinical samples and are automatically assessed on most high-volume chemistry analyzers. Because interferences may be method or analyzer dependent, it is important to verify manufacturer interference claims when validating new instrumentation and developing interpretive comments for affected test results. The approach and level of detail provided to the consumer may vary by manufacturer. The objective of this study was to evaluate the presence, direction, and degree of interference from hemoglobin, bilirubin, Intralipid, and human lipids on 41 common chemistry assays on the Beckman AU 5800 chemistry analyzer.

Methods: Hemolysate, Unconjugated Bilirubin, and Triglyceride-rich lipoprotein interferents were purchased from Sun Diagnostics (New Gloucester, ME). Intralipid was acquired from the hospital pharmacy. Pooled patient samples were spiked with varying concentrations of the interferents corresponding with the LIH indices and run on the AU5800. Significant deviation from the original result was assessed for each analyte using CLIA limits (if available) or a difference of 10% as recommended in the AU5800 IFU assay-specific interference criteria.

Results: 22 of 41 analytes tested required appending of a comment due to interference exceeding the defined acceptable limit for that analyte. Interference thresholds were established for 5 analytes.

Conclusions: This study validated the degree of lipemia, icterus, and hemolysis interference outlined in the Beckman AU5800 IFUs for 41 chemistry analytes as well as established the directionality of the interference. Once defined, interpretive comments were developed to guide clinical decisions using affected test results.

| **LIPID** | mg/dL | | **BILIRUBIN** | mg/dL | | **HEMOLYSIS** | mg/dL | |
|---|---|---|---|---|---|---|---|---|
| Ammonia | >40 | Inc | Alk. Phos. | >20 | Inc | Albumin | >300 | Inc |
| AST | >300 | Inc | Ammonia | >20 | Inc | Ammonia | >50 | Inc |
| T. Bilirubin | >300 | Inc | Magnesium | >20 | Inc | AST | >50 | Inc |
| | | | | | | BHB | >50 | Inc |
| Prealbumin | >200 | Dec | Lactate | >2.5 | Dec | Potassium | >50 | Inc |
| Ferritin | >300 | Dec | Tot. Chol | >5.0 | Dec | Iron | >50 | Inc |
| ALT | >300 | Dec | Lipase | >10 | Dec | LDH | >50 | Inc |
| Iron | >300 | Dec | Creatinine | >20 | Dec | Magnesium | >100 | Inc |
| | | | Amylase | >20 | Dec | Phosphorus | >300 | Inc |
| | | | | | | | | |
| | | | | | | D. Bilirubin | >50 | Dec |
| | | | | | | Haptoglobin | >50 | Dec |
| | | | | | | Amylase | >200 | Dec |
| | | | | | | Alk. Phos. | >300 | Dec |
| | | | | | | GGT | >300 | Dec |

Table 1: Degree and direction of interference determined via spiking with Intralipid, Triglyceride-rich lipoproteins, Bilirubin, or Hemolysate for chemistry analytes using the Beckman AU 5800. (Inc= increase, Dec = decrease)

## A-280

### Evaluation of Biotin Interference on Abbott ARCHITECT Assays

J. Jaraczewski, M. Hauptman, R. Schneider. *Abbott Diagnostics, Abbott Park, IL*

**Background and Relevance:**

The use of biotin as a supplement has increased significantly in recent years and many health care professionals do not realize their patients are taking high doses. The increase has resulted in an increased prevalence of people being exposed to levels much higher than the recommended daily dose and as a consequence, inaccurate lab results for assays that utilize the free capture biotin-streptavidin methodology. The purpose of this study was to identify any Abbott assays that may be susceptible to biotin interference based on assay design and then evaluate the performance of these assays with high concentrations of biotin. After a comprehensive review of Abbott's current on market ARCHITECT clinical chemistry and immunoassay methods, no assays were identified that utilize the free capture biotin-streptavidin; however, 5 assays were identified for subsequent interference testing as they contain streptavidin or biotin in the assay design.

**Materials and Methods:** For each of 5 ARCHITECT assays: (Methotrexate; Active B12; Vitamin D; 2nd Generation Testosterone; Anti-CCP), sample pools were created and spiked with concentrations of biotin between 30 – 1000 ng/mL. For Active B12, a single sample in the normal range was utilized. For the other assays, two sample pools were used for testing with one negative/near the lower end of the assay or medical decision point and one positive/near the upper end of the assay or medical decision point. The biotin spiked samples were tested against a control sample preparation (no biotin) to determine if there was a statistical difference between the untreated and biotin containing specimens.

**Results:**

Table 1 ARCHITECT Assays

| **Assay** | **Concentration of untreated Sample pool** | **Specimen** | **% Difference From Untreated Sample Pools (95% Confidence)** |
|---|---|---|---|
| Active B12 (LN 3P24) | 87.91 U/mL | Normal | -3.51 to 3.70 |
| Anti-CCP (LN 1P65) | 0.66 U/mL | Low (Negative) | -3.59 to 7.40 |
| | 10.70 U/mL | High (Positive) | -0.15 to 3.80 |
| Methotrexate (LN 2P49) | 0.051 umol/L | Low | -3.80 to 3.71 |
| | 0.877 umol/L | High | 0.36 to 6.15 |
| Testosterone – 2nd Gen (LN 2P13) | 6.92 nmol/L | Low | -4.59 to 0.52 |
| | 21.36 nmol/L | High | -4.84 to 1.15 |
| Vitamin D LN 3L52 | 25.76 ng/mL | Low | -0.14 to 8.02 |
| | 35.16 ng/mL | High | -0.35 to 7.32 |

**Conclusions:** Five ARCHITECT assays potentially susceptible to biotin interference, based on assay design, were tested at increasingly high concentrations of biotin. No Abbott ARCHITECT assay evaluated as part of this study yielded result variability due to biotin interference at concentrations up to 1000ng/mL.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Hematology/Coagulation

## A-281

### Spectrum of Mutations in *Hbb* Gene among Thalassemia major patients in a cohort of Nepalese population

S. Thapa[1], N. Thakur[1], A. Jang Kunwar[1], S. Kafle[1], K. Singh[2]. *[1]Kathmandu Center for Genomics and Research Laboratory(KCGRL), Lalitpur, Nepal, [2]Nobel College, Kathmandu, Nepal*

**Background**

The thalassemias are the most common monogenic disorders with a genetically determined reduction in the rate of one or more types of normal haemoglobin polypeptide chain resulting in a decrease in the amount of haemoglobin involving the affected chain. Beta thalassemia is a highly heterogeneous disorder in its phenotype, geographical distribution and molecular mechanism[1, 2].

**Method**

DNA was extracted from the 26 clinically diagnosed blood sample and Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS PCR) was used for amplification to analyze mutations in *hbb* gene and 2% gel electrophoresis was used for visualization of PCR products[3].

**Results**

Among 26 β-thalassemia major patients, 13 (50%) had IVS 1-5 (G>C) mutation, 8 (30.76%) had 619bp deletion, 2 (7.69%) CD 8/9 (+G), 1 (3.84%) CD 15 (G>A), 1 (3.84%) had -88 (C>T) mutation whereas CD41/42 (-TCTT) was not detected in any of the patients. Among the patients with 619 bp deletions 2 (25%) were homozygotes and 6 (75%) were heterozygotes.

**Conclusions**

This is the baseline study to assist in the regulation of proper new health policies which will impact in the proper diagnosis and treatment.

**Keywords**

ARMS-PCR, Beta-Thalassemia, Nepal

**References**

1.Khattak SA, Ahmed S, Anwar J, Ali N, Shaikh KH. "Prevalence of various mutations in beta thalassaemia and its association with haematological parameters", JPMA The Journal of the Pakistan Medical Association., 62(1): pp .40-3, 2012.

2.Kumar R, Singh K, Panigrahi I, Agarwal S. "Genetic Heterogeneity of Beta Globin Mutations among Asian-Indians and Importance in Genetic Counselling and Diagnosis", Mediterranean journal of hematology and infectious diseases., vol 5, no 1, 2013.

3. Urvashi Bhardwaj, Yao-Hua Zhang, Fred Lorey, Linda L. McCabe, Edward R.B. McCabe. "Molecular Genetic Confirmatory Testing from Newborn Screening Samples for the Common African-American, Asian Indian, Southeast Asian, and Chinese β-Thalassemia Mutations", American Journal of Hematology., vol 78, pp. 249-255, 2005.

## A-282

### Effects of 100-km ultramarathon on haematological variables in runners with hepatitis B virus carriervariables in runners with hepatitis B virus carrier

W. JEN[1], W. Kao[2], L. Li[3], C. How[4]. *[1]Department of Pathology and Laboratory Medicine, Teipai Veterans General Hospital, Taipei, Taiwan, [2]Department of Emergency Medicine, School of Medicine, Taipei Medical University, Taipei, Taiwan, [3]Department of Pathology and Laboratory Medicine, Teipai Veterans General Hospital and School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [4]Emergency Department .Teipai Veterans General Hospital and Department of Emergency Medicine,School of Medicine, Yang-Ming University, Taipei, Taiwan*

**Background:** Ultramarathon is known to cause immediate post-race erythropoietin response, followed by substantial sports anemia. Liver and kidney are the two major organs to produce erythropoietin (EPO). Kidney and Liver injury with augmented production of many cytokines may influence EPO synthesis and response. The aim of the study is to explore whether haematological change might be different between hepatitis B (HBV) carrier and non-HBV ultramarathon runners.

**Methods:** Blood samples were collected from eight asymptomatic HBV carriers and eighteen non-HBV individuals who finished a 100-km ultramarathon race. For each subject, the samples were collected at three different times: (1) one week before race, (2) immediately following the race and (3) 24 hours after the race. Samples were analyzed and compared between these 2 groups for red blood cells (RBC) counts, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV) and plasma EPO levels.

**Results:** HBV carrier runners had a less variation on hematological change. The Hct, RBC counts, and Hb values in HBV carrier group were only slightly elevated immediately following the race and dropped to a lesser extent 24 hours after the race, compared to those of non-HBV subjects. There was no difference on change of MCV values in both groups. In HBV carrier runners, plasma EPO levels were relatively higher at baseline, and increased significantly in the same fashion in response to ultramarathon.

**Conclusions**: This is the first study to explore how hematological change specifically for ultramarathon runners with HBV carrier runners. The hemoconcentration by the end of the run was due to EPO production. Ultramarathon increased EPO production in both HBV carrier and non-HBV runners. Although HBV carrier runners have an increase of EPO immediately following the race, their change on Hct, RBC count, and HGB values or Hct had a less variation, implying that HBV carrier runners might have EPO hyporesponsiveness.

**Keywords:** EPO, hepatitis B carrier, hyporesponsiveness, hemoconcentration

## A-283

### Analysis of the relationship between the difficult and rare inclusion bodies and clinical prognosis in patients with various hematological diseases

J. Zhang. *Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu,sichuan, China*

**Background:** In the light of the situation that the morphology, the name and the composition of the inclusion bodies of various blood diseases in the world are not uniform, the purpose of this paper is to summarize the relationship between the inclusion bodies and the clinical prognosis of various blood system diseases.

**Methods:** We collected hematological diseases cases from January 2015 to December 2016 in our hospital ,which included 259 cases of myeloid leukemia, 206 cases of lymphoproliferative disease and 336 cases of multiple myeloma, the intracellular inclusion shape, proportion and its correlation with clinical prognosis were also retrospectively analyzed.

**Results:** All kinds of hematological diseases can be found in different forms of inclusion bodies. The appearance of Auer's bodies in AML-M3 was the most common, accounting for 36.2%, followed by multiple myeloma, up to 6.2%, the inclusions morphological changes of the maximum again for all types of other leukemia cells. It can be a kind of Auer's like body, spindle shape, coarse granular, spherical, crystalline, amorphous and multiple forms etc. The ratio of AML-M2 inclusion is 3.2% while the AML-M4 inclusion proportion is 2.4%, the inclusions can be Chediak-Higashi-like granules, spherical purple red structure, golden square crystal and so on. However, lymphoproliferative disease inclusions in the lowest rates of only 1% and appeared like Auer's body. Besides, there is no direct relationship between inclusion bodies and clinical prognosis in patients with various hematological diseases.**Conclusion:**Inclusion bodies can be found in nearly every series of hematological diseases. However, the frequency of inclusion bodies, the morphological characteristics of each inclusion body and its correlation with the diagnosis and prognosis of the disease still need to be verified by a large number of clinical data.

## A-284

### Performance evaluation of Nanopia® PAI-1 for measurement of plasminogen activator inhibitor-1

Y. Tanaka, S. Katagiri, E. Suzuki, N. Maruyama, Y. Sakai, A. Sato, T. Nonaka, A. Yoshihara, T. Yamada. *Nagaoka Red Cross Hospital, Nagaoka, Japan*

**Background:** Plasminogen activator inhibitor-1 (PAI-1), produced from the vascular endothelium, inhibits fibrinolytic reactions intravascularly. PAI-1 forms complexes

with tissue type plasminogen activator (t-PA)—the fibrinolysis factor—resulting in t-PA losing its activity. PAI-1 levels are useful to determine the state of coagulation and intravascular fibrinolysis and are elevated in disseminated intravascular coagulation, sepsis, etc. This study evaluated the performance of Nanopia® PAI-1—a newly developed general-purpose reagent from Sekisui Medical—as well as the correlation between Nanopia PAI-1 and the LPIA-tPAI test from LSI Medience Corporation. **Methods:** A precision study was conducted using the CP3000 analyzer. Within-day reproducibility was evaluated by measuring two concentration-control samples ten consecutive times. Between-day reproducibility was evaluated by measuring two concentration-control cryopreserved samples for ten days. To evaluate dilution linearity, we prepared eight dilution series of high-concentration samples (2000 ng/mL), using physiological saline, and measured nine series of samples, including blanks, in duplicate. A correlation study between Nanopia PAI-1, using the CP3000 analyzer, and the LPIA-tPAI test, using the LPIA-NV7 analyzer, was conducted with 50 patients' plasma samples. **Results:** Within-day coefficients of variation (CVs) were 4.23% and 2.96% and between-day CVs were 5.11% and 2.93% for low-concentration control and high-concentration control, respectively. The graph of dilution linearity showed a convex upward curve up to 2000 ng/mL. It was inferred that there was linearity up to 300 ng/mL. Hence, we examined the precise linearity. We prepared ten dilution series of high-concentration samples (350 ng/mL), using physiological saline, and measured 11 series of samples, including blanks, in duplicate. As a result, good linearity that passed through a point near the origin was obtained. The correlation study (n = 50, range 8.9 to 184.3 ng/mL) showed good correlation ($r^2 = 0.966$). The regression formula was: $y = 0.92x + 6.58$. **Conclusion:** This study demonstrated good precision and correlation. It showed that high-concentration samples exceeding 300 ng/mL, which is the upper measurement limit of Nanopia PAI-1, can be measured accurately by dilution. Based on these considerations, it seems plausible that Nanopia PAI-1 has sufficient performance in routine laboratory tests.

## A-285

### A case of therapy-related acute leukemia with mixed phenotype with *BCR-ABL1* after treatment of diffuse large B-cell lymphoma

D. Yang[1], S. Cho[2], J. Lee[3], Y. Kim[1], J. Choi[4], M. Kim[1]. *[1]Kosin University College of Medicine, Busan, Korea, Republic of, [2]Ajou University School of Medicine, Suwon, Korea, Republic of, [3]Yeongam-gun Public Health Center, Yeongam, Korea, Republic of, [4]Yonsei University College of Medicine, Seoul, Korea, Republic of*

**Background:** Although therapy-related acute leukemia (tAL) is a well-recognized clinical syndrome and is increasing owing to the prolonged survival of patients treated with chemoradiotherapy, tAL with mixed phenotype is extremely rare. Here, we report a rare case of tAL with mixed phenotype with *BCR-ABL1* after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

**Methods:** A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.**Results:** Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/L; platelet count, $129\times10^9$/L; and white blood cell count, $2.41\times10^9$/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspiration smears revealed 40.7% leukemic blasts with medium cell size, oval to round shape, vesicular nuclei, fine chromatin patterns, and basophilic cytoplasm. On cytochemical staining, these blast cells were not positive on PAS and NSE staining, but were weakly positive for MPO staining. Flow cytometric analysis showed that the blasts were positive for both T-lymphoid and myeloid markers (cytoplasmic CD3, 87%; CD5, 90%; CD7, 96%; cytoplasmic myeloperoxidase, 20%; CD13, 91%; CD33, 87%) and negative for CD2, CD10, CD11b, CD14, CD15, CD19, CD20, CD61, CD117, and TDT. Immunophenotyping fulfilled the diagnostic criteria of T/myeloid biphenotypic leukemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcription PCR using HemaVision kit (Bio-Rad Laboratories) revealed the presence of minor *BCR-ABL1* (*e1a2*) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirates.

**Conclusion:** Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5-1% of leukemia. The T/myeloid phenotype is rarer and represents 35% of all MPAL cases The risk of secondary malignancies after lymphoma treatment is relatively increased for leukemia. AML, ALL, MDS, CML and chronic myelomonocytic leukemia are reported secondary hematologic malignancies. Until now, only one case of tAL with mixed phenotype after lymphoma has been reported worldwide. To the best of our knowledge, this is the second case of tAL with mixed phenotype after DLBCL. This case is also unique because the *BCR-ABL1* has not been described in the literature for patients with tAL with mixed phenotype, after hematologic malignancy. According to the 2008 WHO classification, tAL can be attributed to radiation, alkylating agents, or topoisomerase II inhibitors. Our patient did not receive radiation therapy but previously received cyclophosphamide and doxorubicin. Therefore, this is the first case of tAL with mixed phenotype and *BCR-ABL 1* after alkylating agent and topoisomerase II inhibitor therapy for DLBCL.

## A-286

### Preliminary results from an international comparative laboratory field study using BAY 94-9027, a site-specifically PEGylated recombinant factor VIII product

N. Church[1], L. Leong[2], Y. Katterle[3], Y. Rao Danda[1], H. Ulbrich[3], I. Noerenberg[3], S. Bruns[4], L. A. Michaels[1]. *[1]Bayer, Whippany, NJ, [2]Bayer, US Innovation Center, San Francisco, CA, [3]Bayer AG, Berlin, Germany, [4]Winicker Norimed GmbH, Nuremberg, Germany*

**Background:** Accurate measurement of factor VIII (FVIII) activity in patients with hemophilia A is important for patient monitoring and treatment decisions. Discrepancies in results using different assays or reagents to measure prolonged–half-life factor products have been recognized and highlight that effective monitoring of patient response to these products may require adjustments in clinical laboratory practices. A global field study was conducted to assess the ability of clinical laboratories to measure BAY 94-9027 activity in spiked hemophilic plasma samples using their in-house or specific assays. BAY 94-9027 is a prolonged–half-life FVIII product site-specifically conjugated with a 60-kDa polyethylene glycol molecule (2×30 kDa branched). **Methods:** In this 2-part study, laboratories received sample sets (3–4 per laboratory) of 26 blinded samples in randomized order for analysis. Each set consisted of triplicate test samples of BAY 94-9027 or a comparator (antihemophilic factor [recombinant] plasma/albumin-free method [rAHF-PFM (Advate®); Shire]) spiked at low (<10 IU/dL), medium (10–50 IU/dL), and high (50–100 IU/dL) concentrations in pooled hemophilic plasma. Normal control plasma and unspiked hemophilic plasma in triplicate were positive and negative controls, respectively. Two additional blinded samples matching 2 of the other 24 samples in the set were included in each set to decrease predictability of the sample sets. Laboratories analyzed test samples using their in-house assays, reagents, and standards (part 1). An additional sample set was provided if laboratories used both the one-stage and chromogenic assays. In part 2, all laboratories tested 2 additional sample sets using 2 activated partial thromboplastin time kits (Pathromtin® [Siemens] and HemosIL® SynthASil [Instrumentation Laboratory]) previously shown to accurately measure BAY 94-9027 and full-length FVIII. FVIII recovery and FVIII levels were primary and secondary endpoints, respectively. Results were analyzed for intra- and interlaboratory variation. **Results:** 52 laboratories in North America, Europe, and Israel participated in the field study. In part 1, 49 laboratories tested samples using the one-stage assay, 16 used the chromogenic assay, and 13 used both assays. The reagents routinely used for measuring FVIII activity varied among participating laboratories. Mean FVIII recovery ranged from 75.1%–103.2% for BAY 94-9027 and 94.6%–114.7% for rAHF-PFM across all concentrations and reagents using the one-stage assay. As expected based on previously published data, the PTT-A (Stago) and HemosIL® APTT-SP kits (Instrumentation Laboratory) underestimated BAY 94-9027 at all concentrations. More accurate one-stage results were generated using the Pathromtin® and HemosIL® SynthASil kits as shown in part 2 of the study. For the chromogenic assay, mean FVIII recovery ranged from 104.4%–117.1% for BAY 94-9027 and 87.7%–107.8% for rAHF-PFM across all concentrations. Interlaboratory variability was low for measurement of BAY 94-9027 with chromogenic assays. **Conclusions:** Results from this global field study indicate that chromogenic assays are an accurate method for measurement of plasma FVIII levels of BAY 94-9027. FVIII activity in patients receiving BAY 94-9027 can also be accurately monitored using many commonly used one-stage assay kits without need of a conversion factor. Understanding the limitations and advantages of specific assay kits is important for choosing the correct systems to measure FVIII products in clinical practice.

## A-287

### Complete Blood Count (CBC) Reference Intervals across Pediatric, Adult, and Geriatric Ages: Indirect Establishment from Health Checkups Data

E. Nah. *Korea association of Heath Promotion, Seoul, Korea, Republic of*

**Introduction:** Comprehensive studies on the health-associated reference interval (RI) for complete blood count (CBC) are uncommon. The conventional approach of sampling a healthy reference population for the generation of RI is not commonly available in children and geriatrics, either. Thus, we have established the age- and gender-specific RI across pediatric, adult, and geriatric ages for the 17 routine CBC items by indirect method.

**Methods:** We utilized the database of hospital information system to pre-exclude some subjects by exclusion criteria and to obtain CBC results from our health checkups database. Data derived from more than eight trillion reference population with gender- and age-specific subgroups comprising between 4,367 and 442,002 persons. After applying exclusion criteria and removing outliers, we have determined statistically relevant partitions of age and gender, and calculated RI, including 90% CIs according to CLSI C28-A3 guidelines.

**Results**: CBC values showed dynamic changes not only between gender but also ages. Most items were needed partitions by gender and ages. Gender difference was observed for hematocrit, hemoglobin, red blood cell count, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Hematocrit, hemoglobin and red blood cell count increased with age until adulthood except for puberty and decreased in geriatrics. White blood cell and platelet counts were highest in early childhood and early adolescence, respectively. Both of them decreased with ages. Neutrophil counts were lowest in early childhood and increased with ages. Otherwise, lymphocyte counts were highest in early childhood and decreased with ages.

**Conclusions**: This study provided comprehensive age- and gender-specific RIs for routine CBC items, which are expected to contribute for assessment of health and disease status.

## A-288

### Performance evaluation of a new generation of automated analyzer for pleural and peritoneal fluids cytology

P. V. Bottini, C. R. Garlipp, B. F. Nogueira, D. B. Pompeo, M. I. Souza. *Univesity of Campinas, Campinas, SP, Brazil*

**Background:** Body fluids are generally sent for urgent analysis. Traditional manual cytology is a time-consuming and low precision procedure, subjective and prone to interoperator variability. Therefore, the use of an automated analyzer improves the TAT, reducing the time to report a preliminary result to the clinician. The objective of this study was to evaluate the performance of the recently installed Sysmex XN-3000 and compare it with the Sysmex XE-5000, which has been in use for some years in our laboratory for pleural and peritoneal fluids cytological analysis.

**Methods:** We studied 108 pleural and peritoneal fluids. All samples were sent in an anticoagulant-treated tube and analyzed up to 2 hours after collection. The laboratory routine included automated total and differential cell counts (Sysmex XE-5000 and Sysmex XN-3000) and manual differential counts (cytocentrifuged air-dried hematological staining of May-Grunwald). Sysmex XN-3000 validation protocol included precision, carryover, linearity studies and comparison with traditional microscopic differential counts and with the analyzer in use (Sysmex XE-5000). Simple linear regression (least-square method), paired t-test, and kappa agreement were used to the statistical analysis.

**Results:** Sysmex XN-3000 met all the requirements for analytical quality regarding precision (CVs < desirable specifications for imprecision) and linearity (r>0.99). Carryover effect was minimal (<0.1%). Sysmex XN-3000 demonstrated a strong correlation with microscopy regarding WBC differential counts (r=0.95 for both MN and PMN), with an agreement of 93% (kappa=0.813, p<0.0001). Comparison between both analyzers revealed no significant differences from a clinical or statistical point of view. Sysmex XN-3000 WBC and RBC counts were highly correlated with that of the Sysmex XE-5000 reference method (r>0.98 in both cases). An excellent agreement between both analyzers was also observed for mononuclear cells (MN) and polymorphonuclear cells (PMN, r=0.99 in both cases).

**Conclusion:** Our data demonstrated that the performance of both analyzers is equivalent, allowing both to be interchangeable without impact on the final report. Additionally, Sysmex XN-3000 showed strong correlation and agreement with traditional microscopy.

## A-289

### Evolution in the incidence of monoclonal gammopathies in a southern Spain tertiary hospital in the last thirteen years

J. Maesa[1], M. de Toro-Crespo[1], P. Menendez-Valladares[1], N. Barbosa[2], I. Rodriguez-Martin[1], C. Bermudo-Guitarte[1]. *[1]Hospital Universitario Virgen Macarena, Sevilla, Spain, [2]The Binding Site, Barcelona, Spain*

**Background:**

Monoclonal gammopathy (MG) is the most common plasma cells disorder. It affects around 3% of the population older than 50 years. The great majority of MG are monoclonal gammopathies of undetermined significance (MGUS), which is a premalignant disorder defined to present less than 3 g/dL of serum monoclonal protein, less than 10% of clonal bone marrow cells and absence of end-organ damage. MGUS is easily detected in laboratory tests and should be monitored because 1% of MGUS per year progress to Multiple Myeloma (MM).

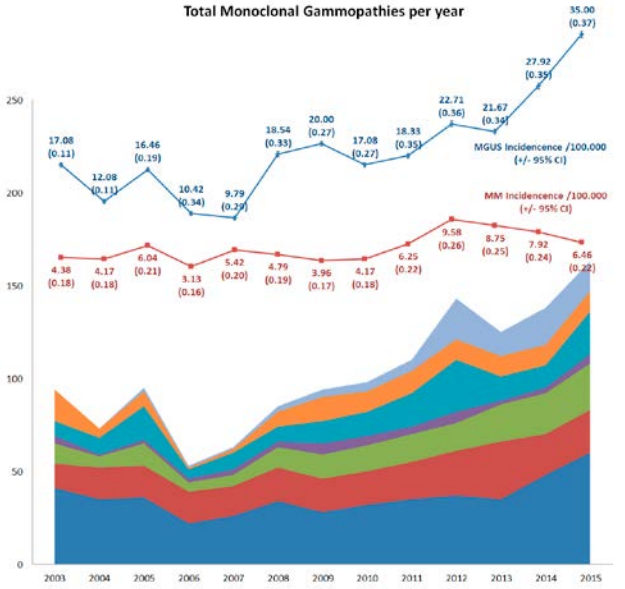
Incidence of MGUS and MM is not always easy to determine, but there is a general perception of an increasing incidence that can be attributed to different causes. One is the aging of the population. Another reason is the contribution of clinical laboratories, which count on new determinations (free light chains) or improved techniques in electrophoresis, nephelometry or immunofixation, allowing them to support the diagnose of MGUS that years before remained undiagnosed.

The aim of this study is to determine the incidence of MGUS, MM and its different types in the reference population of a tertiary hospital in southern Spain between 2003 and 2015.

**Methods:**

In a retrospective study, we determined the total number of MG and its different types diagnosed in our hospital between 2003 and 2015. We calculated the incidence per 100.000/year of MGUS and MM, with 95% confidence intervals. Our reference population, in 2015, was 480.851.

**Results:**




| | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| kappa/lambda | 0 | 0 | 2 | 1 | 1 | 3 | 4 | 5 | 6 | 22 | 13 | 20 | 16 |
| IgA/lambda | 17 | 5 | 8 | 1 | 2 | 8 | 13 | 11 | 12 | 11 | 11 | 11 | 11 |
| IgA/kappa | 8 | 9 | 18 | 5 | 9 | 8 | 12 | 13 | 18 | 28 | 13 | 12 | 23 |
| IgM/lambda | 4 | 1 | 2 | 2 | 3 | 3 | 6 | 5 | 4 | 6 | 2 | 3 | 5 |
| IgM/kappa | 11 | 6 | 12 | 5 | 6 | 11 | 13 | 14 | 15 | 15 | 20 | 22 | 25 |
| IgG/lambda | 13 | 17 | 17 | 17 | 16 | 18 | 18 | 18 | 20 | 24 | 31 | 22 | 23 |
| IgG/kappa | 41 | 35 | 36 | 22 | 26 | 34 | 28 | 32 | 35 | 37 | 35 | 48 | 60 |

**Conclusion:**

The aging of population and the higher sensitivity of laboratory techniques for diagnosing of MG is reflected in the incidence of MGUS, which increased from 17.04 cases per 100.000 in 2003 to 35.00. MM incidence in our area did not increased in parallel.

## A-290

### Usefulness of JAK2 Exon 12, MPL W515, and CALR Exon 9 Mutation Tests for Clonality Identification for Philadelphia Chromosome-negative Myeloproliferative Neoplasms: Single-institution Comparison Study

S. Hong, H. Jeong, H. Chung, E. Lee, H. Lee, H. Eom, D. Lee. *National Cancer Center, Goyang, Korea, Republic of*

**Background:** Clonality identification using molecular methods enhance the accuracy of the diagnosis of hematologic malignancies. Recently introduced molecular marker, calreticulin (CALR) gene mutation affect improving the diagnosis of BCR/ABL-negative myeloproliferative neoplasms (MPNs) after Janus kinase 2 (JAK2) V617F mutation discovered in 2005. The frequency of CALR and myeloproliferative leukemia (MPL) genes mutations have been reported differently according to the category of MPNs and ethnicity. However, practical data from a clinic are limited. Therefore, we conducted a study comparing the diagnostic value of additional molecular tests (JAK2 exon 12, MPL W515, and CALR exon 9) to JAK2 V617F test for BCR/ABL-negative MPNs.

**Methods:** We included adult patients (≥ 18-year-old) who were diagnosed as polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), or myeloproliferative neoplasm, unclassifiable (MPN-U) between January 2009 and December 2016 at National Cancer Center, Korea. Medical records of the patients were retrospectively reviewed. Genomic DNA was extracted from bone marrow or peripheral blood of all patients. Allele-specific PCR (AS-PCR) was used for JAK2 V617F mutation from 2009 to June 2014, and Sanger sequencing was used to detect JAK2 V617F and exon 12, MPL W515, and CALR exon 9 from July 2014. The chi-square test was used for statistical analysis, and $p < 0.05$ was considered statistically significant.

**Results:** Total patients were 41 (M/F=23/18, mean age=60.2 ± 11.5 years); the number of patients tested for JAK2 V617F only was 12, and the others (29) were performed additional tests. The MPN subtypes was following as [total (ET, 19; PV, 12; PMF, 9; MPN-U, 1), before (ET, 7; PV, 2; PMF, 3) and after (ET, 12; PV, 10; PMF, 6; MPN-U, 1) Sanger sequencing]. Any molecular clonalities were detected in 68.3% (28/41); JAK2 V617F in 53.7% (22/41), JAK2 exon 12 in 0%, CLAR exon 9 in 17.2% (5/24), and MPL W515 in 3.4% (1/28). The positive rates of JAK2 V617F by detection methods were not statistically different, though the rate of Sanger sequencing was slightly higher than those of AS-PCR (41.7% vs. 56.7%). The detection rates of molecular clonalities significantly increased in the period conducted additional mutation tests compared to the period performed V617F only ($p < 0.05$); 41.7% (JAK2 V617F, 5/12) vs. 76.7% (JAK2 V617F, 17; CALR, 5; MPL, 1). The positive rates of CALR and MPL mutations among the JAK2 -negative MPNs patients were 41.7% (5/12: ET, 4; PMF, 1), 8.3% (1/12: PMF, 1).

**Conclusion:** Based on our result, conducting additional molecular tests using Sanger sequencing (JAK2 exon 12, MPL W515, and CALR exon 9) is helpful to identify clonal evidence of BCR/ABL-negative MPNs. Especially CALR exon 9 sequencing is useful to diagnose Philadelphia chromosome-negative MPNs.

## A-291

### The Growth Differentiation Factor-15 Levels Are Increased in Patients with Compound Heterozygous Sickle Cell and Beta-Thalassemia, Correlate with Hepcidin-25-Ferritin Molar Ratio and with Markers of Hemolysis, Angiogenesis, Endothelial and Renal Dysfunction

I. Papassotiriou[1], K. Larrisi[1], A. Margeli[1], M. Politou[2], E. Voskaridou[3], E. Terpos[4]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Laboratory of Hematology and Blood Bank Unit, "Aretaieio" Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, [3]Thalassemia and Sickle Cell Disease Center, "Laikon" University General Hospital, Athens, Greece, [4]Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece*

**Background**: The clinical manifestations of Sickle Cell Disease (SCD) include episodes of vascular occlusion, chronic hemolytic anemia and frequent infections. SCD is also characterized by the presence of chronic inflammation manifested by leukocytosis and monocytosis and increased circulating levels of pro-inflammatory cytokines and chemokines. Growth Differentiation Factor-15 (GDF-15), also known as macrophage-inhibitory-cytokine-1 (MIC-1) or non-steroidal-anti-inflammatory-drug-activated-gene (NAG-1) is a member of the transforming-growth-factor superfamily. Expression of the GDF-15 gene in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury. Also GDF-15 has been proposed as an erythroblast-derived factor, although not erythroblast specific, mediates Hepcidin-25 suppression under conditions of increased erythropoietic activity, and high levels of GDF15 associate with ineffective erythropoiesis and may reflect a certain type of bone marrow stress or erythroblast apoptosis. **Aims**: The aim of this study was to evaluate the GDF-15 levels in patients with compound heterozygous HbS and beta-thalassemia (HbS/$\beta^{thal}$) and to explore possible associations with disease features, such as Hepcidin-25 production, hemolysis, inflammation, endothelial dysfunction and angiogenesis. **Methods**: Seventy-five adult Caucasian patients with HbS/$\beta^{thal}$ were included in the study, while 20 healthy individuals served as controls. Patients with HbS/$\beta^{thal}$ divided in two groups: group A included 36 patients under hydroxycarbamide (HC+) treatment and group B included 39 patients without hydroxycarbamide (HC-) treatment. Along with hematologic and blood chemistry parameters determination, measurements of circulating levels of GDF-15, hepcidin-25, hs-CRP, vWF-antigen, hs-TnT and Placental Growth Factor (PlGF) were performed in both patients with HbS/$\beta^{thal}$ and controls using immunoenzymatic techniques. **Results**: GDF-15 levels were elevated in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.0001$). Regarding hydroxycarbamide treatment, GDF-15 levels were elevated in (HC+) patients compared to (HC-) patients ($p=0.002$), or 30/36 vs 21/39 patients had elevated GDF-15 levels ($p=0.002$). In contrast, Hepcidin-25 levels were significantly lower in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.01$). In addition, a markedly low Hepcidin-25/Ferritin molar ratio was observed in patients with HbS/$\beta^{thal}$ compared to controls ($p<0.001$). Whilst, no direct correlation was found between GDF-15 and hepcidin-25 levels, a significant negative correlation between GDF-15 levels and Hepcidin-25/Ferritin molar ratio was detected in patients with HbS/$\beta^{thal}$ ($p=0.002$). GDF-15 levels also correlated significantly with markers of erythropoiesis, such as Hb, HbF, ferritin and reticulocytes ($p<0.05$), with markers of hemolysis, such as LDH and uric acid ($p<0.05$), and with markers of endothelial dysfunction and angiogenesis such as vWF-antigen and PlGF ($p<0.05$). Surprisingly, no correlation was found between GDF-15 and hs-CRP levels. GDF-15 and $eGFR_{(Cystatin\text{-}C\text{-}based)}$ correlated negatively ($r=-0.421$, $p<0.001$). **Conclusions**: These findings demonstrate a multifactorial role of GDF-15 in patients with HbS/$\beta^{thal}$ as it correlates with erythropoiesis, hemolysis, angiogenesis, endothelial and renal dysfunction. Interestingly, the higher GDF-15 levels measured in patients treated with hydroxycarbamide may reflect possible drug induced sub-clinical cardiotoxicity, although this has not been described to-date. To this end, our knowledge is restricted only to doxorubicin-induced cardiotoxicity, where GDF-15 up-regulation seems to be more sensitive than that of hs-TnT, LDH and NT-proBNP. Further studies will reveal the role of GDF-15 in the biology of HbS/$\beta^{thal}$.

## A-292

### Clinical significance of new blood coagulation indicators in patients with lung cancer

Y. Jin, Q. Niu, Y. Fu, Y. Zhou, H. Jiang. *West China Hospital, Sichuan University, Chengdu, China*

**Background and objective** Being one of the most important complications and also one of the leading cavses of death, venous thromboembolism (VTE) could significantly increase the morbidity and mortality of patients with malignant tumors. Accurate risk evaluation of VTE and early prophylaxis according to the risk level are important to reduce the incidence of VTE, as well as to improve the quality of life and disease prognosis in patients with cancer. D-dimer has been included in guidelines of diagnosis and treatment of VTE. Accumulating evidences have demonstrated the great value of new blood coagulation indicators, including thrombomodulin (TM), thrombin-anti-thrombin complex (TAT), plasmin-anti-plasmin complex (PIC) and tissue-type plasminogen activator-plasminogen activator inhibitor complex (t-PAIC), in evaluating the risk of VTE. However, their roles in risk assessment of tumor-related VTE were still unclear. Therefore, we aimed to investigate the changes of these four items and their relationships with D-dimer in lung cancer patients and to explore their value in evaluating risk of lung cancer-related VTE. **Methods** In this case-control study, we enrolled 90 patients with lung cancer and 92 healthy volunteers. Routine coagulation screening test (including PT, APTT, FIB, TT), D-dimer, TM, TAT, PIC and t-PAIC were measured with automatic blood coagulation analyzer Sysmex CS5100. **Results** Compared with health controls**,** D-dimer, TM, TAT and PIC in cancer patients all significantly increased ($P<0.05$); while t-PAIC showed no difference between cancer patients and health subjects ($P>0.05$) (Fig 1). In addition, TM, TAT and PIC all showed positive correlation with D-dimer level in lung cancer patients ($r=0.385$, $P<0.01$; $r=0.424$, $P<0.001$; $r=0.504$, $P<0.001$, respectively). **Conclusions** The results suggested that both coagulation and fibrinolysis system were activated in lung cancer patients, who were at high risk for developing VTE. Preventative anticoagulant

therapy might be necessary for patient with lung cancer, especially for those with increased D-dimer, TM, TAT or PIC.

Table 1 blood coagulation items compared with patients of lung cancer

| group | cases | D-Dimer (ng/ml) | TM (Iu/ml) | TAT (ng/ml) | PIC (ug/ml) | TPAI-C (ng/ml) |
|---|---|---|---|---|---|---|
| control | 92 | 0.5900 | 7.45700 | 1.0710 | 0.50100 | 6.07900 |
| cancer | 90 | 1.8700 | 8.92800 | 1.9995 | 0.83450 | 5.60000 |
| p | | 0.016 | 0.012 | 0.003 | 0.001 | 0.951 |

## A-293

### Traditional osmotic fragility test comparison with flow cytometric osmotic fragility test under the same protocol and definition of single NaCl concentration that provides an accurate hereditary spherocytosis screening by flow cytometric analysis

C. S. Nobre, A. C. Dias, J. A. Silva, R. H. Jácomo, L. F. A. Nery, G. B. Barra. *Laboratório Sabin, Brasília, Brazil*

Introduction

In the Traditional Osmotic Fragility test (TOF) the lysis of red blood cell (RBC) is measured colorimetrically in different tubes with decreasing concentrations of NaCl. In the Flow Cytometric Osmotic Fragility test (FCOF) the fraction of not-lysed RBCs is counted in a single tube spiked with deionized water. Although both tests are wildly accepted for hereditary spherocytosis (HS) screening, their equivalence in the same conditions is unknown. Moreover, some flow cytometers can not overlay analysis of before and after deionized water spiking. Thus, the aims of this study was to compare the FCOF with the TOF using multiple tubes with decreasing concentrations of NaCl in different pre-analytic conditions and to find a single NaCl concentration for each tested condition that allows an accurate HS screening by FCOF.

Methods

Twenty adult subjects (10 females) with HS (cases) and 20 healthy individuals (controls) matched 1:1 by gender and age were recruited to the study. To confirm the laboratorial diagnosis of HS or not, all subjects were submitted to complete blood count, reticulocyte count, blood smear. Haptoglobin, bilirubin, lactate dehydrogenase and direct antiglobulin tests were also performed. TOF (performed in E-225D, CELM) and FCOF (performed in FACSCanto II, Becton-Dickinson) were executed in fresh/incubated (24 h at 37°C degrees) heparinized/EDTA whole blood by using the 17-tube method with NaCl concentrations ranging from 1 to 0.1 g/L. Whole blood was diluted by 1:5000 for FCOF and 1:200 for TOF. Osmotic fragility curves and Median Corpuscular Fragilities (MCF) were used to compare all tested conditions and ROC curves were used to define the (%) lysis cut-off that provided a reliable discrimination between cases and controls.

Results

Laboratorial diagnosis of HS could be confirmed in all cases and none of the controls. The average age was 37.3±13.2 years for cases and 36.9±12.7 years for controls (p=0.24). The osmotic fragility curves in FCOF assumed the traditional sigmoid shape observed in the TOF and the curves of cases and controls were clearly distinguished in all tested condition. Incubation for 24h at 37°C degrees increased the discrimination between cases and controls in both heparinized and EDTA whole blood. The MCF for cases was significantly different from controls in all tested condition. However, the MCF values varied by technology, anticoagulant and incubation time. NaCl concentrations and (%) lysis cut-off that allowed reliable discrimination between cases and controls in FCOF were of 6g/L and 21.5% for heparinized fresh blood, 7g/L and 33.5% for heparinized incubated blood, 6g/L and 22.5% for EDTA fresh blood and 7.5g/L and 37.5%) for EDTA incubated blood, respectively. The sensitivity and specificity were 100% (95%CI 83 - 100%) for all instances, except for incubated heparinized blood, which the specificity was 95% (95%CI 75 - 99.8%).

Conclusion

The FCOF with multiple tubes and decreasing concentrations of NaCl was feasible and could discriminate cases and controls as well as TOF. Moreover, we found single NaCl concentration that allows for a reliable HS screening by using FCOF in heparinized/EDTA and fresh/incubated whole blood.

## A-294

### Development of a Wearable Device to Monitor Heparin Anticoagulation Therapy.

J. Wang, J. Hartanto, S. Ananthakrishnan, J. V. Jokerst. *University of California, San Diego, La Jolla, CA*

**Background.** Heparin anticoagulation therapy has a narrow therapeutic window and is the second most common medication error. The partial thromboplastin time (PTT) monitors heparin, but suffers from long turnaround times, a variable reference range, and limited utility with low molecular weight heparin (**LMWH**). Here, we describe a photoacoustic imaging technique to monitor heparin anticoagulation therapy in real time and catheter than can monitor heparin.

**Methods.** We first surveyed five phenothiazinium dyes at five concentrations for their sensitivity to heparin and found that 0.4 mM methylene blue offered the highest signal to background ratio. *In vitro* experiments used fresh human blood stabilized with sodium citrate. First, 10 uL of 1 to 400 U/mL heparin was added to 90 uL of fresh human blood followed by 20 µL of 2 mM methylene blue. Samples were loaded into capillary tubes and imaged with a Visualsonics LAZR photoacoustic scanner from 680 – 900 nm. For *in vivo* experiments, mice (n=3) were injected with 100 µL of 0 or 200 U/mL of heparin dissolved in sterile PBS by tail vein. Thirty minutes later, the animals were injected with 100 µL of 50 mM methylene blue via tail vein. Blood was collected via cardiac puncture and imaged within 4 hours. We covalently linked methylene blue derivatives to a polyurethane catheter.

**Results.** Initial experiments showed strong correlation between heparin concentration and signal ($R^2$>0.90) with stability for at least 15 minutes. The signal increased within 20 seconds of heparin addition. We showed that heparin concentrations as low as 1 U/mL in blood produced statistically significant signal increases versus heparin-free samples ($p<0.02$), and the signal decreased with protamine sulfate treatment. This approach also has utility with LMWH with a detection limit of 0.1 mg/ml. The *in vivo* experiments showed a 2.8-fold photoacoustic signal increase in animals treated with MB versus PBS ($P<0.0001$). The order of addition was important—animals injected with MB first followed by heparin showed little signal. We also used the catheter to measure heparin in human blood with a detection limit of 1 U/mL.

**Data Validation.** The data was validated by comparison to the PTT and protamine sulfate treatment. Mice without heparin treatment had PTT values of 30.1 ± 8.9 s and photoacoustic signal of 16,870 ± 1200 a.u. Mice treated with 200 U/mL (100 µL) heparin had PTT values over 400 s and photoacoustic signal of 40,320 ± 7460. This shows that the signal is indicative of a functional response. Next, we showed that the photoacoustic signal decreased when animals were treated with protamine sulfate—a known heparin antagonist. Mass analysis showed that 10 g of protamine were needed to neutralize 1 U of heparin, which is the clinically used dose (see Supplementary). We also correlated the photoacoustic signal from six human samples to the PTT and showed a Pearson's R of 0.86.

**Conclusion.** To the best of our knowledge, this is the first report to image anticoagulation therapy. We are building a wearable sensor in tandem with a smart intravenous catheter to monitor anticoagulation in real time.

## A-295

### Association of hOCT1 gene expression with hematological & molecular response to imatinib in patients with chronic myeloid leukaemia in chronic phase

N. Bhutani[1], S. A. Guru[2], P. Yadav[2], N. Gupta[2], A. Saxena[2]. *[1]Vardhman Mahavir Medical College, New Delhi, India, [2]Maulana Azad Medical College, New Delhi, India*

**Background:** Human organic cation transporter1(hOCT1,SLC22A1),an influx transporter,is responsible for the uptake of Imatinib into chronic myelod leukemia (CML)cells.Some patients fail to achieve optimal molecular response to Imatinib, defined as major molecular response (MMR) i.e.BCR-ABL 1 ≤ 0.1% within 12 months of therapy. Pretherapeutic expression of hOCT1 may be beneficial in predicting the response to imatinib in CML patients. **Methods:** 30 newly diagnosed BCR-ABL positive CML patients in chronic phase&30 healthy control subjects, all ethnic Indians ,were recruited in the study. hOCT1 gene expression in PBMCs was quantified by SYBR Green based qRT-PCR,using the 2-DDCtmethod. After initiation of imatinib therapy ,hematological response was monitored at regular intervals ,and molecular response (BCR-ABL1/ABL1 ratio) assessed after 6 or 12 months **Results:** The cases were divided into two groups, high expression(n=15) and low expression (n=15) groups, based on the median value of fold change in hOCT1 gene expression. (median =5.6). 11 (73.33%) patients with low expression achieved CHR by the end

of 3 months, whereas 4(26.66%) did not.On the contrary, all 15 patients (100%) with high hOCT1 gene expression achieved CHR by the end of 3 months. (p=0.10).It was also observed that the mean THR (time to CHR) in low expression group was higher than in high expression group.(p=0.046)It was seen that while all 15 patients with high expression had an optimal response , only 13.33 % (n=2) patients with low expression had it. In the low expression group, 40% patients had treatment failure(n=6)while remaining 23.33 % (n=7)were categorized as warning. (p=0.000)(ELN 2013 guidelines) **Conclusion:** Hence it was concluded that high expression of hOCT1 gene leads to early achievement of CHR. In case of molecular response, it was observed that high expression of hOCT1 gene was significantly associated with achievement of on optimal response to imatinib. These findings emphasize that knowledge of pretherapeutic level of hOCT1 could be a useful marker to predict imatinib therapy outcome in CML patients, and the prospects of personalized therapy in such patients.

## A-296

### Evaluation of the High sensitivity CRP assay for use on the Binding Site Optilite® turbidimetric analyser

E. Proctor, F. Murphy, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

C-reactive protein (CRP) is a biomarker of systemic inflammation produced by hepatocytes. It is activated by inflammatory cytokines from a wide variety of stimuli including inflammation, infection, tissue damage and neoplasty. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. Minor CRP elevation has been associated with various disorders and clinical conditions in different demographic and socioeconomic groups. These mild increases in serum CRP concentrations can have prognostic implications and can be utilised to stratify patient risk. Here we describe the performance of an immunoassay for the detection and quantification of High Sensitivity CRP on the Binding Site Optilite® analyser. A linearity study was performed according to CLSI EP06-A guidelines; the assay was linear over a measuring range of 0.2-10 mg/L at a 1/1 analyser dilution. Limit of quantitation (LoQ) based on CLSI EP17 was 0.50 mg/L. Correlation to the Roche C501 assay demonstrated good agreement using 148 clinical samples ranging from 0.23-10.2 mg/L (Passing and Bablok analysis slope y=1.00x+0.15). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on three analysers. Samples were selected to cover the medical decision point, pathological concentrations, the reference interval and the minimum dilution. The total precision coefficients of variation (CVs) were as follows: 5.6% at 0.98 mg/L, 5% at 1.55 mg/L, 4.5% at 3.0 mg/L, 4.4% at 5.4 mg/L and 3.1% at 8.5 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 8 potential drug and metabolite interferents, including ibuprofen, caffeine and intralipid at four serum concentrations (0.97, 1.49, 3.13 & 5.70 mg/L). No significant interference was observed (maximum difference in the control samples was 8.67%). In conclusion, the Optilite High Sensitivity CRP assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-297

### Performance of the High sensitivity CRP assay for use on the Binding Site SPAPLUS® turbidimetric analyser

J. Seibaka, K. Samuels, F. Murphy, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

C-reactive protein (CRP) is a biomarker of systemic inflammation produced by hepatocytes. It is activated by inflammatory cytokines from a wide variety of stimuli including inflammation, infection, tissue damage and neoplasty. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. Minor CRP elevation has been associated with various disorders and clinical conditions in different demographic and socioeconomic groups. These mild increases in serum CRP concentrations can have prognostic implications and can be utilised to stratify patient risk. Here we describe the evaluation of a High Sensitivity CRP serum assay (The Binding Site Ltd, UK) for the Binding Site SPAPLUS® analyser. A linearity study was performed according to CLSI EP06-A guidelines; the assay was linear over a measuring range of 0.5-10 mg/L at a 1/1 analyser dilution. Limit of quantitation (LoQ) based on CLSI EP17 was 0.50 mg/L. Correlation to the Roche C501 assay demonstrated good agreement using 218 clinical samples ranging from 0.49-9.99 mg/L (Passing and Bablok analysis slope y=1.00x+0.08). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on four analysers. Samples were selected to cover the medical decision point, pathological concentrations, the reference interval and the minimum dilution. The total precision coefficients of variation (CVs) were as follows: 3.6% at 1.0 mg/L, 2.2% at 1.6 mg/L, 2.0% at 3.0 mg/L, 2.5% at 5.3 mg/L and 3.5% at 8.5 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 8 potential drug and metabolite interferents, including ibuprofen, penicillin and intralipid at four serum concentrations (0.98, 1.57, 3.04 & 5.46 mg/L). No significant interference was observed (maximum difference in the control samples was -4.70%). In conclusion, the HS CRP assay for the SPAPLUS assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-298

### Performance of the CRP assay for use on the Binding Site Optilite® turbidimetric analyser

O. Nevill, J. Seibaka, K. Samuels, V. L. Poole, F. Murphy, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Detection of C-reactive protein (CRP) within serum is widely regarded as the most reliable biomarker for systemic inflammation. CRP functions by readily binding damaged cell membranes and microbial polysaccharides, and is involved in the agglutination and precipitation of invasive bacteria. It is also capable of activating the complement cascade, resulting in opsonisation and phagocytosis of cell debris and bacteria. Normal serum contains <10 mg/L CRP, an increase in circulating CRP levels can be detected within 6 hours post onset of inflammation. Moderately elevated serum levels (10 - 40 mg/L) are associated with mild inflammation and viral infections whereas high levels (>40 mg/L) are indicative of acute phase inflammation and bacterial infections. Here we describe the performance of an immunoassay for the detection and quantification of serum CRP on the Binding Site Optilite® analyser. The Optilite CRP assay displayed good agreement with the Roche Modular P CRP assay in a comparison of 193 serum samples ranging from 3.87 - 498.23 mg/L (Passing-Bablok analysis; Y=1.00x+5.56). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing five serum levels on three kit lots and three analysers over 21 days. Samples were targeted to the medical decision point, pathological concentrations, the reference interval and the minimum dilution. Total precision gave CVs of 3.9% at 9.99 mg/L, 2.9% at 22.33 mg/L, 2.8% at 63.88 mg/L, 3.1% at 146.73 mg/L and 2.8% at 258.28 mg/L. Interference testing was performed using 18 potential drug and metabolite interferents including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.50, 60.06 & 170.73 mg/L). No significant interference was observed (maximum difference from control samples was -8.69%). The assay was also observed to be linear over the measuring range of 5 - 300 mg/L at the standard 1/1 dilution using a serially diluted sample pool (recovery was ≤ ±7.4% for all samples). In conclusion, the Optilite CRP assay provides a reliable and precise method for quantifying CRP content in human serum and correlates well with existing methods.

## A-299

### Evaluation of the CRP assay for use on the Binding Site SPAPLUS® turbidimetric analyser

O. Nevell, E. Proctor, F. Murphy, K. Samuels, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

C-reactive protein (CRP) is a nonspecific inflammatory biomarker of hepatic origin that is commonly quantified in the detection and monitoring of infection and acute phase inflammation. Baseline serum CRP levels are typically <10 mg/L, but are observed to increase 1000-fold with infection, trauma or chronic inflammatory disorders. CRP concentrations reach a peak within two days on acute phase response, having a half-life of approximately 18 hours. Here we describe the evaluation of The Binding Site CRP serum assay for SPAPLUS® analyser. The assay has been validated, using a linearity study performed to CLSI EP06-A guidelines, to have a measuring range spanning 5-250 mg/L at a 1/1 analyser dilution. A limit of quantitation (LoQ) study based on CLSI EP17 confirmed a limit of 5 mg/L. Correlation to the Roche Modular P assay demonstrated good agreement using 225 clinical samples ranging from 4.66-498.23 mg/L (Passing and Bablok analysis slope y=1.02x+3.22). Precision studies were performed according to CLSI EP5-A2 guidelines over a period of 21 days using three reagent lots on four analysers. The coefficients of variation (CVs) were as

follows: 7.8% at 8.7 mg/L, 5.7% at 13.5 mg/L, 2.6% at 60.0 mg/L, 3.6% at 147 mg/L and 6.2% at 225.2 mg/L. Interference testing was performed following CLSI EP7-A2 guidelines using 18 potential drug and metabolite interferents, including ibuprofen, penicillin, intralipid and fluconazole at three serum concentrations (9.5, 63.4 & 170.0 mg/L). No significant interference was observed (maximum difference in the control samples was -9.86%). In conclusion, the SPAPLUS CRP assay provides an accurate method for quantifying human serum CRP.

## A-300

### Evaluation of the Anti-Tetanus Toxoid Immunoglobulin assay for use on the Binding Site Optilite® turbidimetric analyser

D. G. McEntee, C. Campbell, F. Murphy, A. Kaur, V. L. Poole, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

The serological measurement of anti-tetanus toxoid antibodies produced in response to vaccination with tetanus toxoid protein aids the assessment of a patient's immune response. Here we describe the evaluation of the Anti-Tetanus Toxoid Immunoglobulin assay for use on the Binding Site Optilite® analyser. The measuring range of the assay is 1.67 - 50 IU/mL at the standard 1/10 analyser dilution. Correlation to the Binding Site Anti-Tetanus Toxoid Immunoglobulin assay for the SPAPLUS® was performed using 115 plasma samples ranging from 1.74 - 47.72 IU/mL. This demonstrated good agreement when analysed by Passing and Bablok regression (y=0.98x + 0.51). The assay also demonstrated good agreement between serum and plasma matrices using 107 paired serum and EDTA plasma samples ranging from 1.585 - 48.363 IU/mL (Passing and Bablok analysis: y=0.98x + 0.06). Precision studies were performed based on the CLSI approved guideline EP5-A2, testing six serum levels (2.77, 3.15, 4.27, 7.43, 8.85 and 17.04 IU/mL) on a single kit lot over three analysers and 21 days. All levels gave total precision CV values of <9%. Linearity studies were performed following the CLSI EP6-A, using a serially diluted plasma sample. The assay was linear across the measuring range (all results were within 10% of expected values). Interference testing was performed according to CLSI EP7-A2, using serum samples with anti-tetanus toxoid antibody concentrations both close to the medical decision point and at an elevated level. No significant assay interference was observed with triglyceride (1000mg/dL), Intralipid (1000mg/dL), bilirubin (200mg/L) or haemoglobin (5g/L). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and gave a limit of 0.15 IU/mL. The antigen excess capacity of the assay was determined to be equivalent to 120 IU/mL at the standard dilution. In conclusion, the Anti-Tetanus Toxoid Immunoglobulin assay for the Optilite analyser provides a reliable, accurate and precise method for quantifying anti-tetanus toxoid antibodies in serum and plasma.

## A-301

### Performance of the Total Protein assay for use on the Binding Site Optilite® turbidimetric analyser

Z. Reading[1], H. Baethies[2], M. Schneider[2], F. Murphy[1], V. L. Poole[1], D. J. Matters[1], M. Grimmler[3], S. J. Harding[1]. *[1]The Binding Site Group Ltd, Birmingham, United Kingdom, [2]DiaSys Diagnostic Systems GmbH, Holzheim, Germany, [3]University of Applied Science, Idstein, Germany*

Quantification of total protein in serum provides a useful tool for the assessment of the synthesis and maintenance of circulating proteins. Abnormal total protein levels acts as a key indicator for multiple disease states; elevated TP levels are a marker for bone marrow disorders, liver cirrhosis and inflammation. A decrease in total protein concentration can be detected in disorders associated with defective protein synthesis, impaired kidney function, malnutrition and malabsorption. Here we describe the performance of the Total Protein assay for use on the Binding Site Optilite® Analyser. The measuring range of the assay was determined as 0.12-15 g/dL. Linearity was assessed using a serially diluted serum sample, following the CLSI approved guideline EP6-A. The assay was linear across the measuring range (all results were within 10% of expected values). Correlation to the Roche Hitachi 917 assay demonstrated good agreement using 94 clinical samples ranging from 0 - 14.3 g/dL by Passing and Bablok analysis (y=1.017x-0.038). A precision study was performed over a period of 5 days. Total coefficients of variation (CVs) were as follows: 0.77% at 5.8 g/dL, 0.57% at 7.0 g/dL, and 0.54% at 10.8 g/dL. Interference testing was performed according to CLSI EP7-A2 guidelines. No significant assay interference was observed in the presence of triglycerides (1000mg/dL), L-ascorbic acid (60mg/dL), unconjugated bilirubin (60mg/dL), conjugated bilirubin (60mg/dL) and haemoglobin (500mg/dL). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and reported a limit of 0.03g/dL. In conclusion, the Optilite Total Protein assay provides a reliable and precise method for quantifying total protein in human serum.

## A-302

### Identification and quantification of urinary monoclonal proteins by capillary electrophoresis.

P. Milani[1], T. Bosoni[2], F. Russo[1], V. Valentini[1], M. Basset[1], G. Ferraro[1], L. Pirolini[2], A. Foli[1], M. Bozzola[1], F. Lavatelli[1], F. Belvisi[2], M. Nuvolone[1], G. Consogno[2], F. Li Bergolis[2], R. Albertini[2], G. Palladini[1], G. Merlini[1]. *[1]Amyloidosis Research and Treatment Center, University of Pavia, Pavia, Italy, [2]Clinical Chemistry Laboratory, Foundation IRCCS Policlinico San Matteo, Pavia, Italy*

**Background:** Identification and quantification of urinary monoclonal proteins (uMPs) is of utmost importance in the diagnosis and monitoring of monoclonal gammopathies. We prospectively assessed the performance of the Sebia Capillarys 2 Flex Piercing Urine protein capillary electrophoresis (UPCE) and immunotyping in patients affected by light chain (AL) amyloidosis and other plasma cell dyscrasias.

**Methods:** Samples were tested with: (a) homemade high-resolution agarose gel immunofixation electrophoresis (hr-IFE) of serum and concentrated (10 times) urine; (b) commercial semi-automated agarose gel immunofixation of urine (Sebia Hydragel Bence Jones on Hydrasys 2); (c) UPCE and immunotyping (Sebia Capillarys 2 Flex Piercing); (d) quantification of circulating free light chains (FLC) by *Freelite* and *N latex FLC*. Urinary MPs were quantified using Sebia Phoresis software tools. Only patients in whom uMPs were detected by hr-IFE were included in the study.

**Results:** 119 patients with a uMP were included. Ninety (76%) subjects suffered from systemic AL amyloidosis, 17 (14%) had a MGUS and the remaining 12 (10%) had other plasma cell disorders. Estimated glomerular filtration rate was <30 mL/min in 13 (11%). A uMP was detected by UPCE in 94 patients (79%), and was quantifiable in 84 cases (81%). The median uMP excretion in the whole cohort was 50 mg/24h (range 10-1360 mg/24h) as assessed by Phoresis tool. Interestingly, 9 of the 12 patients with AL amyloidosis and a difference between involved and uninvolved FLC (dFLC) <50 mg/L (not measurable for hematologic response with current criteria) had a quantifiable uMP (median 90 mg/24h). The uMP was also quantifiable on Hydragel HR agarose gel in 90 patients (76%). There was a good correlation between measurements of uMP excretion on Capillarys and Hydragel (Pearson's r = 0.90, 95%CI 0.86-0.93). So far, 25 patients with AL amyloidosis and quantifiable uMP and dFLC (*Freelite*) >50 mg/L were treated and had response data at 3 months. Eleven subjects responded (1 complete response, 5 very good partial response and 5 partial response) with a median 88% dFLC decrease (range 55-98%). In all of them uMP excretion decreased (median 100%, range 30-100%). Post-treatment UPCE was also available in 5 patients with baseline dFLC (*Freelite*) <50 mg/L. In 2 of them the uMP was still visible but was no longer quantifiable, in 2 it remained stable and in one patient uMP increased from 20 to 40 mg/24h.

**Conclusion:** Capillarys 2 Flex Piercing Urine protein electrophoresis can identify uMPs in patients with plasma cell dyscrasias with a good sensitivity, and can quantify uMP excretion as low as 10 mg/24h. Changes in uMP excretion can be monitored during treatment in patients with AL amyloidosis, including some patients without dFLC-measurable disease. Further studies are warranted to evaluate this tool in response assessment.

## A-303

### All Automated Analyzers for CSF Testing are Not Created Equal

J. MacKenzie[1], R. Kassab[1], C. Vilk[2], J. Baker[2], G. Hong[1]. *[1]Advanced Instruments, Norwood, MA, [2]Multicare Health Systems - Tacoma General Hospital, Tacoma, WA*

**Objective:** The main purpose of the study was to compare the manual hemocytometer method for performing cerebrospinal fluid (CSF) cell counts with the GloCyte Automated Cell Counter for CSF and the Iris iQ200 body fluid module. **Background:** Traditionally, CSF cell counts have been performed using a manual counting chamber. Clinical laboratories are beginning to look to automated methods for increased precision, shortened turnaround time, and reduced interoperator variability. However, not all automated hematology analyzers on the market provide accurate counts for both total nucleated cells (TNC) and red blood cells (RBC). Clinicians and their patients rely on accurate results making bias an important consideration for clinical laboratories. **Methods:** CSF specimens that were obtained for clinical purposes and sent for analysis to the laboratory at Multicare Health System - Tacoma General Hospital were used for the study. In total, there were 61 specimens including

60 specimens from 45 patients and 1 spiked specimen. All 61 specimens were counted using manual and GloCyte methods. Iris counts were performed on 50 of the 61 specimens. Pearson correlation and Passing-Bablok regression analysis were used to compare methods. Clinical diagnoses were also reviewed. **Results:** There was a strong linear relationship between the manual and automated methods for TNC (R = 0.984 for GloCyte; R = 0.982 for Iris). Overall, the Iris overcounted TNCs; the analyzer had a positive proportional bias of 38% while GloCyte showed no bias. Bias was absent for cell counts ≤30 TNC/µL for both analyzers. For RBC, there was a strong linear relationship between the manual and automated methods (R = 0.998 for GloCyte; R = 0.997 for Iris). Neither automated method showed bias for RBC. **Conclusion:** This study demonstrates that not all automated analyzers are created equal. The Iris exhibited a 38% bias for TNC whereas the GloCyte did not show any bias for TNC or RBC. The absence of bias for the GloCyte shows that GloCyte and manual counts can be used interchangeably and indicates that diagnostic accuracy is not compromised. The presence of bias for Iris TNC counts warrants further studies incorporating larger samples sizes and clinical outcomes to determine if there is any impact on clinical decision making.

## A-304

### Lupus Anticoagulant: Choosing the Right Testing Strategy in the Era of New Oral Anticoagulants

A. Vivero, A. Runge, J. Vallone, S. Kitahara, O. Volod. *Cedars-Sinai Medical Center, Los Angeles, CA*

**Background:**

Antiphospholipid syndrome (aPL) is an acquired autoimmune disorder associated with arterial/venous thrombosis or pregnancy loss. The syndrome can occur in the setting of autoimmune diseases or can occur as a primary entity. Laboratory diagnosis guidelines require testing for the presence of antiphospholipid antibodies (lupus anticoagulant (LA), anticardiolipin, anti Beta 2GP1).

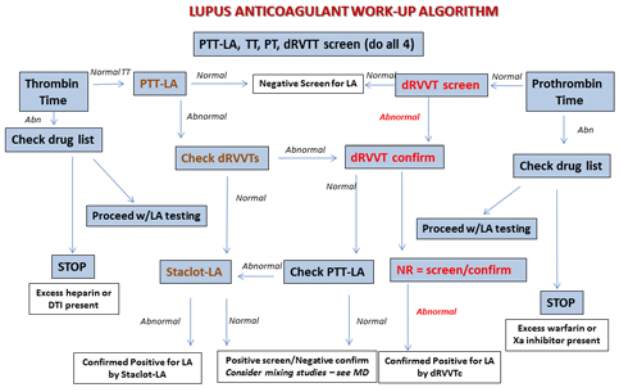
A retrospective study of our testing practices revealed a high incidence of LA false positive results. This was an impetus for us to evaluate our current testing protocol. As a result, we developed a new algorithmic approach to LA testing which significantly decreased our false positives.

Our objective was to develop a new approach to LA testing taking into consideration the current following guidelines: International Society of Hemostasis and Thrombosis (ISTH), British Committee for Standards in Haematology (BCSH), and Clinical and Laboratory Standards Institute (CLSI) to maximize detection of LA in the era of new oral anticoagulants in a cost effective way.

**Methods:** We tested 20 purchased normal plasma samples (LA negative) and established new reference ranges for the following tests: prothrombin time (PT), thrombin time (TT), lupus sensitive partial thromboplastin time (PTT-LA), dilute Russel Viper Venom screen and confirm (DRVV) times and Staclot-LA. After the implementation of the new algorithmic approach (see Figure 1), 1146 patient results tested between April 2014 to March 2015 were retrospectively reviewed.

**Results:** The following results were obtained based on our proposed algorithmic approach: DRVV positive LA 111/1146 (10%), Statclot LA positive and DRVV negative 228/1146 (20%).

**Conclusion:** The algorithmic approach to LA testing was found to be useful in decreasing our false positive rates and the cost of testing for our institution and the patients while increasing the accuracy of detection.




## A-305

### Oligosecretory light chain myeloma with cytoplasmic expression of IgE

J. Garcia de Veas Silva[1], M. López Vélez[1], A. Espuch Oliver[1], L. Pérez Albaladejo[1], R. Rios Tamayo[1], N. Barbosa de Carvalho[2], J. García Lario[1], T. De Haro Muñoz[1]. *[1]Complejo Hospitalario Universitario de Granada, Granada, Spain, [2]The Binding Site, Barcelona, Spain*

**Background**: IgE Multiple Myeloma (MM) is a rare entity with fewer than 50 cases reported in the literature and a prevalence of 0.01% in all cases. Serum free light chain assays presents greater sensitivity in the diagnosis and management of monoclonal gammopathies compared with classical techniques as serum protein electrophoresis and Bence Jones proteinuria. With the introduction of serum free light chains assay, the non secretory MM could be identified as measurable oligosecretory MM. We reported the clinical findings of a woman patient with IgE MM with elevated levels of serum free light chains at diagnosis.

**Case report:** A 55 years old woman was admitted to the hospital with intense back pain of six months of evolution. In the CT scan, a pathological fracture was observed in D12 vertebral body. PET/CT scan showed a weak uptake throughout the axial skeleton with two foci at D8 and D12 with the presence of multiple focal lesions in all bones. The laboratory findings showed two small peaks in gamma region of serum protein electrophoresis (0,15 g/dL) and the serum immunofixation detected only kappa chains. Immunoglobulins were decreased (IgA = 35 mg/dL, IgG = 356 mg/dL, IgM = 39 mg/dL) with a very altered serum free light chain ratio (kappa=2860 mg/L, lambda=5.36 mg/L and a ratio of 535). Furthermore, the patient presented impaired renal function (creatinine=2.8 mg/dL), anaemia (haemoglobin of 9.2 g/dL) and severe hypercalcemia with serum levels of 13 mg/dL of calcium. The beta-2-microglublin level was of 18.7 mg/L. The bone marrow biopsy showed a 14% of plasma cells with 4.5% with pathological immunophenotype. Biopsy study of pathological tissue at D12 showed positive expression of CD138, CD38 and CD56 with cytoplasmic expression of IgE and restriction for kappa chains. The patient was diagnosed with oligosecretory light chain MM with cytoplasmic IgE in ISS stage 3 and extensive bone injury. She began treatment with Bortezomib-Lenalidome-Dexamethasone and received one cycle. After first cycle, the patient presented a severe neutropenia with fever and she was admitted in the Intensive Care Unit. The patients presented a poor outcome and she died six days after.

**Conclusions**: Immunohistochemical studies of the D12 tumour biopsy showed the presence of cytoplasmic IgE in the plasma cells. Although, the plasma cells produced both IgE and free light chains, only the free light chains were detected in the serum of the patient. The patient was diagnosed of oligosecretory light chain MM with cytoplasmic expression of IgE in ISS stage 3. IgE MM in advanced stages is a disease with an aggressive course and it is associated with severe complications and high rate of mortality.

## A-306

### Serum free light chains in the evaluation of the response of non secretory multiple myeloma

J. Garcia de Veas Silva[1], M. López Vélez[1], A. Espuch Oliver[1], L. Pérez Albaladejo[1], C. Bermudo Guitarte[2], N. Barbosa de Carvalho[3], J. García Lario[1], T. De Haro Muñoz[1]. *[1]Complejo Hospitalario Universitario de Granada, Granada, Spain, [2]Hospital Universitario Virgen Macarena, Sevilla, Spain, [3]The Binding Site, Barcelona, Spain*

**Background**: Serum free light chains (sFLC) are used in the diagnosis, prognosis and therapy monitoring of patients with Multiple Myeloma (MM). Non-Secretory MM (NSMM) accounts for 1-5% of all MM cases and is characterized by the absence of detectable monoclonal proteins in serum and urine by EPS and IFX. Therefore, invasive bone marrow examinations are required for monitoring disease activity. Quantification of serum free light chains (sFLC) is a sensitive method to diagnose many of these patients. The objective of our study is to show the utility of sFLC assay also in the monitoring of a NSMM patient.

**Methods:** A 63 year old man with NSMM, in complete response after treatment with VAD (Vincristine/Adriamycin/Dexamethasone) and autologous stem cell transplant (ASCT). He was monitored regularly after ASCT to ensure remission or detect a possible relapse. sFLC were measured using the assay Freelite (The Binding Site, UK).

**Results**: During the monitoring after ASCT, sFLC lambda levels began to increase with abnormal ratio (month+46: 51.2 mg/L with ratio=0.12; month+47: 144 mg/L with ratio=0.08) suggesting recurrence of NSMM at this moment. In month+50

(lambda=572 mg/L, ratio=0.02) the bone marrow showed a 4% of plasma cells and the serum protein electrophoresis and Bence Jones proteinuria were still negative. The patient began treatment with Lenalidomide/Dexamethasone (13 cycles) achieving a reduction of sFLC lambda to 20.1 mg/L and normalization of the ratio (0.58) at month+58. Seven months after this treatment, sFLC levels began to increase again with values of 231 mg/L at month+65 (ratio=0.09), 893 mg/L at month+67 (ratio=0.01) predicting a new relapse. At month+69, the patient presented a clinical relapse with presence of new osteolytic lesions, starting a new treatment with Bortezomib/Dexamethasone.

**Conclusions**: Freelite is a noninvasive assay potentially useful for monitoring the disease activity in NSMM patients that present with abnormal sFLC. Due to its high sensitivity, this assay can predict a relapse months before evidence of clinical relapse improving monitoring and helping managing NSMM patients. Furthermore, Freelite reduces the number of bone marrow biopsies for this group of patients avoiding patient anxiety and the risk of associated complications.

## A-307

### The precision and accuracy of low Factor VIII levels by one stage clotting.

F. Helmich[1], I. van Moort[2], M. van Wershoven-Passier[3], M. de Maat[2], F. van der Graaf[3]. *[1]Zuyderland Medisch Centrum, Sittard-Geleen, Netherlands, [2]Erasmus Universitair Medisch Centrum, Rotterdam, Netherlands, [3]Maxima Medisch Centrum, Veldhoven, Netherlands*

**Background:** The safe and cost-effective treatment of severe Hemophilia requires a readily-available, robust, sensitive and precise method for low-level Factor VIII activity (FVIII:C) testing. According to guideline procedures based on one-stage clotting, patient plasma is diluted with diluent and FVIII-deficient plasma (FDP) and the Activated Partial Thromboplastin Time (APTT) provides a measure for FVIII:C. Low FVIII:C levels induce weak clots at long APTTs, which are often too imprecise and inaccurate for classification or optimal dosing. Here, we investigate the effect of plasma dilution on the accuracy and precision of low FVIII:Cs in deficient patients. **Methods:** On a STA-R Evolution, 50 µl of diluted patient plasma, 50 µl FDP and 50 µl Kaoline activator were incubated for 240 s after which 50 µl $CaCl_2$ was added and the APTT was started. The reference method, based on a 10-fold dilution of patient plasma, was compared with investigated method based on a 2-fold dilution; in the latter, the effect of manual (dilutions with FDP) versus automated (dilutions with diluent) preparation of calibrators was investigated. According to standard evaluations protocols, the precision and accuracy of samples in the range of <0.01-0.10 IU/ml was obtained and the methods were compared by Passing and Bablok regression in 22 patients with FVIII:C <0.15 IU/ml. **Results:** Relative to the reference method, shorter APTTs are acquired and a stronger response is evidenced from the calibration curves using 2-fold dilution. Unlike the reference method, there is a marked difference between calibration curves that are based on standards prepared by water-dilution and standards prepared by FDP-dilution. Herein, different APTTs are acquired at the same FVIII:C revealing the influence of the other clotting factors on the APTT in the less-diluted method. While the variance at FVIII:C = 0.09 IU/ml is similar between both methods (CV = 7-8%), better precision is found at FVIII:C = 0.01 IU/ml in the investigated method (CV = 5-8% versus 14% in the reference method). At higher concentrations of the other clotting factors, firm clots are formed within uniform clotting times; given the higher response of the calibration curve, uniformity is further enhanced upon converting APTTs to FVIII:Cs. The method comparison reveals that compositional similarity between calibrators and low FVIII:C samples is crucial at lower dilutions, hence falsely elevated FVIII:Cs are found when using calibration standards based on water-dilution ($FVIII:C_{NEW}$ = 1.49 x $FVIII:C_{REF}$ - 0.51). Proper correlation between the reference method and the 2-fold diluted method is achieved by using calibrators prepared with FDP ($FVIII:C_{NEW}$ = 0.98 x $FVIII:C_{REF}$ - 0.77). **Conclusion:** The quantification of FVIII:C by one-stage clotting is more precise using a less-diluted APTT. Considering the influence of other clotting factors on the APTT, calibrators having similar concentrations of the other clotting factors should be used. At extremely low FVIII:C levels and in the absence of interfering species, the properly calibrated and less-diluted APTT may result in higher accuracies as well.

## A-308

### Individual Erythrocyte Soret Band Absorption For Cell Type Discrimination

D. Zelmanovic. *Diatron, Budapest, Hungary*

Background

We sought to determine whether the hemoglobin in individual erythrocytes absorbs enough light in the Soret band to produce robust signals for detection, and whether these signals can discriminate erythrocytes from other cell types. Since hemoglobin is found in erythrocytes but not in thrombocytes or leukocytes, a robust absorption signal should discriminate among these cell types. In order to measure erythrocyte absorption it is necessary to account for all of the light intercepted by a cell. This requires a knowledge of the scattering behavior of erythrocytes, thrombocytes, and leukocytes. Using Mie scattering theory as a guide, and treating all cells and nuclei as homogeneous bodies, we attempted to measure absorption by collecting the light scattered by individual cells, including erythrocytes, into a 17 degree cone around the incident radiation, since the theory predicts that almost all of the light that is scattered by all cell types falls within this cone, and consequently only light lost to absorption will register. This technique is not subject to a limitation of standard light-scattering-based flow cytometry for blood samples; that erythrocyte light-scatter coincidence signals overlap leukocyte signals, rendering a single-dilution, non-lytic measurement impossible. A single dilution system is less expensive relative both to hardware and reagent usage than multi-dilution techniques, and so is a desirable alternative.

Methods

Blood samples were diluted in a medium that spheres and fixes erythrocytes. Erythrocyte fractions were prepared by passing whole blood through Pall Acrodisc leukocyte filters and collecting the leukocyte depleted fractions. Leukocyte fractions were prepared by back flushing used filters with $NH_4Cl$ to lyse residual erythrocytes. Whole blood and erythrocyte fraction samples were diluted 50-fold to demonstrate insensitivity to erythrocyte coincidence. Leukocyte samples were undiluted. Samples were run on a modified hematology analyzer; light source replaced by a 406 nm laser, sheath replaced by sphering and fixing diluent. Two measurements were made on each cell; light scattered over 17° and orthogonally (80-100°). 20000 cells were analyzed for each sample. Data was collected in FCS format and displayed as right angle vs. 17° (absorption) plots.

Results and Conclusions

The plots are of erythrocytes, leukocytes, erythrocytes + leukocytes, and a normal whole blood sample. They show that the erythrocyte absorption signal is robust, but that the absorption channel alone does not discriminate between erythrocytes and polymorphonuclear leukocytes which have numerous and relatively large granules that cause scattering loss outside of 17 degrees. They also show that in combination with right angle scattering intensity, these cell types are discriminated. Thrombocyte signals are below detection threshold. We conclude that 406 nm absorption by individual erythrocytes generates robust signals, distinct from leukocyte and thrombocyte signals on right angle vs. absorption plots. It applies at high erythrocyte concentration, and is therefore suitable for automated hematology analyzers requiring high sampling rates.

## A-309

### Multicenter Study of the High-volume Sysmex CS-5100 System* Compared to the Sysmex CA-1500 System Using Siemens Healthineers Reagents

R. Barten[1], M. Kahl[1], M. Weik[1], I. Birschmann[2], C. Eby[3], J. L. Francis[4], E. Hod[5], B. Kemkes-Matthes[6], S. W. Pipe[7]. *[1]Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, [2]Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany, [3]Washington University School of Medicine, Saint Louis, MO, [4]Florida Hospital, Orlando, FL, [5]Columbia University, Medical Center, New York, NY, [6]Haemostasis Center, University Hospitals Giessen and Marburg, Giessen, Germany, [7]University of Michigan, Ann Arbor, MI*

**Background:** The objective of this study was to compare the performance of two automated coagulation analyzers, the high-volume Sysmex® CS-5100 System and the Sysmex CA-1500 System, using Siemens Healthineers reagents. Performance characteristics of the systems for factor V deficiency (FV), factor VII deficiency (FVII), protein C deficiency clotting (PC-cl), and protein C deficiency chromogenic (BCPC) were compared.

**Methods:** Three U.S. and one German laboratory participated in method comparison (MC) studies. Result comparability was investigated using leftover samples.† MC of the Sysmex CS-5100 System versus the Sysmex CA-1500 System was based on a total of 2269 results (sum of results over all parameters). Precision studies were performed according to CLSI guideline EP05-A3 and followed the scheme of 20 x 2 x 2 testing at three clinical sites. Twenty-two samples (FV: n = 6, FVII: n = 6, PC-cl: n = 5, BCPC: n = 5) covering important medical decision points and the total clinical reportable range CRR were used. The complete dataset contained 5209 results. In addition, performance data for the Sysmex CS-5100 System regarding limit of quantitation (LoQ) for FV, FVII, and both PC applications were determined according to CLSI guideline EP17-A2.

**Results:** Analysis of MC data was done by Passing-Bablok regression and difference plot and revealed very good agreement to the Sysmex CA-1500 System, showing slopes between 0.95 and 1.05 and correlation coefficients ≥0.984 (depending on application). CVs for within-run (repeatability) precision varied from 2.4 to 3.2% for FV, 1.3 to 2.0% for FVII, 2.5 to 3.2% for PC-cl, and 1.5 to 6.9 % for BCPC (depending on the sample).

**Conclusion:** Results for the Sysmex CS-5100 System were in good agreement with those for the Sysmex CA-1500 System. Precision for the new devices/reagents combination showed low CV values. Based on the data collected during these studies, in combination with improved functionality and ease of use, the high-volume Sysmex CS-5100 System provides high performance, quality, and efficiency to coagulation laboratories.

*Product availability may vary from country to country and is subject to varying regulatory requirements.

†Donors gave informed consent, and review boards were involved.

Sysmex is a registered trademark of Sysmex Corporation.

## A-310

### Multicenter Study of the Mid-volume Sysmex CS-2100*i*/2500 System* Compared to the Sysmex CA-1500 System Using Siemens Healthineers Reagents

R. Barten[1], M. Kahl[1], M. Weik[1], I. Birschmann[2], C. Eby[3], J. L. Francis[4], E. Hod[5], B. Kemkes-Matthes[6], S. W. Pipe[7]. *[1]Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, [2]Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany, [3]Washington University School of Medicine, Saint Louis, MO, [4]Florida Hospital, Orlando, FL, [5]Columbia University, Medical Center, New York, NY, [6]Haemostasis Center, University Hospitals Giessen and Marburg, Giessen, Germany, [7]University of Michigan, Ann Arbor, MI*

**Background:** The objective of this study was to compare the performance of two automated coagulation analyzers, the mid-volume Sysmex® CS-2100*i*/2500 System† and the Sysmex CA-1500 System, using Siemens Healthineers reagents. Performance characteristics of the systems for factor V deficiency (FV), factor VII deficiency (FVII), protein C deficiency clotting (PC-cl), and protein C deficiency chromogenic (BCPC) were compared.

**Methods:** Three U.S. and one German laboratory participated in method comparison (MC) studies. Result comparability was investigated using leftover samples.‡ MC of the Sysmex CS-2100*i*/CS-2500 System versus CA-1500 System was based on a total of 2172 results (sum of results over all parameters). Precision studies were performed according to CLSI guideline EP05-A3 and followed the scheme of 20 x 2 x 2 testing at three clinical sites. Twenty-two samples (FV: n = 6, FVII: n = 6, PC-cl: n = 5, BCPC: n = 5) covering important medical decision points and the total clinical reportable range (CRR) were used. The complete dataset contained 5259 results. In addition, performance data for the Sysmex CS-2100*i*/CS-2500 System regarding limit of quantitation (LoQ) for FV, FVII, and both PC applications were determined according to CLSI guideline EP17-A2.

**Results:**Analysis of MC data was done by Passing-Bablok regression and difference plot and revealed very good agreement to the Sysmex CA-1500 System, showing slopes between 0.94 and 1.04 and correlation coefficients ≥0.977 (depending on application). CVs for within-run (repeatability) precision varied from 3.2 to 5.0% for FV, 1.9 to 2.5% for FVII, 2.5 to 4.8% for PC-cl, and 1.2 to 4.6 % for BCPC (depending on the sample).

**Conclusion:** Results for the Sysmex CS-2100*i*/CS-2500 System were in good agreement with those for the Sysmex CA-1500 System. Precision for the new devices/reagents combination showed low CV values. Based on the data collected during these studies, in combination with improved functionality and ease of use, the mid-volume Sysmex CS-2100*i*/2500 System provides high performance, quality, and efficiency to coagulation laboratories.

*Product availability may vary from country to country and is subject to varying regulatory requirements.

† Performance characteristics presented have been established using the Sysmex CS-2100i Automated Blood Coagulation Analyzer, which is the representative member of an instrument family. The performance can be applied accordingly to the Sysmex CS-2500 Automated Blood Coagulation Analyzer, which is a member of the same instrument family.

‡Donors gave informed consent, and review boards were involved.

Sysmex is a registered trademark of Sysmex Corporation.

## A-311

### Importance of free ligth chains in the rapid diagnosis of multiple myeloma

M. de Toro Crespo[1], J. Maesa Márquez[1], P. Menéndez Valladares[1], J. Garcia de Veas Silva[2], N. Barbosa[3], C. Bermudo Guitarte[1]. *[1]Virgen Macarena Hospital, Sevilla, Spain, [2]Complejo Hospitalario Universitario de Granada, Granada, Spain, [3]binding site, Barcelona, Spain*

**Background:** A 70 year old male with a medical history significant for hypertension, diabetes type 2 and alcohol abusepresented to the emergency department with asthenia, decreased appetite and dark stools.Initial laboratory tests results revealed macrocytic anemia with decreased Hb 8.9 g/dL (reference interval 13-17.5), thrombocytopenia with platelets 38 x $10^3$/µL (reference interval 140-450), hypertriglyceridemia 321 mg/dL (reference interval 70-170) and hyperuricemia 9.3 mg/dL (reference interval 2.6-7.2).Other routine biochemical parameters were normal. Physical examination revealed no clinically significant findings.The peripheral smear showed bicytopenia. Structural changes include:pronounced red cells anisopoikilocytosis and macrocytosis; hyposegmented neutrophils and some metamyelocytes; thombopenia with anisocytosis, giant platelets and fewmicroaggregates; and isolated blastic cells. The patient was transfused with platelets and red blood cells. His medication list included quinapril-hydrochlorothiazide 20/12.5 mg/24 h, metfomin 850 mg/24 h, diazepam 5 mg/24 h, acetaminophen 325 mg/8 h, vitamine B12 and folate supplements were added. **Further investigation:** The patient was referred for further investigations. Routine biochemical parameters revealed creatinine 1.52 mg/dL (reference interval 0.7-1.5), hypercalcemia 14.4 mg/dL (reference interval 8.5-10.5), hyperuricemia 11.7 mg/dL, gamma-glutamyl-transferase 189 U/L (reference interval 11-49), Ferritine 698.4 ng/mL (reference interval 22-322). Serum vitamine B12 was within range butfolate levels were low, 2.3 ng/mL (reference interval 4.6-18). Tumor markers panel showed Ca 125 63.9 U/mL (reference interval 5-35), Ca 15.3 208.2 U/mL (reference interval 2-37), Ca 19.9 1.3 (reference interval 2-37), β-2-microglobulin 12.55 mg/L (reference interval 0.8-2.2). Turbidimetric assay revealed very high IgA 2128 mg/dL (reference interval 40-350), IgG 303 mg/dL (reference interval 650-1600), IgM 23 (reference interval 50-300), serum λ FLC concentration 1005.81 mg/L (reference interval 5.71-26.3), serum κ FLC concentration 8.73 mg/L (reference interval 3.3-19.4), kappa-lambda ratio 0.01 (reference interval 0.26-1.65).Serum protein electrophoresis showed an M component (1.8 g/dL) in the beta zone. Results are consistent with multiple myeloma. A leukoerithroblastic reaction was shown in a new peripheral blood smear. The results of other tests were within their respective reference intervals. Two unsuccessful bone marrow aspirations lead to a biopsy.Computed tomography scans showed multiple lytic lesions in the skull, spine, ribs and pelvis consistent with multiple myeloma or metastasis.Biopsy evidenced bone marrow collagen fibrosis with infiltration of 50% plasma cells CD138 positive. His hematologist recommended initiation of treatment consisting of bortezomib/ dexamethasone protocol. **Conclusion:** Weakness and fatigue are common manifestations of multiple myeloma often associated with anemia.Serum protein electrophoresis and, especially, the determination of free light chain κ and λ were fundamental for the faster diagnosis of multiple myeloma that was made difficult by unsuccessful two bone marrow aspiration. The biopsy evidenced bone marrow collagen fibrosis.Reticulin fibrosis is associated with many benign and malignant conditions while collagen fibrosis is particularly prominent in late stages of severe myeloproliferative diseases like multiple myeloma or following tumour metastasis to the bone marrow. Recent evidence has shown that the amount of bone marrow reticulin staining often exhibits no correlation to disease severity, while the presence of type I collagen, is often associated with more severe disease and a poorer prognosis.

## A-312

### Loss of *Rnase1* produces coagulation abnormalities in mice

E. R. Garnett, R. T. Raines. *University of Wisconsin-Madison, Madison, WI*

**Background:** Use of anticoagulant drugs carries a risk of bleeding, and there is significant interest in developing new therapies that do not present this risk. This requires a better understanding of the factors that contribute to coagulation and thrombosis *in vivo*. The contact activation pathway has been a subject of recent research to this end, with studies highlighting the ability of polyphosphate and RNA to stimulate coagulation in plasma and demonstrating inhibition of the coagulation proteases factor XI (fXI) and factor XII (FXII) as possible antithrombotic therapies. At the same time, studies in mice have demonstrated antithrombotic properties for the enzyme RNase A, a nonspecific endoribonuclease. The objective of our research is to clarify the role of RNA and RNase in regulation of coagulation *in vivo* through study of a mouse model that lacks RNase 1, the murine homolog of this RNA-degrading protein. **Methods:** *Rnase1*-null mice were generated in our laboratory, and evaluated in comparison with wild-type littermates. Plasma coagulation was evaluated *in vitro* using kinetic clotting assays with Thromborel® S or Dade Actin® FSL Activated PTT Reagent, as well as with mixing tests using human factor-deficient plasma. *In vivo* analysis of bleeding and clotting behavior was conducted using lipopolysaccharide-stimulated thrombin-antithrombin complex assay, tail-vein bleeding test, and ferric chloride-induced arterial thrombosis assay. Studies were conducted with a minimum of three biological replicates per group, and statistical significance was evaluated using Wilcoxon rank-sum test.**Results:** *In vitro* coagulation assays reveal shortened clotting times for *Rnase1*-null plasma relative to wild-type, with significantly shorter times for unstimulated plasma and when stimulated with Thromborel S. Yet, *Rnase1*-null mice did not exhibit increased thrombin-antithrombin complex formation in response to lipopolysaccharide challenge, did not bleed less than wild-type mice in a tail-vein bleeding test, and did not form thrombi more quickly than did wild-type mice in a ferric-chloride induced arterial thrombosis model. Mice that lack expression of contact pathway coagulation factors, such as fXI and fXII, also do not exhibit perturbed in vivo coagulation behavior despite prolongation of coagulation in vitro. Additionally, these factors are activated in vitro by RNA. Accordingly, and because *Rnase1*-null plasma contains significantly more RNA than does wild-type plasma, we are evaluating whether the loss of RNase 1 permits increased activation of the contact activation pathway. Indeed, preliminary factor activity assays indicate that fXII is strongly activated in *Rnase1*-null plasma, and experiments are underway to demonstrate that this is an RNA-dependent process.

**Conclusion:** Our results suggest that RNase 1 is an endogenous negative regulator of contact pathway activation in mice via RNA degradation. This finding provides insight into the function of RNA in the pathophysiology of coagulation, and could inform future development of anticoagulant therapeutics.

## A-313

### Optical platelet counts by Abbott prototype hematology analyzer show high level of agreement with CD61 immunoplatelet results

S. Mulla[1], Y. A. Leslie[1], M. Rogriguez[1], W. L. Rebekah[1], W. A. Bryan[1], A. M. Jones[1], O. Heldt[1], R. Chandran[2], G. Lakos[3], A. A. Mohammad[1]. *[1]Baylor Scott & White Healthcare, Temple, TX, [2]Abbott Laboratories, Lake Forest, CA, [3]Abbott Laboratories, Santa Clara, CA*

**Background:** The CELL-DYN Sapphire (Abbott Laboratories, Santa Clara, CA) hematology analyzer is able to enumerate platelets (PLT) based on CD61 labeling in addition to optical and impedance PLT measurements. The CD61 immunoplatelet (PLTim) method is considered to be a surrogate reference method for the ICSH/ISLH recognized flow cytometric assay (CD41/CD61). We utilized this immunoplatelet assay to verify the performance of a novel optical PLT counting method on a prototype hematology analyzer (Abbott), focusing on samples with low PLT counts.

**Methods:** One hundred and sixty-two EDTA-anticoagulated samples with platelet counts of 5.1-442.8 x $10^9$/L were selected from the routine workload of the hematology laboratory. Samples were analyzed with two prototype systems and with one CELL-DYN Sapphire analyzer within eight hours of collection. PLT counts obtained with the prototype systems and the CELL-DYN Sapphire optical PLT method (PLTo) were compared to the PLTim method, as well as with each other. Agreement was evaluated using regression statistics and Pearson's correlation. **Results:** Results by all methods and on each analyzer were available for 110 samples, ranging 5.1-88.8 x$10^9$/L PLT. Pearson's correlation coefficients, slope and intercept of the regression line (Passing Bablok), and mean differences between the results are shown below:

| | n | r | Slope | Intercept | Mean difference |
|---|---|---|---|---|---|
| Prototype#1 vs PLTim | 110 | 0.98 | 0.92 | -1.6 | -2.9 |
| Prototype#2 vs PLTim | 110 | 0.97 | 0.94 | 0.22 | -2.7 |
| Prototype#1 vs Prototype#2 | 110 | 0.98 | 0.99 | -0.26 | 0.2 |
| Sapphire PLTo vs PLTim | 110 | 0.99 | 0.90 | 0.63 | -3.4 |

**Conclusion:** Results obtained by the prototype analyzers showed excellent correlation with the PLTim measurement, and also with each other. The mean difference between PLT results by either prototype system and the PLTim results was smaller than that of between the Sapphire PLTo and PLTim methods. These data demonstrate that the new prototype hematology analyzer provides results in low PLT ranges that are equivalent with those obtained with the PLTim method.

## A-314

### Establishment of Reference Intervals for Whole Blood Luminescent Platelet Aggregometry

A. L. Pyle-Eilola[1], C. M. Carter[1], J. Cucci[2], W. L. Chandler[3]. *[1]Nationwide Children's Hospital, Columbus, OH, [2]St. Luke's Boise Medical Center, Boise, ID, [3]Seattle Children's Hospital, Seattle, WA*

**Background:** Platelet aggregometry may be employed for the diagnosis of platelet function disorders such as von Willebrand's disease and monitoring response to certain anti-platelet drugs. Though traditionally performed on platelet rich plasma, many laboratories, especially in pediatric institutions aiming to reduce blood volume collected, adapt assays for whole blood. There is a paucity of published normal range studies for whole blood platelet aggregation analysis by impedance and luminescent ATP release assays. The objective of this study is to generate reference intervals for whole blood platelet aggregometry by impedance and luminescence. **Methods:** Whole blood samples were collected by venipuncture into sodium citrate vacutainer tubes from 77 normal, drug-free donors recruited at Nationwide Children's Hospital (Columbus, OH), Seattle Children's Hospital (Seattle, WA), and St. Luke's Hospital (Boise, ID). Various concentrations of agonists were introduced to elicit aggregation, and impedance whole blood aggregometry and luminescent ATP release measured by Chronolog aggregometer according to manufacturer's recommendations (ChronoLog, Havertown, PA). Reference intervals were generated using either parametric or transformed parametric analysis, as appropriate, in EP Evaluator 10 (Data Innovations, Burlington, VE). **Results:** Reference intervals and 90% confidence intervals were generated for whole blood aggregometry by both impedance and luminescent ATP release assays in response to several agonists (see table). **Conclusion:** This study fills an important gap in the literature which currently has a paucity of reference intervals for this methodology. These may serve as guidance for laboratories running these assays.

| Agonist | Agonist Concentration | Lower Limit (confidence interval) | Upper Limit (confidence interval) |
|---|---|---|---|
| **Impedance Aggregation** | | | |
| Arachadonic Acid | 0.5 mM | 6.9 Ω (5.1-8.7) | 28.7 Ω (26.9-30.4) |
| ADP | 5 µM | 5 Ω (3.0-7.0) | 26 Ω (24-28) |
| ADP | 10 µM | 5.5 Ω (4.5-6.6) | 33.4 Ω (29.5-37.6) |
| Collagen | 1 µg/mL | 12.1 Ω (11.1-13.2) | 32.6 Ω (29.8-35.6) |
| Collagen | 5 µg/mL | 10.5 Ω (7.8-13.1) | 42 Ω (39.3-44.6) |
| **Luminescent ATP release** | | | |
| Arachadonic Acid | 0.5 mM | 0.45 nM (0.37-0.54) | 2.78 nM (2.46-3.13) |
| ADP | 5 µM | 0.11 nM (0.06-0.17) | 2.01 nM (1.58-2.53) |
| ADP | 10 µM | 0.21 nM (0.16-0.27) | 1.95 nM (1.66-2.27) |
| Collagen | 1 µg/mL | 0.43 nM (0.35-0.51) | 2.22 nM (1.96-2.50) |
| Collagen | 5 µg/mL | 0.54 nM (0.45-0.64) | 2.93 nM (2.61-3.28) |
| Ristocetin | 0.25 mg/mL | 0.0 nM (-0.7--0.5) | 0.8 nM (0.7-0.9) |
| Ristocetin | 1.0 mg/mL | 4.3 nM (3.6-5.1) | 33.6 nM (28.3-39.8) |
| Thrombin | 1 Unit | 0.80 nM (0.71-0.90) | 3.03 nM (2.68-3.43) |

## A-315

### The Changes Of Coagulation Parameters In Plasma Samples Reflecting Different Hematocrit Values

I. Ozen Kucukcetin, G. Yucel, S. Ozdem. *Akdeniz University, Antalya, Turkey*

**Background:** There are many preanalytical variables affecting routine coagulation tests. Elevated hematocrit (Htc) levels are one of these variables. Studies have shown that relative decrement in the amount of plasma in samples with high Htc levels caused an increment in citrate concentrations with the resultant defects in coagulation

test results. However, no study has been conducted to determine the effect of low Htc levels in plasma samples on coagulation tests. Therefore, in the present study, we aimed to evaluate whether the low plasma Htc levels affect the results of coagulation tests.

**Methods:** Standard plasma was injected into coagulation tubes containing 3.2% citrate to obtain plasma samples with hematocrit rates of 5% to 75% which were grouped as A (5-25%), B (30-50%) and C (55-75%) according to the Htc levels. Prothrombin time (PT), active partial thromboplastin time (aPTT), thrombin time (TT) and fibrinogen measured in these plasma samples. **Results:** The results were given as mean ± SD. PT (s) were 13.7±0.30, 14.88±0.57 and 20.16±4.66 in groups A, B and C, respectively. aPTT (s) values were measured as 35.79±1.39 in group A, 42.48 ± 3.51 min in group B and 76.47±31.55 in group C. TT (s) were measured as 26.42±0.77 (group A), 28.24±1.17 (group B) and 32.02±2.60 (group C). Fibrinogen levels (mg/dL) were determined as 230.0±5.24, 221.4±7.26 and 189.7±19.65 in groups A, B and C, respectively. The values for all measured parameters, were significantly($p < 0,0001$) different in group A reflecting low Htc levels compared to other groups.

**Conclusion:** Previous studies have reported an advers effect of Htc levels on routine coagulation tests, and suggested that the citrate concentration should be adjusted for it contains >55% of Htc to remove this adverse effect. In our study, low Htc (5-25%) levels were also shown to cause erronous results in routine coagulation tests suggesting that a similar citrate level adjustment is necessary in plasma samples with low Htc levels.

## A-316

### Longitudinal monitoring of MDS by Flow cytometry reveals rapid transformation of MDS in remission into AML: a case report

N. Ozcan, N. Duzkale, A. H. Kaya, A. &. E. Tekgunduz, F. Altuntaş. *Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, ankara, Turkey*

**Background:** Myelodisplastic syndromes (MDS) are a heterogeneous group of disorders with ineffective hematopoesis. Flow cytometric immunophenotyping (FC) of hematopoietic progenitor and maturing cells in dysplastic bone marrow has proven as a sensitive method in the diagnosis and prognostication of myelodysplastic syndromes. In this study, using FC we follow up a case with MDS who was in remission but rapidly transformed into AML within a year and passed away.

**Methods:** Conventional cytogenetics, fluorescence in situ hybridization (FISH) and FC were used for evaluation of bone marrow.

**Results:** Bone marrow examination by FC demonsrated myelodisplastic changes with 12% of myeloblasts and was diagnosed as refractory anemia with excess of blasts (RAEB II). After he was given low dose chemotherapy, the bone marrow biopsy material showed %5 myeloblast, hence was considered in remission. However, the patient did not follow up for a year. When he was readmitted for evaluation and treatment 40% myeloblasts were demonsrated in bone morrow by FC. Cytogenetic study was performed on his bone marrow but not there was not enough number and quality of metaphase, however only one metaphase revealed 92,XXYY,t(4;11)(q21;q23).

**Conclusion:** In this case, we emphasize the significance of MDS immunophenotyping by FC longitudinally to determine acute transformation of MDS to AML. Most common use of FC in MDS has been primarily restricted to the characterization of blast cells in secondary acute leukemia following MDS, however more frequent monitoring might be helpful to determine the trasformation on a more timely manner and adjust the treatment accordingly.

## A-317

### Monosomy 7 might be associated with poor prognosis in Periferal T-cell lymphoma not otherwise specified: a case with nervous system involvement

N. Duzkale, N. Ozcan, H. Batgi, A. &. E. Tekgunduz, F. Altuntaş. *Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, ankara, Turkey*

**Background:** Periferal T-cell lymphomas not otherwise specified (PTCL-NOS) include a heterogeneous group of diseases involving lymph nodes and extra nodal sites deriving from the clonal expansion of mature T-lymphocytes. Many affected patients present with nodal involvement, and there is also potential for extranodal involvement of the liver, gastrointestinal tract, bone marrow, and/or skin. In this study, we report a case with PTCL-NOS, with nervous system involvement, a poor prognostic parameter, and a novel cytogenetic abnormality.

**Methods:** Conventional cytogenetic and fluorescence in situ hybridization (FISH) tecnique was used for evaluation of bone marrow. Flow cytometry was used for evaluation of bone marrow and cerebrospinal fluid (CSF).

**Results:** Bone marrow biopsy materials revealed infiltration by the PTCL-NOS, and based on that, the patient was classified as stage IVB according to the Ann Arbor classification. We identified monosomy 7, {nuc ish(D7Z1×1, D7Z1 dim×1)[137/171]/nuc ish(D7Z1×1)[26/171]} abnormality by FISH analysis. Cerebrospinal fluid examination by flow cytometry showed central nervous system infiltration.

**Conclusion:** Monosomy 7 is a common abnormality identified in myelodysplastic syndrome and acute myeloid leukemia, particularly in therapy-related cases and is a poor prognostic indicator. Despite it is not associated with subtypes of non-Hodgkin's lymphoma, we propose that monosomy 7 can be a novel poor prognostic factor in PTCL-NOS. However, its significance is uncertain in non-Hodgkin's lymphoma currently and further investigation is warranted to determine it more definitively.

## A-318

### Protein S deficiency diagnosis: analysis from a large laboratory in Latin America

A. K. Zeinad-Valim, R. M. Cosentino, J. R. Alves, G. A. Campana. *DASA - Diagnosticos da America, Sao Paulo, Brazil*

**Background:** The diagnosis of hereditary protein S (PS) deficiency is the most difficult of the hereditary thrombophilias to document with certainty, particularly in the setting of an acute thrombosis or anticoagulant administration, especially vitamin K antagonists. Many other acquired conditions are associated with reduced levels: pregnancy, oral hormonal contraceptive use and several conditions associated with acute illnesses, in which there is an increase of C4b-binding protein. Protein S (for Seattle) is a negative regulator of coagulation. It circulates in two states: free, and bound to the complement component C4b-BP. When elevated, the C4b-BP leads to a shift of the PS from the free form (the only form that is active) to the bound (inactive) form, leading to an erroneous diagnosis. Free PS serves as a cofactor for protein C, which inactivates procoagulant factors Va and VIIIa, reducing thrombin generation. The frequency of PS deficiency may be less than 1 percent of individuals with venous thromboembolism (VTE). Free PS level (measured with an immunoassay) is probably the best screening test. The objective of this study is to evaluate the profile of total and free PS requests for the investigation of thrombophilia in a large Brazilian laboratory. **Methods:** Between October and December 2016 a survey of all samples with total and free PS was performed. The results were analyzed to identify the reduction of total and free PS and the reduction of free PS with normal total PS. **Results:** In 3227 PS applications, in only 304 (9.4 percent) of them there was also the request for free PS. Of these, in 58 there was a reduction of the total and free PS, in 53 reduction of the free PS only (in 10 samples the reduction constituted a severe deficiency). In that same period, only 851 requests (26.3 percent) were of free PS (associated or not to the requests of total PS). **Conclusion:** PS deficiency is an autosomal dominant condition, and the major clinical feature is VTE. Although the description of the best method of evaluation of PS deficiency is the dosage of free PS, a large number of requests still consider only the total PS for diagnosis. The presence of free PS reduction, with normal total PS levels, underscore the importance of the specification of free PS for the diagnosis of this thrombophilia, since only the free form is active in reducing the thrombin generation. Such a situation leads to misleading results, false negatives and manly false positives, because it is a rare condition with several acquired interfering factors. This demonstrates that a continuing education of the teams is necessary, either through information leaflets, lectures or meetings, in order to reduce the anxiety of patients and relatives and the unnecessary use of anticoagulants, which can have serious consequences for the community and health systems.

## A-319

### Performance of Hemoglobin A1c and Fructosamine on Estimating Glycemic Control in Diabetes Patients with Hemoglobin Variant Hope

Y. Wang, C. Fluharty, D. Andrews, M. Constantin, F. Nolte. *Medical University of South Carolina, Charleston, SC*

**Background:** Hemoglobin A1C (HbA1c) and fructosamine are commonly used to estimate glycemic control in diabetes patients. Hemoglobin (Hb) variant Hope is prevalent in Southeast Asian population, but has been reported in the United States.

This case study aims to evaluate the clinical performance of HbA1c and fructosamine on estimating glycemic control in 4 diabetes patients with Hb variant Hope.

**Methods:** HbA1c in patients' whole blood were measured via high-performance liquid chromatography (HPLC) performed on Turbo Variant™ II (Bio-Rad) and immunoassay on DCA Vantage Analyzer (Siemens), respectively. The presence of Hb Hope was analyzed by Variant™ II β-Thalassemia Short Program and acid gel electrophoresis. Fructosamine from the same samples were quantified using spectrophotometry (ARUP Laboratories). These patients' recent fasting glucose levels (2 weeks - 3 months) were obtained through retrospective chart review.

**Results:** Spuriously elevated HbA1c (39.7%-55.3%) as determined by HPLC (Fig. 1A, D) was observed in all patients. The interference from Hb Hope was suggested based on the elevated P2 on Hb chromatograph (Fig. 1B) and intensive band corresponding to HbF on acid gel (Fig. 1C). In contrast, normal A1c% (3.5%-5.3%) from the same samples were obtained via immunoassay, which were consistent with their corresponding normal fructosamine levels (184-264 µM) (Fig. 4D). Surprisingly, patient chart review revealed that these patients encountered multiple episodes of elevated fasting glucose (110-258 mg/dL) in the past 2 weeks to 3 months, with an average glucose level of 160 mg/dL (Fig. 4D).

**Conclusion:** Our data demonstrate that Hb Hope causes significant positive bias on HbA1c HPLC assay but not HbA1c immunoassay, which displayed a good agreement with patient's fructosamine level. However, the performance of HbA1c and fructosamine in estimating glycemic control in patients with Hb Hope might be questioned, in view of the discrepancy between the suggested in-control glycemic status and the elevated fasting glucose.

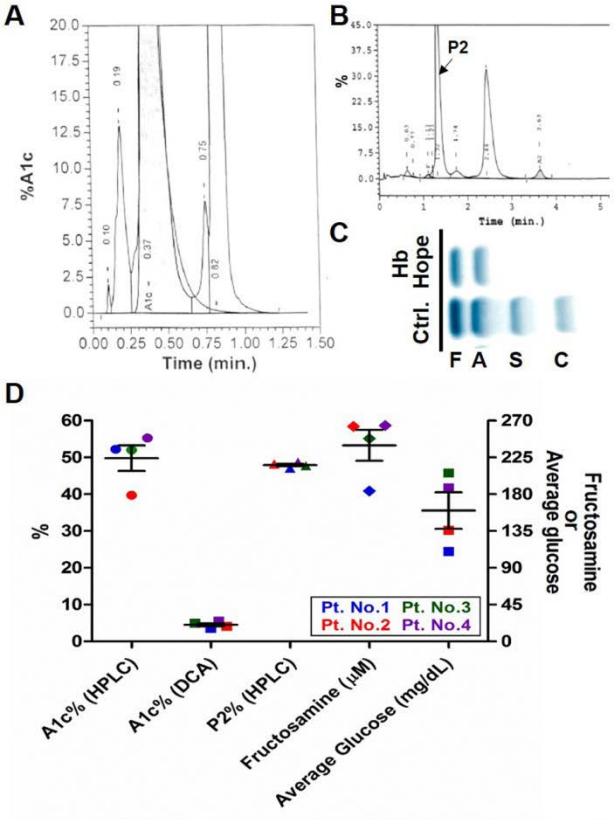



**Figure 1.** (A) HbA1c chromatograph on BIORAD Turbo Variant II. (B) Hb variant chromatograph on BIORAD variant II β-thalasima program. (C) Acid gel electrophoresis of patient with Hb Hope and loading control (Ctrl.) Hb. (D) Quantifications of A1C% by HPLC and DCA immunoassay, Hb P2% by HPLC, fructosmaine, and the average glucose in the previous 2 weeks to 3 months.

## A-320

### Discrepancies in measured fibrinogen concentration using low and high thrombin content commercial fibrinogen reagents

A. M. Wockenfus, S. W. Smeets, S. D. Carter, L. J. Donato, B. S. Karon. *Mayo Clinic, Rochester, MN*

**Background:** Commercial fibrinogen reagents with lower thrombin content are more susceptible to interference from direct thrombin inhibitors, anti-thrombin antibodies, and other interferants. We compared fibrinogen results between low and high thrombin content fibrinogen assays to determine the rate and magnitude of discrepant fibrinogen results in an acute care patient population.

**Methods:** As part of the evaluation of a new coagulation analyzer, we measured fibrinogen using the higher thrombin content (80 UNIH/mL) FIB 5 reagent on a Stago Compact (Diagnostica Stago); and the lower thrombin content (35 UNIH/mL) FIB C assay on an IL ACL TOP 500 (Instrumentation Laboratory). The initial comparison was done using 50 frozen samples submitted to the stat laboratory. In a follow-up experiment using 50 fresh samples, we compared FIB 5 on the Compact to both FIB C and the QFA (high thrombin content of 100 UNIH/mL) fibrinogen reagents on the IL TOP. The stat laboratory performs fibrinogen measurement primarily for patients undergoing cardiovascular surgery. The number/percent of discrepant results (>25% difference between assays) was determined.

**Results:** In the first experiment using frozen samples, 5 of 50 (10%) FIB C results were >25% lower than the corresponding FIB 5 value. For these samples fibrinogen concentration measured by the lower thrombin content FIB C reagent ranged from 74 to 350 mg/dL lower than corresponding FIB 5 (higher thrombin content) value. In the second experiment using fresh samples, 12 of 50 (24%) of FIB C values were >25% lower than the corresponding FIB 5 value, while 1 sample had FIB C value >25% greater than FIB 5. Using the higher thrombin content IL QFA reagent, 4 of 50 (8%) QFA values were >25% lower than FIB 5, while 1 was >25% higher than the corresponding FIB 5 value. Selected chart review of patients with discrepant fibrinogen results demonstrated that 4 of the discrepant fresh sample comparisons were from 2 infants who had received topical thrombin, suggesting that anti-thrombin antibodies may have caused the discrepancies. Another discrepant result came from an adult post-myocardial infarction that was on a direct thrombin inhibitor (bivalirudin). Remaining discrepant samples had no obvious explanation.

**Conclusion:** In an acute care patient population (mostly patients following cardiovascular surgery), discrepancies between different commercial fibrinogen reagents are common. Using reagents with higher thrombin content reduces but does not eliminate discrepancies between commercial fibrinogen reagents.

## A-321

### Comparative Study of the Point-of-care Xprecia Stride Coagulation System to the BCS XP, Sysmex CS-2500, and Sysmex CA-1500 Systems

N. C. Vanderslice[1], A. Hodges[2], C. Lessard[1]. [1]*Siemens Healthineers, Norwood, MA,* [2]*Universal Biosensors, Melbourne, Australia*

**Background:** The objective of the study was to compare the performance of the point-of-care quantitative prothrombin time (PT) test for the monitoring of oral anticoagulant therapy with a vitamin K antagonist (VKA) using four lots of Xprecia Stride™ test strips to the results of central-lab PT/INR testing using plasma of the same samples on the BCS® XP, Sysmex® CA-1500, and Sysmex CS-2500 Systems.

**Methods:** Capillary fingerstick samples were obtained from approximately 90 patients receiving VKA therapy and 30 healthy patients at two sites. A fingerstick was used to obtain blood that produced results on four lots of Xprecia Stride test strips. In addition to the capillary fingerstick, a venous sample was obtained from each patient, which was separated into a plasma fraction. The plasma was frozen and sent to a central lab on dry ice for PT/INR testing. Method comparison and outlier removal were performed per CLSI guideline EP09-A3.

**Results:** Passing-Bablok regression analysis showed exemplary agreement between the Xprecia Stride analyzer and the central-lab devices. The Xprecia Stride analyzer demonstrated slopes of 0.95–0.98 against the Sysmex CA-1500 System, 0.94-0.97 against the Sysmex CS-2500 System, and 0.90–0.94 against the BCS XP System.

**Conclusion:** All method comparisons between the Xprecia Stride analyzer and the laboratory devices showed good agreement. The data demonstrates that the point-of-care device can provide results that will lead to similar medical decisions across the therapeutic ranges of warfarin (VKA) monitoring. With this performance, the Xprecia Stride analyzer can be used to provide clinically relevant results in a timely manner at the point of care.

Product availability may vary from country to country and is subject to varying regulatory requirements

BCS XP and Xprecia Stride are trademarks of Siemens Healthcare; Sysmex is a trademark of Sysmex Corporation.

| Site/ Operator | Strip Lot | Xprecia Stride Analyzer vs. Sysmex CA-1500 System($r^2$) | Xprecia Stride Analyzer vs. Sysmex CS-2500 System ($r^2$) | Xprecia Stride Analyzer vs. BCS XP System($r^2$) |
|---|---|---|---|---|
| Site 1, Operator 1 | 1 | 0.966 | 0.966 | 0.966 |
| | 2 | 0.966 | 0.964 | 0.962 |
| | 3 | 0.964 | 0.966 | 0.963 |
| | 4 | 0.953 | 0.955 | 0.953 |
| Site 2, Operator 2 | 1 | 0.939 | 0.937 | 0.928 |
| | 2 | 0.948 | 0.947 | 0.939 |
| | 3 | 0.932 | 0.933 | 0.923 |
| | 4 | 0.944 | 0.943 | 0.932 |

## A-322

### New MiniCollect® 9NC Coagulation Blood Collection Tubes for pediatric sample testing

S. Griebenow, M. Holzer. *Greiner Bio-One GmbH, Kremsmünster, Austria*

**Background:** Drawing blood from infants or children is mostly critical, particularly when the amount needed to fill a standard coagulation tube by ensuring the correct ratio of blood to additive can't be guaranteed. The MiniCollect Coagulation Tube is intended for collection of citrate anticoagulated whole blood samples for coagulation assays and allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity.

**Methods:** Two clinical studies were carried out to compare the performance of the new pediatric tube to a standard VACUETTE Coagulation tube by taking venous blood. Altogether, 20 healthy and 75 hospitalized subjects (Laboratory Rainbach and Hospital Steyr, Upper Austria) were recruited. Informed consent was given by all donors and the study was approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, common coagulation parameters were tested using an ACL Top 500 (Laboratory Instruments). Analysis was done with the instrument's accompanying reagents (precision aPTT ≤2.5%; PT ≤ 3%, Fibrinogen ≤8%). Statistical evaluation was done by STATISTICA 13.

**Results:** Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of pediatric tubes with the new design did not reveal any clinically nor statistically significant deviations ($p<0.05$). The values in both tubes resulted in maximum deviations of 7.1% for aPTT.

**Conclusion:** From a clinical perspective, the MiniCollect Coagulation tube with the new design is substantially equivalent to a VACUETTE Coagulation tube. The newly designed tube provides an essentially enhanced blood collection device for pediatric sample testing.

## A-323

### Hematological sample testing in new MiniCollect® Blood Collection Tubes

S. Griebenow, M. Holzer. *Greiner Bio-One GmbH, Kremsmünster, Austria*

**Background:** Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. MiniCollect® $K_2$EDTA and $K_3$EDTA Blood Collection Tubes are used to collect, transport, store and evaluate capillary blood specimens for hematology tests.

**Methods:** Studies considering venous and capillary collection were done at Steyr Hospital and Laboratory Rainbach (Austria) using MiniCollect tubes with the old design vs. new design. Altogether, 65 hospitalized and 90 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. Complete blood counts including 15 parameters were tested using a DxH800 (Beckman Coulter, precision WBC ≤ 3%/RBC ≤ 1.5%). Comparison testing to Microtainer (BD) was done. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.

**Results:** Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design did not reveal any clinically nor statistically significant deviations ($p<0.05$). Comparing the initial values of the old and new design for venous collection, both EDTA tubes resulted in a highest deviation of 3.0% for RBC. Comparable highest deviations for initial values in relation to 48h values were obtained for $K_2$EDTA (WBC 0.4%; RBC 0.1%) and $K_3$EDTA (WBC 2.6%; RBC 0.1%). Capillary collection led to a highest deviation for WBC of 0.7% for $K_2$EDTA and of 2.2%.the $K_3$EDTA tubes.

**Conclusion:** From a clinical perspective, the MiniCollect $K_2$EDTA and $K_3$EDTA tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

## A-324

### A proposition for Total error evaluation of fetal hemoglobin

M. Vanin. *DASA, Cascavel, Brazil*

Background: At birth, fetal hemoglobin comprises 65 to 90% of total hemoglobin concentration, and after the second trimester, this percentage decreases to less than 2%. Fetal hemoglobin (HbF) is formed by two gamma globin chains combined with two alpha globin chains and is represented by the formula α2 γ2 with expression of the genes γG γA, located on the short arm of chromosome 11. The permanent increased percentage of HbF can occur due to some hereditary abnormalities, as in: delta-beta thalassemia characterized by reduced synthesis or absence of delta and beta chains, with consequent increase in Hb F; in beta thalassemia, when syntesis of beta chains is reduced with increased of A2 and fetal hemoglobins; and, in hereditary persistence of Hb F (HPFH), a genetic disorder characterized by continuous production of HbF in adulthood. HbF also influences the clinical manifestation of other hemoglobinopathies, working as an important protective factor against sickling phenomenon, due higher affinity for oxygen.

Objective: We aimed to propose a total error limit in HbF dosage by the sum of random error with systematic error, evaluate how the results can vary and also to define a target value, for a clinical acceptable performance for this analyte, thus helping the continuous improvement of quality.

Methods: Total error of the analyte fetal hemoglobin (VARIANT ™ II - ß-thalassemia Short Program Bio-Rad®) was calculated as the sum of random and systematic errors, obtained from January 2013 to December 2016. As random error, we used coefficient of variation (CV) of the test multiplied by 1.65 to a desired confidence interval of 90%. For the systematic error we used in the calculation the results from the proficiency testing provider Control Lab® (hemoglobinopathies).Results: During this period, we obtained the medium CV of 2.76% and total error of 14.05%, for fetal hemoglobin.

Conclusion: We compared average CV observed at this study to those reported by the kit manufacturer's labeling, and found that the CV obtained was very close to the informed by Bio-Rad® (2.47%). We also noted that the results of the Proficiency Test were within the acceptable limit stated by the provider, Control Lab®. Whereas until the present moment there is no suggestion in the literature to the total error of this analyte, we conclude that a total error of 14.05% should be acceptable for fetal hemoglobin.

## A-325

### Avoiding Unnecessary Plasmapheresis in Suspected Thrombotic Thrombocytopenic Purpura Using Stat Testing of ADAMTS13 Activity in Blood

E. A. Korte, R. Valdes, M. T. Ayyoubi. *University of Louisville, Louisville, KY*

We document a significant number of unnecessary plasma exchange procedures to treat suspected thrombotic thrombocytopenic purpura (TTP) which could have been

avoided with rapid measurement of ADAMTS13 activity in blood. ADAMTS13 is a metalloproteinase that cleaves von Willebrand factor (vWF) to maintain coagulation homeostasis. Reduced ADAMTS13 activity results in large vWF multipliers, hypercoagulability, extensive microthrombus formation, and severe end-organ damage. TTP is a rare disease (annual incidence 4-11 cases per million) characterized by reduced ADAMTS13 activity. Diagnosis of TTP hinges on the ability to differentiate it from other primary or drug-induced thrombotic microangiopathies. Rapid diagnosis and initiation of therapy is paramount due to the high mortality rate if untreated (>90%) and severe pathogenic complications, including renal dysfunction, dysrhythmias, and neurological manifestations. The first-line therapy is daily plasma exchange which is both expensive and presents its own set of risks to patients.

Laboratory testing for ADAMTS13 activity is diagnostic for TTP if below 10% of expected. However, as a send-out test, turnaround time for ADAMTS13 activity is often days or weeks. Due to the high mortality of TTP when untreated, in the absence of another etiology for microthrombotic angiopathy, all patients with suspected TTP will undergo plasma exchange though less than half will have a final diagnosis of TTP upon reporting of ADAMTS13 activity (literature reports 10-45% and our present study shows 42% (5/12) of suspected cases to be true TTP).

We posit that stat testing for ADAMTS13 would benefit patients and hospitals through a reduction in the risks and high cost of therapy. To that end, we examined the effect of laboratory turnaround time on plasma usage in all patients suspected of having TTP over an 18-month period. We report that 1210 units of plasma were unnecessarily transferred while awaiting ADAMTS13 activity results, representing 76 individual apheresis encounters. Average laboratory turnaround time was 5.8 days for the three reference laboratories used during the study period. We also examined the potential costs and benefits if the laboratory offered testing of ADAMTS13 activity. Infrequency of requests for ADAMTS13 activity testing means that as a stat test, samples will not be batched, resulting in the utilization of many laboratory resources, including 1.5-4 personnel hours and a complete set of control reagents for every analyzed sample. The average price per test offered by 3 different manufacturers would result in laboratory direct costs of $362.50 per sample if no batching of samples was possible compared to reference laboratory testing at less than $200 per sample. The hospital and patient, however, would see a reduction in inappropriate plasmapheresis amounting to reduced direct costs of $17,425 per patient or $121,030 for the facility over the 18-month study period. Further, the reduction in inappropriate therapy removes unnecessary complications and expedites appropriate therapy. We conclude that availability of a rapid in-house assay for ADAMTS13 activity would reduce unnecessary plasma exchanges. Although the test will likely represent a loss of revenue for the laboratory, losses should be offset when compared to the cost-savings to the patient and hospital.

## A-326

### Age and Gender Specific Complete Blood Count Reference Intervals for a Community-based Patient Population in Ontario, Canada

H. Dunn, W. Hui, D. Tkachuk, J. Dhaliwal, A. Ptolemy, H. Li. *Dynacare, Brampton, ON, Canada*

**Objective:** Establish age and gender specific reference intervals (RIs) for complete blood count (CBC) testing of community-based patients on Beckman Coulter DxH analyzers.

**Methods:** RIs were identified through a retrospective review of 84584 male and 119050 female CBC results from our regional reference laboratory information system**.** CBC results were initially partitioned by year for patients ≥ 1Y and by month for patients < 1Y. Final stratification of age and gender RIs was based on clinical and statistical significance. Statistical software (EP Evaluator) was then used to establish each RI using a central 95$^{th}$ percentile criterion. All proposed age and gender specific RIs were subsequently verified by testing *N*=20 normal patient specimens.

**Results:** Except for MCHC (RI: 308-340g/L) and MPV (RI: 7.5-11.7fL) the CBC analytes were stratified into multiple age groups.

The derived RIs revealed potential linkages between age/gender and CBC results: (1) relatively high concentrations in childhood which decrease with age and stabilize in adulthood (WBC; RDW; platelets; lymphocytes; monocytes; eosinophils; and basophils); (2) relatively low concentrations in childhood which increase with age and stabilize in adulthood (neutrophils and RBC); (3) high concentrations at birth that drop after 1M then increase with age and stabilize in adulthood (hemoglobin; hematocrit; MCV; and MCH); (4) no change with age (MCHC and MPV); (5) RBC, hemoglobin and platelets RIs drop significantly in patients >75Y; (6) Male and female <15Y CBC RIs are identical. After adulthood, male and female RIs of RBC, hemoglobin, hematocrit and MCV are significantly different and require stratification.

**Conclusion:** Using lab specific patient population data to establish central 95$^{th}$ percentile intervals helps to label abnormalities appropriately within Ontario's ethnically diverse population and avoid unnecessary further investigation.

| Age Stratification of Reference Intervals | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 - 3D | 4D – 1M | 2 M - 1Y | 2 - 9Y | 10 – 14Y | 15 - 75Y | > 75Y |
| WBC (10E9/L) | 8.7 - 29.0 | 5.0 - 16.7 | | 3.7 - 13.7 | 3.5 - 11.8 | | |
| RBC (10E12/L) | 3.9 - 6.0 | 2.2 - 5.7 | 3.5 - 5.4 | 4.0 - 5.6 | | M:3.7 - 6.0<br>F:3.6 - 5.4 | M:2.9 - 5.5<br>F:3.0 - 5.2 |
| HEMOGLOBIN(g/L) | 136 - 196 | 71 - 194 | 87 - 138 | 102 - 143 | 108 - 155 | M:110 –169<br>F:101 - 152 | M:89 – 162<br>F:88 - 151 |
| HEMATOCRIT (L/L) | 0.42 - 0.64 | 0.20 - 0.59 | 0.27 - 0.41 | 0.32 - 0.43 | 0.33 - 0.47 | M:0.31 - 0.51<br>F:0.30 - 0.46 | |
| MCV (fL) | 101 - 122 | 86 - 113 | 58 - 89 | 63 - 91 | 68 - 94 | M:75 – 103<br>F:72 - 102 | |
| MCH (pg) | 29 - 39 | | 18 - 30 | 20 - 30 | 22 - 31 | 23 - 33 | |
| RDW | 12.3 - 23.7 | | | 12.4 - 18.8 | | | |
| PLATELETS (10E9/L) | 178 - 535 | | | | 165 - 424 | 136 - 400 | 109 - 429 |
| RETICULOCYTES (10E9/L) | 8 - 153 (0-3M) | 17 - 146 (>= 4M) | | | | | |
| NEUTROPHILS (10E9/L) | 0.8 - 7.2 | | | 1.1 - 8.4 | 1.3 - 7.4 | 1.7 - 8.3 (>= 14Y) | |
| LYMPHOCYTES<br>EOSINOPHILS (10E9/L) | 2.0 - 9.5<br>0.0 - 1.6 | | 2.4 - 9.6<br>0.0 - 1.1 | 1.3 - 6.4<br>0.0 - 0.9 | 1.2 - 4.2<br>0.0 - 0.8 | 0.8 - 3.4 (>= 14Y)<br>0.0 - 0.6 (>= 14Y) | |
| MONOCYTES<br>BASOPHILS (10E9/L) | 0.1 - 2.4<br>0.00 - 0.24 | | 0.2 - 1.5<br>0.00 - 0.16 | 0.2 - 1.0<br>0.00 - 0.11 | | | |

## A-327

### Estimating short- and long-term reference change values for tests of platelet function

B. M. Katzman, A. M. Wockenfus, R. J. Scott, S. C. Bryant, A. S. Jaffe, B. S. Karon. *Mayo Clinic, Rochester, MN*

**Background:** To balance the risks of perioperative bleeding and thrombosis, protocols for mechanical circulatory support placement often require titration of antiplatelet agents using laboratory tests of platelet function. A relative change value (RCV) or "delta", based upon analytic and biologic variability, would be useful to define significant changes in platelet function. Platelet function tests with higher RCVs are more likely to cross defined thresholds for high platelet reactivity due to analytic and biologic variability, rather than changes in patient condition. However, variable platelet activation occurs with each blood draw, making separate measurement of analytic and biologic variability (the traditional approach to RCV calculation) difficult for platelet function tests. We estimated short-term and long-term RCVs for two tests of platelet function to facilitate antiplatelet agent titration and monitoring. **Methods:** A total of 16 healthy volunteers (8 male and 8 female) were recruited to have arachidonic acid-induced and adenosine 5'-diphosphate (ADP)-induced platelet function measured by whole blood impedance aggregometry using Multiplate (Diapharma Group Inc., West Chester, OH) and VerifyNow (Accumetrics, San Diego, CA) devices. Study volunteers had blood drawn on 3 occasions on the first study day and returned for a single blood draw 1, 2, and 3 months after the initial measurements. All measurements were performed in duplicate following each blood draw, for a total of 96 duplicate measurements. Analytic variability ($CV_A$) was estimated from the average variability observed among the 96 duplicate measurements. Short-term RCV is a function of variability attributable to imprecision ($CV_A$) and pre-analytic factors (platelet activation with each blood draw); and was estimated from $CV_A$ and within person biologic variability ($CV_I$) observed among the 6 measurements per subject performed on study day 1. Long-term RCV is a function of total (analytic and biologic) variability and was calculated from $CV_A$ and $CV_I$ observed from the first measurement on day 1 and from measurements 1, 2, and 3 months later. Short-term and long-term RCVs were calculated according to the following equation: $RCV = 2.77*(CV_A^2 + CV_I^2)^{1/2}$. **Results:** Estimated short-term and long-term RCVs for arachidonic acid-induced platelet function by VerifyNow were 4%; compared to short- and long-term RCVs of 19% and 32% for Multiplate. Short-term and long-term RCVs for ADP-induced platelet function by VerifyNow were 16% and 23%; while short- and long-term RCVs by Multiplate were 25% and 40%. **Conclusion:** Small (~ 5%) changes in arachidonic acid-induced platelet function by VerifyNow can be interpreted as a change in patient condition or status and not a function of analytic and biologic variability. In contrast, by Multiplate changes up to 20% are anticipated due to analytic variability and effects of drawing blood. Over longer periods of time, Multiplate changes under ~30% may represent analytic and biologic variability. For ADP-induced platelet function, relevant "deltas" are approximately 20% (VerifyNow) and 25-40% (Multiplate). Lower RCVs on the VerifyNow should allow for more consistent classification of platelet function over time for any individual patient.

## A-328

### Hemoglobin Greenville-North Carolina: A Novel Hemoglobinopathy Diagnosed At East Carolina University/Vidant Medical Center.

A. Thombare[1], W. Tang[1], S. Patil[1], E. Bray[1], J. Oliveira[2], R. Baltaro[1]. *[1]East Carolina University/Vidant Medical Center, Greenville, NC, [2]Mayo Clinic, Rochester, MN*

**Background and Objective**: Abnormal hemoglobin variants resulting in hemoglobinopathy pose serious health problems leading to wide range of morbidities throughout the world. A large number of such hemoglobin variants have been discovered. We present a novel hemoglobin variant that has not been described in literature or hemoglobin variant database. After confirmation at Mayo Clinic, Rochester, MN, the hemoglobin was registered as hemoglobin Greenville-NC in Hemoglobin Variant (HbVar) Database of Human Hemoglobin Variants.

**Methods and Results**: A sixteen year old African American female presented with positive pregnancy test with estimated gestation of 12 weeks. Patient's mother had a history of sickle cell trait and father's -hemoglobinopathy status was unknown. During routine prenatal work up, mild anemia with normocytic and normochromic red blood cells and increased red blood cell distribution width (RDW) were detected. High Performance Liquid Chromatography (HPLC) was performed as screening test for detection of hemoglobinopathy. By HPLC, hemoglobin A, A2, S and two other hemoglobins were identified. Hemoglobin A, S and other hemoglobin that migrated in between Hb S and Hb C position were confirmed by acid gel electrophoresis. The two alpha variants could not be positively characterized by hemoglobin electrophoretic methods. Molecular testing, capillary electrophoresis and mass spectrometry were performed at Mayo Clinic. Alpha globin gene sequencing identified two heterozygous mutations: *HBA1*: alpha1, 63 GCC>ACC, Ala>Thr (HGVS c.190G>A, p.A64T), a novel variant; and *HBA2*: alpha2, 48 CTG>CGG, Leu>Arg, (HGVS c.146T>G, p.L49R), hemoglobin Montgomery. Beta globin gene sequencing confirmed heterozygous hemoglobin S [*HBB*: Beta 6, GAG>GTG, Glu>Val (HGVS: c.20A>T, p.E7V)]. Mass spectrometry confirmed the novel variant mass of 15156 amu. Relative percentages by mass spectrometry of different hemoglobins in this patient were estimated to be Hb Montgomery 18%, Hb Greenville-NC 19%, and Hb S 40%. *In silico* analysis by computation methods are mixed with some showing a possible cryptic splice site enhancement and others suggest no significant effect.

**Conclusion**: The novel variant, Hb Greenville-NC, has not previously been reported in population genetics databases, and its clinical significance is unknown. It is possible that this mutation is inherited and can be identified in other family members. Hemoglobinopathies may not have clinical manifestations in heterozygotes, even when compounded by additional hemoglobinopathies. For example, our patient also had hemoglobin Montgomery. In heterozygous individuals, hemoglobin Montgomery is not associated with clinical manifestations or hematologic abnormalities. When combined with Hb S, it is expected to behave similarly to Hb S trait. The patient's mild anemia on prenatal workup may have been the result of her hemoglobinopathy, but could also have resulted from nutritional or other factors. We intend to pursue hemoglobin testing and correlation with any relevant clinical history of the patient's family members, to better determine patterns of inheritance and clinical manifestations of this novel hemoglobinopathy.

## A-329

### CELL-DYN Emerald 22 results are equivalent with those obtained with CELL-DYN 3700 and CELL-DYN Sapphire

S. Crespo Rubio[1], J. A. Royo Blasco[1], J. J. Hoffmann[2], G. Lakos[3]. *[1]Megalab S.A., Madrid, Spain, [2]Abbott Laboratories, Hoofddorp, Netherlands, [3]Abbott Laboratories, Santa Clara, CA*

**Background:** The CELL-DYN Emerald 22 (E22) is a compact hematology analyzer designed for small and medium-sized laboratories. It provides a complete blood count, including a 5-part WBC differential. The analyzer combines impedance technology for cell counts with UNI-FLOW dual-angle light scatter for the differential. The goal of the study was to assess the performance of E22 in comparison with CELL-DYN CD3700 (CD37) and CELL-DYN Sapphire (SAP). **Methods:** Five hundred and one routine blood samples were tested on all three analyzers. Due to invalidations and results being outside of the analytical measuring range, results were available for analysis on 461 to 501 samples, depending on the measurand. Data were processed by Passing-Bablok regression, Pearson correlation and Bland-Altman bias plots. A subset analysis was performed on samples with WBC <2.0x$10^9$/L (n=42) and with PLT <50x$10^9$/L (n=30).

**Results:** Correlation coefficients for WBC, PLT, HGB, RBC and MCV ranged from 0.96 to 0.99 between E22 and CD37, and from 0.96 to 1.00 between E22 and SAP. In the subset analysis of cytopenic samples, the correlation coefficients for WBC and PLT were 0.98 and 0.87 between E22 and CD37, and 0.97 and 0.82 between E22 and SAP. In this subpopulation the mean (±SD) and median (inter-quartile range) differences between E22 and CD37 results were -7.36 (± 7.67) and -5.60 (8.27) for PLT, and -0.06 (± 0.13) and -0.06 (0.16) for WBC, respectively. The mean and median differences between E22 and SAP results were -0.24 (± 7.38) and 1.60 (6.33) for PLT and -0.04 (± 0.13) and -0.02 (0.15) for WBC. The predicted bias for WBC at 1.0 x$10^9$/L and for PLT at 20.0 x$10^9$/L were -0.01 and -2.47 when compared to CD37, and 0.00 and -1.70 when compared to SAP. **Conclusion:** Results generated by E22 were substantially equivalent with those generated by CD37 and SAP. WBC results in the low range were very consistent among the three analyzers. PLT results by E22 below 50 x$10^9$/L tended to be lower than those reported by CD37 and SAP. CELL-DYN Emerald 22 is a suitable backup instrument for labs using CELL-DYN 3700 or CELL-DYN Sapphire.

## A-330

### Characterization of Hematologic Malignancies with Anchored Multiplex PCR and Next-Generation Sequencing

L. Johnson[1], M. Bessette[1], A. Berlin[1], J. Haimes[1], L. Griffin[1], K. Trifilo[1], N. Manoj[1], H. Wang[1], R. Ryan[2], M. Hussaini[3], B. A. Kudlow[1]. *[1]ArcherDX, Boulder, CO, [2]Massachusetts General Hospital, Boston, MA, [3]Moffitt Cancer Center, Tampa, FL*

**Introduction:** Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutation type. We developed targeted next-generation sequencing (NGS) assays based on Anchored Multiplex PCR (AMP™) for simultaneous detection of mutations and gene expression levels relevant in hematologic malignancies.

**Methods:** AMP is a library preparation method for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification. AMP-based Archer® VariantPlex™ and FusionPlex® assays enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as internal tandem duplications (ITDs) with VariantPlex assays. MBC adapters ligated to RNA fragments prior to amplification enable relative gene expression analysis.

**Results:** We show that open-ended amplification from KMT2A GSPs enabled detection of a KMT2A-MLLT3 fusion through breakpoint identification, with reads extending 6 exons into MLLT3. We also detected a novel RUNX1 fusion, RUNX1-G6PD, in a case of acute unclassifiable leukemia. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected ITDs of varying sizes and insertion points, with simultaneous point mutation detection, in AML-positive blood samples. Finally, MBCs used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples.

**Conclusions:** Our results demonstrate that AMP-based NGS enables comprehensive detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of known and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Immunology

## A-331

### CXCL10 Gene Promoter Polymorphism -1447A>G is Associated with malaria in Ghanaian Children

F. A. Botchway[1], F. A. Botchway[1], C. E. Lekpor[2]. *[1]Department of Chemical Pathology/ korle Bu Teaching Hospital/ University of Ghana, Accra, Ghana, [2]Korle-Bu Teaching Hospital/ University of Ghana/Department of Pathology, Accra, Ghana*

Background: *Plasmodium falciparum* malaria kills nearly a million people annually. Over 90% of these deaths occur in children under five years of age in sub-Saharan Africa. In Ghana, malaria accounts for about 60% of all outpatient visits in public health facilities, with 40% of the affected being children under age 5 years. The disease accounts for 13.2% of all mortalities in Ghana and ranks fifth as the commonest cause of death in children under 5 years of age. The risk factors for severity of malaria pathogenesis and the wide variation in clinical manifestations of malaria are poorly understood. The influence of host genetics on susceptibility to *P. falciparum* malaria has been extensively studied over the past twenty years. Recent studies indicate that interferon gamma inducible chemokine, CXCL10, is a predictor of both human and experimental cerebral malaria severity. In addition, polymorphisms in the CXCL10 gene promoter has been associated with increased CXCL10 production, which is linked to severity of malaria in Indian malaria patients. In the present study, we hypothesized that in a subset of Ghanaian malaria patients, susceptibility to malaria is associated with different variants of the CXCL10 gene. Method: We determined whether polymorphisms in the CXCL10 gene are associated with the clinical status of malaria patients. We tested several known polymorphisms and identified one reported single nucleotide polymorphism in the CXCL10 promoter (-1447A>G [rs4508917]) and compared 43 malaria and 111 non malaria cases using PCR-restriction fragment length polymorphism method. Results: The median age for malaria patients was 6 years and that for non-malaria patients was 4 years. There was no significant difference with regards to hemoglobin level between malaria patients (9.5g/dL) and non-malaria patients (10.0g/dL), p=0.588. The -1447A>G genotype of the CXCL10 gene was significantly associated with malaria (adjusted odds ratio =2.55, 95% CI=1.13-5.74, p=0.024). Conclusion: These results suggest that the -1447A>G polymorphism in CXCL10 gene promoter could be partly responsible for malaria outcomes in Ghanaian malaria children.

## A-332

### Paroxysmal, but not persistent, atrial fibrillation is associated with increased levels of transforming growth factor beta1

A. E. Stanciu[1], R. G. Vatasescu[2], M. M. Stanciu[3], M. Dorobantu[2]. *[1]Institute of Oncology, Bucharest, Romania, [2]Clinic Emergency Hospital, Bucharest, Romania, [3]University Politehnica of Bucharest, Bucharest, Romania*

**Background:** Inflammation plays an important role in the genesis and maintenance of atrial fibrillation (AF). The precise mechanism and signaling pathways involved in structural remodeling and atrial fibrosis are still unknown. We aimed to assess the extent of inflammation in pathogenesis of different types of AF.

**Methods:** Twenty-five patients with paroxysmal AF, 30 patients with persistent AF and 20 healthy control subjects were enrolled in the study. Peripheral blood samples were collected before catheter ablation of pulmonary veins. Serum levels of NT-proBNP, IL-6, TGF-beta1, MMP-9 and TIMP-1 were measured by ELISA.

**Results:** NT-proBNP, IL-6, TGF-beta1 and MMP-9/TIMP-1 ratio were higher in AF patients than in controls (P<0.001). NT-proBNP and IL-6 levels were higher in persistent AF than in paroxysmal AF (172.5±67.6 pmol/L vs. 122.2±56.7 pmol/L, P=0.02 and 14.7±9.8 pg/mL vs. 8.5±4.2 pg/mL, P=0.003). TGF-beta1 and MMP-9/TIMP-1 ratio were lower in persistent AF than in paroxysmal AF (16.7±1.8 ng/mL vs. 25.2±3.2 ng/mL, P=0.006 and 3.1±1.2 vs. 5.2±3.4, P=0.002). TGF-beta1 inversely correlated with NT-proBNP (r= - 0.84, P<0.001). Higher levels of IL-6 and NT-proBNP in persistent AF than in paroxysmal AF, suggest that IL-6 and NT-proBNP may be related to the burden of AF. Atrial fibrogenesis is accompanied by a biphasic response, an early increase of TGF-beta1 and MMP-9/TIMP-1 ratio in paroxysmal AF and a later loss of TGF-beta1 and MMP-9/TIMP-1 ratio in persistent AF.

**Conclusion:** The later loss of TGF-beta1 may result from the reduction of pulsatile stretch of atrial cardiomyocytes, due to the spreading of cardiac fibrosis or from antifibrotic functions of NT-proBNP. Our data suggest that fibrosis progresses, despite compensatory changes in the TGF-beta1 signaling pathway.

## A-333

### Association of ERAP1 with Ankylosing Spondylitis in Chinese Han population.

P. Deo[1], H. Lihua[2]. *[1]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia, [2]Department of Laboratory Medicine, Union Hospital, Wuhan, China*

**Background:** Although HLA-B27 is recognized to be the major gene associated with AS, a role for genes outside the HLA region (non-MHC) is increasingly being recognized, among which interlukin23R and ERAP1 are recently been suggested to be associated with AS by studied performed on a gene-targeted association study of 14500 ncSNP in 1000 AS cases and 1500 controls by (WTCCC-TASC) in 2007. We here aim to see the relationship between HLA-B27, IL23R and ERAP1 polymorphism in Chinese AS patients.

**Methods**: A total of 248 Patients attending orthopedics, rheumatology, and physiotherapy clinic of union Hospital and Tongji medical College were selected. Among these 248, 84(74 Male/ 10 Female) patients satisfied the modified New York criteria of AS.360 ethnically related healthy controls that did not have any previous autoimmune diseases were also included for our study. The AS populations included of 84 patients (35±8.79) years (mean ± standard deviation). The mean BASDAI Score (Bath AS Disease Activity Index) and BASFAI (Bath AS Function al Index) was 3.7 Years (SD 2.0) and 2.3 (SD 2.2) respectively. The mean BASDAI, BASFI, and ESR and CRP for as patients were 5.4(SD 1.7), 4.7(SD 2.5), 56.97 mm/h (SD 11.45) and 38.33 mg/L (SD 27.03) respectively. **Results**: The 6 types of HLA-B27 found were (B*2702, 03,04,05,06 and B* 2713). Four alleles were detected both in AS patients and in controls (B*2702, 03, 04 and B*2705). B*2704 and B*2705 were predominant alleles in the AS group and controls. In total 5 SNP within the IL23R were genotyped. The result showed no association of IL23R polymorphism with AS. The four genotypes selected for ERAP1 (rs30187, rs10050860, rs27044, rs30187) for genotyping were in Hardy Weinberg equilibrium. Significant positive association of AS with ERAP1 SNPS rs27044 (p=9.36X10-7) and rs30187 (p=7.32X10-6) was seen. The four SNPrs17482087.rs10050860, rs27044 and rs30187 formed a LD Block with D ranging from 95 to 100, indicating strong or complete association. Two haplotype showed association with AS(GCCT:P=4.71x10-7, CCCC:P=8.56x10-6). **Conclusion**: Our results confirm IL23R polymorphism is not associated with AS in Chinese Han patients while ERAP1 polymorphism is associated with AS similar to that of Caucasians.

## A-334

### Discrepancies between two immunoassays for the determination of MPO and PR3 autoantibodies

Q. Sun[1], B. Calderon[2], Z. Zhao[1]. *[1]NIH, Bethesda, MD, [2]Eli Lilly and Company, Indianapolis, IN*

**Background:** Testing for autoantibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) is part of an anti-neutrophil cytoplasmic antibodies (ANCA) panel test that aids the diagnosis of small-vessel vasculitis, inflammatory bowel disease, as well as systemic autoimmune diseases such as Lupus. Several immunoassays have been approved by the FDA for the measurement of MPO-ANCA and PR3-ANCA in patient serum. Here we characterized the differences between two automated immunoassays at three facilities for measuring MPO and PR3 autoantibodies.

**Methods:** 117 patient serum samples were analyzed for MPO and PR3 autoantibodies. The INOVA Quanta Lite IgG assay (INOVA Diagnostics) were performed on the DSX workstation (DYNEX Technologies) at site 1 and site 2 and the BioPlex 2200 Vasculitis Panel were performed on the BioPlex 2200 testing platform (Bio-Rad Laboratories) at site 3. The results were compared both qualitatively (between two methods) and quantitatively (between INOVA assays).

**Results:** Comparison of the INOVA assay at two different facilities employing 36 patient samples demonstrated high concordance (97.2% for MPO and 94.4% for PR3) and quantitative correlation (Deming regression $R^2$=0.973 for MPO and $R^2$=0.935 for PR3). Conversely, INOVA and BioPlex methods showed relatively poor concordance

at 70.4% for MPO (n=81; 95%CI: 59.7% to 79.2%) and at 76.5% for PR3 (n=81; 95%CI: 66.2% to 84.4%). The comparison results were shown in Table 1.

**Conclusion:** This study demonstrated low concordance between two methods for MPO-ANCA and PR3-ANCA measurements. INOVA Quanta Lite IgG assays were consistent between two sites; however, comparison of INOVA and BioPlex multiplex system demonstrated differences. This is consistent with the general lack of standardization of antigen-specific immunoassays and different mechanisms employed by these two techniques. Given the discrepancies, the performance of different autoantibody immunoassays should be taken into consideration when interpret the MPO-ANCA and PR3-ANCA results.

Table 1. Concordance of different methods of MPO and PR3 autoantibodies

| Concordance (95% CI) | INOVA (site 1) | |
|---|---|---|
| | MPO | PR3 |
| INOVA (site 2) | 97.2% (85.8 to 99.5%) | 94.4% (81.9 to 98.5%) |
| BioPlex (site 3) | 70.4% (59.7 to 79.2%) | 76.5% (66.2 to 84.4%) |

## A-335

### Investigating the regulatory role of a negative checkpoint molecule, VISTA in endothelial cells.

M. Salehi, I. Radichev, A. Savinov. *Children's department, Sanford Research, Sioux Falls, SD*

**Background:** Peripheral tolerance is crucial to maintain tolerance against self-antigens and fine tune immune responses. The mechanism of immune tolerance and homeostasis is mediated by a family of co-stimulatory and co-inhibitory molecules that regulate T cell function. Targeting these molecules and their pathways have been promising for therapeutic approaches in autoimmune diseases and cancer. V-set domain Ig Suppressor of T Cell Activation (VISTA) is a recently identified co-inhibitory member of this family, which suppresses T cell immune responses. Based on preclinical studies, VISTA deficiency augments acute inflammation and predisposes individuals to autoimmune disorders. In our preliminary investigation, we identified a sub-population of endothelial cells (ECs) with high level of VISTA expression residing exclusively in secondary lymphoid organs. Considering the previous studies that signifies the important role of specific subsets of ECs from lymph nodes in the induction of immune tolerance, we pursued to further characterize the population of VISTA-expressing ECs.

**Methods:** We performed immunofluorescent staining on frozen tissue sections of lymph nodes from mice to characterize VISTA-expressing EC. To separate ECs into subpopulations based on VISTA expression, Fluorescence-activated cell sorting (FACS) was applied. RNA from sorted cells were further analyzed using Real-Time Quantitative Reverse Transcription PCR for expression of peripheral tissue antigens (PTAs). To study the function of VISTA expression by ECs, isolated mouse T cells were labeled with CellTrace Violet fluorescent dye and cocultured with VISTA-expressing ECs. Subsequent proliferation of T cells was analyzed by Flow Cytometry.

**Results:** We found that VISTA-expressing cells belong to both lymphatic and blood subsets of ECs which control immune cells migration to the lymph nodes. In NOD*scid* mice with impaired T and B cell development, VISTA expression was lost on the blood EC subset but was maintained on lymphatic ECs. We also found that VISTA expression is induced postnatally through adulthood contrary to PD-L1, a similar co-inhibitory molecule. Furthermore, FACS-sorted ECs from lymph nodes of NOD (model for Type I Diabetes) and DBA (control) mice were evaluated for the expression levels of various PTAs that are associated with immune tolerance. In RNA of NOD mice, PTAs were up-regulated in VISTA$^{high}$ ECs which associates with their inflammatory phenotype. Finally, coculture of VISTA-expressing ECs with T cells resulted in suppression of T cell proliferation.

**Conclusion:** Our results suggest that the lymph node microenvironment could have a role in regulation of VISTA expression by ECs. Moreover, our results show that VISTA-expressing ECs in the lymph nodes are favorably situated to tolerize immune cells particularly T cells during their migration into and out of the lymph nodes. VISTA-expressing ECs could function as a checkpoint regulator for T cell function and therefore play a role in controlling autoimmune disorders.

## A-336

### Serum Anti-PLA2R Antibody and Glomerular PLA2R Deposition in Chinese Patients with Membranous Nephropathy: A Retrospective Study

L. Pang, H. Li, J. Du, N. Duan, Y. Liu, D. Yu. *Peking University First Hospital, Beijing, China*

**Background:** M-type phospholipase A2 receptor (PLA2R) is the major target antigen in primary membranous nephropathy (MN). Previous studies have evaluated the diagnostic value of serum anti-PLA2R antibody. However, the correlation of serum anti-PLA2R antibody and glomerular PLA2R deposition, and their association with clinical characteristics need to be further evaluated.

**Methods**: In total, 960 inpatients who performed serum anti-PLA2R antibody measurement between August 2015 and December 2016 were initially reviewed retrospectively. The patients who did not performed renal biopsy were excluded. Thus, 284 patients with renal biopsy proven MN and 427 patients with biopsy proven non-MN were included. Of all the MN patients, 83 patients were clinically ruled out for secondary MN. Therefore, 136 patients were selected as inception group because serum anti-PLA2R antibody and glomerular PLA2R antigen were simultaneously detected. We examined serum anti-PLA2R antibody by ELISA and glomerular PLA2R deposition by immunohistochemical staining in inception group.

**Results**: Positive serum anti-PLA2R antibody and glomerular PLA2R deposition were seen in 58.8% (80/136) and 95.6% (130/136) patients respectively ($p < 0.001$). None of the patients with other glomerular diseases were positive for serum anti-PLA2R antibody. In our study, the specificity of serum anti-PLA2R antibody for PMN is 100% and the sensitivity is 58.8%. Although the Spearman's correlation coefficient is 0.18 ($P = 0.109$), the high level of serum PLA2R antibody was related to the strong expression of glomerular PLA2R antigen. Proteinuria, serum total protein, serum albumin, serum creatinine and eGFR had significant differences between patients with serum anti-PLA2R antibody and those without. There were no significant differences in any clinical biomarkers between glomerular PLA2R deposition-positive and -negative patients. Serum anti-PLA2R antibody levels were correlated with serum albumin, serum creatinine, eGFR and proteinuria. Glomerular PLA2R deposition intensities were weakly correlated with proteinuria. Unexpectedly, there was a positive correlation rather than a negative correlation between glomerular PLA2R deposition intensity and eGFR.

**Conclusion**: Serum anti-PLA2R antibody is more closely correlated with disease activity and renal function than glomerular PLA2R deposition.

## A-337

### Plasmablasts of HIV-infected Individuals Dominantly Produce Polyreactive Antibodies Including Anti-NMDAR Antibodies Contributing to HIV-Associated Neurocognitive Disorders

H. Liao, J. Su. *West China Hospital, Sichuan University, Chengdu, China*

Background: In the post-HAART stage, a substantial percent of HIV patients develop HIV-Associated Neurocognitive Dysfunction (HAND) and HIV-Associated Dementia (HAD). Finding early biomarkers is essential to diagnose and prevent these diseases. HIV infection causes B cell dysfunctions thus failing in generating effective anti-HIV antibodies. This study aims to evaluate the functional antibody repertoire and the potential antibody-related pathogenesis of HAND and HAD.

Methods: Single-cell PCR was performed to clone the paired IgH and IgL genes from plasmablasts of 5 HAND patients and 5 uninfected healthy donors. 80 recombinant antibodies were expressed from the Ig genes. ELISA and ANA assays were carried out to determine thei antibody reactivities. Immunofluorescence staining was performed to evaluate their interactions with the neuroblastoma(SH-SY5Y) and glioblastoma(U251) cell lines.

Results: 30% of antibodies from HIV-infected individuals bind to gp120. Surprisingly, about 72% of the antibodies cloned from HIV-infected individuals were polyreactive, which is sharp contrast to the 16% of polyreactive antibodies from control group. Up to 60% of the antibodies derived from HIV-infected individuals cross-reacted with the DWDYS peptide present in NMDA receptor. 17% of antibodies bind to SH-SY5Y or U251 cell surface antigens. One of the polyreactive antibody HIV201P5B2 derived from HIV patients induced NMDAR clustering and internalization and eventually caused neuronal cell apoptosis.

Conclusions: Taken together, the dominant production of polyreactive antibodies in the plasmablasts of HIV patients revealed an abnormal alteration of B cell function during HIV infection. Anti-NDMAR antibodies generated in HIV-infected individuals may provide a new diagnostic biomarker for HAND and HAD in AIDS patient.

## A-338

### Variability in Approach to Initial Antinuclear Antibodies (ANA) Testing and Follow-Up Testing: a Survey of Participants in the College of American Pathologist's Proficiency Testing Program

S. J. Naides[1], J. R. Genzen[2], G. Abel[3], M. Shan[4], C. Bashleben[4], M. Q. Ansari[5]. *[1]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, [2]ARUP Laboratories Inc, Salt Lake City, UT, [3]Lahey Hospital & Medical Center Burlington, Burlington, MA, [4]College of American Pathologists, Northfield, IL, [5]Cleveland Clinic, Cleveland, OH*

**Background:** A 2010 American College of Rheumatology (ACR) position paper stated that the indirect immunofluorescence assay (IFA) on HEp-2 cell substrate is the "gold standard" method for ANA testing and that laboratories performing other methods should state the method used and describe its performance parameters. Our aim was to identify current practices in initial non-IFA testing and reflex testing directed by an initial positive IFA screen.

**Methods:** Supplemental questions were sent to laboratories participating in the College of American Pathologist's proficiency testing program for ANA as part of the Special Immunology S-A Survey 2016 to determine the practice of ANA testing. Of 5847 kits distributed, 1206 (21%) responded to the questionnaire; 942 were in the United States and 264 were international.

**Results:** Of 669 laboratories performing an initial ANA IFA, only 33% offer reflex testing. Of those offering a reflex option, the follow-up testing was by specific autoantibody(ies) (multiple responses allowed) by enzyme linked immunosorbent assay (ELISA) in 47%, by IFA in 25%, multibead immunoassay (mbead) in 25%, and by ELISA (but not to a specific analyte) in 6%. In 14%, follow-up testing was reported as "other." Of 669 laboratories reporting, 21% initially screened by ELISA. When an ELISA was used as initial testing, 39% of 216 responding laboratories reported using HEp-2 cell lysate as substrate, 4% a lysate of the HEp-2000 cell line (which is engineered to over express SSA antigen), 25% a mixture of specific antigens and 32% "other" substrate. When an ELISA was used for initial ANA screening, results were reported as positive/negative in 67%, in units by 21%, optical density by 3%, and "other" by 9%; 69% of 178 laboratories reported reflexing a positive ELISA to IFA, 24% to specific autoantibody ELISAs, 7% to specific autoantibody IFA, 4% to specific mbead, and 8.4% to "other." When mbeads were used in 134 reporting laboratories, specificities of antibodies tested were 100% SSB, 99% SSA, 99% Jo-1 and RNP, 98% Scl-70, 96% double stranded DNA, 87% centromere B, 58% ribosomal P, 55 Sm/RNP, 44% histone, 8% centromere A, 2% single stranded DNA,1% centromere F and 7% "other." An internal fluorescence standard in mbead testing was included by only 74% of laboratories, and a positive control in each assay by 52% and daily by 44% of laboratories. When initial ANA testing was performed by mbead, a positive screen result reflexed to specific analytes in only 57% of 133 reporting laboratories.

**Conclusion:** Marked variation exists in reflex strategies following a positive initial ANA IFA. In those labs performing an initial screen by methods other than IFA, choice of method and specificities of autoantibodies tested vary widely, which can affect results reported. With the variability in approach to initial ANA testing and follow-up testing, clinical practice would benefit from the development of uniform laboratory guidelines promulgated collaboratively by the laboratory and rheumatology communities.

## A-339

### Effects of Glycated Albumin on Inducing Injury and Inflammation in Human Proximal Tubular Epithelial Cells

N. Duan, H. Li, Y. Liu, L. Pang. *Peking University First Hospital, Beijing, China*

**Background** Glycated albumin (GA) serves as a clinically useful index for monitoring glycemic status and it is also associated with pathogenesis of renal complications in diabetes mellitus. This study aim to investigate the effects of GA on the expression of kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) and inflammatory cytokines in human proximal tubular epithelial cells (HK-2 cells).

**Methods** The HK-2 cells were treated with the different concentration of GA (0.5 mg/ml; 1.0 mg/ml) and albumin (Alb) (0.5 mg/ml, 1.0 mg/ml; 5 mg/ml, 10 mg/ml) for 12h, 24h and 48h. The mRNA expressions of KIM-1 and NGAL were detected by real-time PCR. The releases of KIM-1 and NGAL in the supernatants were detected by ELISA. The concentrations of vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-α), interleukin-8 (IL-8) and soluble intercellular cell adhesion molecule-1 (sICAM-1) were detected by cytometric beads array method.

**Results** Compared with control and Alb groups, the mRNA levels of KIM-1 and NGAL in HK-2 cells were significantly up-regulated at 12 h, 24 h and 48 h after GA treatment ($P < 0.05$); the protein levels of KIM-1 and NGAL released in supernatants of GA-treated cells were significantly higher than those in control group and Alb groups at the same time points ($P < 0.01$); GA groups also had significantly higher levels of sICAM-1, VEGF and IL-8 than control group at each time point ($P < 0.05$).

**Conclusions** GA can up-regulate the expression and release of KIM-1 and NGAL and promote the secretion of inflammatory cytokines which could cause damage to renal tubules, suggesting that GA may reflect the diabetic renal involvement.

## A-340

### Abbott Alinity i Sigma Metrics and Precision Profiles for Immunoassays

V. Petrides[1], S. Westgard[2], S. Schneider[1], J. Herzogenrath[3], A. Orzechowski[1]. *[1]Abbott, Abbott Park, IL, [2]Westgard QC Inc, Madison, WI, [3]Abbott, Wiesbaden, Germany*

**Introduction:** Assay performance is dependent on the accuracy and precision of a given method. These attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for 13 immunoassays tested on the Alinity i-series. Additionally, precision profile charts were created for a subset of assays to compare the precision performance of the assays tested using the Alinity i-series and the ARCHITECT *i* system.

**Methods:** A sigma value was estimated for each assay and was plotted on a method decision chart. The sigma value was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*. A precision study was conducted at Abbott on each assay using the Alinity i-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, 40-100 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity i-series and ARCHITECT $i2000_{SR}$ systems. The mean concentration of the Alinity i-series results were regressed versus the mean ARCHITECT $i2000_{SR}$ results and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated at a medical decision point. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity i-series and the ARCHITECT *i* system, where the ARCHITECT *i* system within-laboratory %CV and mean concentration values were obtained from the assay package inserts.

**Results:** The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near the medical decision point. The precision profile charts of the within-laboratory %CV results for the Alinity i-series overlaid with the ARCHITECT *i* system showed similar performance across the subset of assays evaluated.

**Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity i-series immunoassays had sigma values greater than 5. The precision performance on the Alinity i-series and ARCHITECT *i* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

## A-341

### Breaking Free From the Ratio: Analytical Performance of an Immunoenrichment-Coupled MALDI-TOF MS Detection Method for Monoclonal Immunoglobulin Free Light Chains

L. Sepiashvili, M. C. Kohlhagen, D. L. Murray. *Mayo Clinic, Rochester, MN*

**Background**: Bence Jones proteins or monoclonal immunoglobulin free light chains (FLCs) hold an important supportive role in diagnosis, prognosis, and monitoring of multiple myeloma. An immunonephelometric serum FLC assay that quantitates kappa (K) and lambda (L) light chains unbound to heavy chains is among the most sensitive assays for monoclonal immunoglobulin (M-protein) detection. However, the presence of an M-protein, represented by an abnormal K/L FLC ratio (K/L<0.26 or >1.65),

often cannot be corroborated by other clinical lab methods, including serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Non-secretory multiple myeloma, light chain multiple myeloma, primary systemic amyloidosis, and light chain deposition disease often are only detected by the K/L FLC ratio (rFLC) due to relatively low abundance M-proteins in circulation. Moreover, the rFLC can be abnormal in the absence of an M-protein or vice versa.

**Objective:** To overcome the above limitations, the objective of this study was to develop and evaluate the analytical performance of an immunoaffinity enrichment-coupled MALDI-TOF mass spectrometry (MS) method for direct detection of monoclonal FLCs.

**Methods:** Residual sera (n=129) were evaluated in 3 cohorts varying based on the presence of an M-protein by IFE and rFLC: IFE-negative and normal rFLC (Cohort 1, n=36), IFE-positive and abnormal rFLC (Cohort 2, n=38) , and IFE-negative and abnormal rFLC (Cohort 3, n=55). For analytical sensitivity assessment, residual sera containing K or L M-proteins (>0.3 g/dL, n=4) were serially diluted into a polyclonal serum pool until undetectable. All sera were analyzed by conventional lab methods for M-protein detection, including: (i) SPEP (Helena laboratories), (ii) IFE (Sebia), and (iii) immunonephelometry for FLCs (Binding Site reagent, Siemens BN II analyzer). Serum FLC immunoenrichment was performed with sepharose beads conjugated with polyclonal antibodies that have high specificity towards FLCs and low cross-reactivity with light chains bound to heavy chains. Total IgG, IgM, IgA, K light chain, and L light chain nanobody immunoenrichments were performed as previously described (Mills JR *et al*, 2016, Clinical Chemistry). Immunoenriched specimens were reduced to dissociate heavy and light chains and subjected to MALDI-TOF MS (Bruker Microflex™) in automated acquisition mode. Mass spectra were interrogated for M-proteins and isotypes.

**Results:** Cohort 1 and 2 MALDI-TOF MS results were 100% concordant with IFE and rFLC results. In Cohort 3, 36 of 55 (65%) sera had evidence of monoclonal immunoglobulins or monoclonal FLCs by MALDI-TOF MS, supporting abnormal rFLC results obtained by immunonephelometry. These results suggest that MALDI-TOF MS can help resolve the majority of cases with dichotomous IFE and rFLC results. However, the remaining 19 sera did not contain detectable M-proteins by MALDI-TOF MS despite abnormal rFLC, corroborating negative IFE. FLC immunoenrichment coupled to MALDI-TOF MS was on average 4.3 (range=2-5) and 34 (range=16-80) times more sensitive than rFLC and IFE towards monoclonal light chain detection, respectively. Notably, FLC M-proteins remained detectable by MALDI-TOF MS when rFLCs became normal following serial dilution.

**Conclusions:** Immunoenrichment using anti-FLC antibodies followed by MALDI-TOF MS is a highly sensitive and specific approach for detection of monoclonal abnormalities frequently undetectable by current routine laboratory methods.

## A-342

### Diagnosis of red meat allergy with antigen-specific IgE tests in serum

J. R. Brestoff, M. A. Zaydman, M. G. Scott, A. M. Gronowski. *Washington University, Saint Louis, MO*

**Background:** Red meat allergy is a rare tick-associated hypersensitivity reaction to galactose-α-1,3-galactose (α-gal) and is characterized by anaphylaxis, angioedema, urticaria and/or gastrointestinal symptoms occurring 3-6 hours after ingesting red meat such as beef, pork, or lamb. Due to the poor sensitivity of skin prick tests with commercial meat extracts (20-40%), the primary diagnostic tools available for diagnosis of red meat allergy are quantification of α-gal-, beef-, pork-, and/or lamb-specific IgE in serum or plasma. However the sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) for these tests have not been previously reported.

**Methods:** To address this, we performed a systematic literature search and identified 22 articles that reported individual patient data for these tests from 135 patients with red meat allergy and 37 controls. The gold standard for diagnosis was expert consensus by the articles' original authors. None of these articles used antigen-specific IgE test results to define disease or control status. These individual patient data were extracted and aggregated in a dataset representing patients from the United States (25.0%), Austria (16.9%), Sweden (12.8%), other European countries (25.6%), Australia (16.9%) and China (0.6%).

**Results:** We found that measurement of α-gal-specific IgE using the bovine thyroglobulin (bTG) ImmunoCAP method had the best overall sensitivty (100%) and specificity (92.3%) for diagnosis of red meat allergy. Measuring biotinylated α-gal-specific IgE (α-gal biotin) using the streptavidin (SA)-CAP method or beef- or pork-specific IgE using ImmunoCAP were also effective tests with high sensitivities (89-92%) and variable specificities (65-82%). Receiver Operating Characteristic (ROC) analyses showed that the areas under the curve (AUC) for α-gal bTG (AUC 0.97), α-gal biotin (AUC 0.93) and beef (AUC 0.86) IgE tests were significantly higher than those of pork (AUC 0.72), lamb (AUC 0.68) and total IgE (AUC 0.42) (all comparisons $P<0.05$). Since the prevalence of red meat allergy is not known and likely varies widely geographically, Bayesian statistics were used to calculate PPVs and NPVs for all possible pre-test probabilities for these tests. This analysis demonstrated that the α-gal bTG test had the highest PPV and NPV for any given pre-test probability, whereas lamb IgE and total IgE had essentially no diagnostic value for red meat allergy.

**Conclusions:** These findings indicate that the α-gal IgE test using the bTG ImmunoCAP method is the most useful for establishing a diagnosis of red meat allergy, although the biotinylated α-gal IgE test using the SA-CAP method and beef IgE by ImmunoCAP are also effective tests.

## A-343

### Alinity i Assay Performance of Representative Immunoassays

C. Rudolph, K. Krishnan. *Abbott Diagnostics Division, Abbott Park, IL*

**Objective:** To evaluate the analytical performance of representative immunoassays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human plasma/serum on the Alinity i, Abbott's next-generation immunoassay analyzer. The Alinity i analyzer is a high throughput instrument testing up to 200 tests per hour. The sample and paramagnetic microparticles are combined in a reaction vessel. Analyte present in the sample binds to the antibody-coated microparticles. The mixture is washed. Antibody acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of antigen in the sample and the RLUs detected by the optical system.

**Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity, and method comparison were assessed per CLSI protocols. The assay measuring interval was defined by the range which acceptable performance for bias, imprecision, and linearity was met.

**Results:** Total imprecision, LOQ, linearity, and defined measuring intervals are shown for the representative immunoassays in the table below. Results versus an on-market comparator assay are also shown.

| Assay | Total %CV | LOQ | Linearity | Measuring Interval |
|---|---|---|---|---|
| CA19-9XR | ≤ 10 | 2.06 U/mL | 1.66 to 1623.36 U/mL | 2.00 to 1200.00 U/mL |
| CA125 II | ≤ 4 | 0.6 U/mL | 1.1 to 1138.4 U/mL | 1.0 to 1000.0 U/mL |
| CA15-3 | ≤ 6 | 0.6 U/mL | 0.3 to 925.4 U/mL | 0.5 to 800.0 U/mL |
| HE4 | ≤ 4 | 2.0 pmol/L | 3.2 to 1741.3 pmol/L | 20.0 to 1500.0 pmol/L |
| BNP | ≤ 5 | 5.0 pg/mL | 2.1 to 5271.1 pg/mL | 10.0 to 5000.0 pg/mL |
| Estradiol | ≤7 | 26 pg/mL | 12 to 1230 pg/m/L | 26 to 1000 pg/mL |

**Conclusion:** Representative immunoassays utilizing Chemiluminescent Microparticle Immunoassay (CMIA) technology tested on the Alinity i immunoassay analyzer demonstrated acceptable precision, sensitivity and linearity. Method comparison data showed excellent agreement with on-market immunoassays.

## A-344

### Performance evaluation of Nanopia KL-6 assay in interstitial lung diseases

E. Cho, K. Park, H. Hong, E. Shin, W. Lee, S. Chun, J. Song, H. Koo, H. Koo, K. Do, W. Min. *Asan medical center, SEOUL, Korea, Republic of*

**Background:** Krebs von den Lungen-6 (KL-6), which is a mucin-like glycoproteins excreted from type II alveolar pneumocytes when these cells are injured. KL-6 has been reported to serve as a sensitive marker for monitoring disease activity and predicting the prognosis of interstitial lung diseases (ILD). The aim of the present study was to evaluate the analytical and clinical performance of Nanopia KL-6 assay (Sekisui Medical Co. Ltd. Tokyo, Japan) based on latex-enhanced immunoturbidimetry method. **Methods:** From March to October 2016, 260 patients diagnosed with ILD were enrolled in this study. All patients with ILD underwent HRCT and pulmonary function test (PFT). We used 113 samples and 200 samples for disease and healthy control, respectively. The evaluation consisted of determination of the precision, linearity, method comparison with ELISA kit (EIDIA, Tokyo, Japan), sensitivity and specificity and correlation with HRCT findings or PFTs. The HRCT findings were graded on a one to six scale based on the classification system. **Results:**

The total CV for low and high level quality control materials were below 2% at each concentration. Acceptable linearity was observed in their respective reportable ranges. Correlation analysis of KL-6 indicated that results of the Nanopia KL-6 assay were comparable to ELISA [correlation coefficients (r) = 0.979]. Using a ROC curve, the optimal cutoff point of KL-6 was 350 U/mL with a sensitivity and specificity of 73.9% and 98.0%, respectively, and the area under the curve was 0.953. Serum KL-6 levels was positively correlated with the extent of involvement, traction bronchiolectasis and ground-glass attenuation on the HRCT. In the comparison of all ILD patients' subgroups, significantly higher levels of KL-6 were determined in the idiopathic pulmonary fibrosis (IPF) or connective tissue diseases-related ILDs (CTD-ILD) than other groups. KL-6 levels were negatively correlated with PFTs [FVC, $DL_{CO}$, TLC and 6MWT]. In IPF patients, there were statistically significant correlations with all PFT results, but in nonspecfic interstitial pneumonia and hypersensitivity pneumonitis groups, some of the test measurements showed a good correlation with KL-6. **Conclusion:** The overall analytical and clinical performance of Nanopia KL-6 assay is acceptable for the monitoring of disease progression in clinical practice. Therefore, KL-6 serve as useful non-invasive biomarker to assess the disease severity in patients with ILD.

## A-345

### MMP-3 serum levels correlate to therapy response in RA patients

T. Matthias[1], S. Reuter[1], P. Jeremias[1], S. Brunner[1], A. Schwarting[2]. *[1]AESKU. KIPP Institute, Wendelsheim, Germany, [2]Department of Internal Medicine, Mainz, Germany*

Introduction: Matrix-Metalloproteinase-3 (MMP-3) is involved in the degradation of matrix proteins in different tissues. Literature shows that MMP-3 is overexpressed in rheumatoid arthritis patients (RA), and is involved in the destructive processes of cartilage and bone.

Aim: To evaluate the relevance of MMP-3 for the disease process and therapy response in patients with RA

Methods: 126 naïve, adult RA patients, F/M 1:0.6, respectively, from the ADAPTHERA study cohort, were studied at entry into the study and longitudinally at follow up visits. ADAPTHERA is a network to improve patient care and to find new biomarkers for RA. Follow-up analyses of serum concentration of MMP-3 were performed in comparison to control groups and between active and patients in remission. Classification of disease activity of RA patients was done according to their DAS 28 values. Serum MMP-3 levels have been determined by using a commercially available ELISA (AESKULISA® MMP-3, AESKU.DIAGNOSTICS, Germany).

Results: Mean MMP-3 levels were significantly increased in RA patients compared to the healthy population (males 44.6 ng/ml vs. 25.4 ng/ml; females 26.5 ng/ml vs. 14.2 ng/ml, $p<0.0001$ respectively). Active RA patients (DAS28>2.6) showed significantly higher MMP-3 levels than inactive patients in remission (DAS28 <2.6; males 55.3ng/ml vs. 36.6 ng/ml; females 29.7ng/ml vs. 24.7ng/ml, $p<0.05$ respectively). In addition, patients in remission (DAS28<2.6) still showed significantly higher MMP-3 levels than the healthy population (males: 36.6 ng/ml vs. 25.4 ng/ml; females: 24.3 ng/ml vs. 14.2 ng/ml; $p<0.0001$ respectively).

Conclusion: MMP-3 serum concentrations correlated with disease activity in RA patients and may be a useful biomarker in therapy management.

## A-346

### New markers for coeliac disease: anti-neo-epitope human and microbial transglutaminases

T. Matthias[1], D. Agardh[2], P. Jeremias[1], S. Neidhöfer[1], A. Lerner[1]. *[1]AESKU. KIPP Institute, Wendelsheim, Germany, [2]Skane University Hospital, Malmö, Sweden*

**Objectives:** Microbial transglutaminase (mTg) and human tissue Tg (tTg) form complexes with gliadin peptides and present neo-epitopes. The aim was to test the diagnostic performance of antibodies against both non-complexed and complexed forms of both transglutaminases in children with celiac disease (CD) and compared with disease controls. **Methods:** Serum samples at day of intestinal biopsy were collected from 350 CD children (mean age 7.4 years) and 215 disease controls (mean age 10.2 years) and tested using the following ELISAs detecting IgA, IgG or both IgA+IgG combined: tTg (for in house research use only), *AESKULISA®s* tTg New Generation (tTg neo-epitope) & mTg neo-epitope (RUO). Results were correlated to the degree of intestinal injury, using the revised Marsh criteria. **Results:**

| Antibody | Sensitivity | Specificity | AUC | p-value |
|---|---|---|---|---|
| **tTg-neo Check** | 82.06 | 97.67 | 0.964 | <0.0001 |
| **mTg-neo Check** | 93.82 | 51.63 | 0.890 | <0.0001 |
| **tTg Check** | 89.38 | 95.73 | 0.973 | <0.0001 |
| **tTg-neo IgA** | 72.65 | 98.60 | 0.943 | <0.0001 |
| **mTg-neo IgA** | 45.29 | 98.60 | 0.813 | <0.0001 |
| **tTg IgA** | 86.18 | 99.53 | 0.969 | <0.0001 |
| **tTg-neo IgG** | 82.35 | 86.05 | 0.921 | <0.0001 |
| **mTg-neo IgG** | 89.41 | 67.44 | 0.886 | <0.0001 |
| **tTg IgG** | 41.76 | 99.07 | 0.941 | <0.0001 |

Comparing the different correlations between antibodies' isotypes, the tTg Check ($r=0.7889$, $p<0.0001$) and tTg-neo check ($r=0.7544$, $p<0.0001$) were the best indicators of intestinal damage in CD. **Conclusion:** It is suggested that the combination of tTg-neo IgA/IgG antibodies should be used preferably to reflect intestinal damage during screening and diagnosing childhood CD. The tTg & tTg-neo assays show similar diagnostic performance and recommended as good screening tests for CD in children. mTg-neo IgG presents a new serological biomarker for CD.

## A-347

### Complete IFA Automation vs Manual IFA reading for Anti-Nuclear Antibody testing. An Evaluation of the AESKU HELIOS Instrument and software

T. Alexander, L. Define, P. Agner. *Summa Health, Akron, OH*

**Background:** In 2009, the American College of Rheumatology (ACR) issued a position statement for anti nuclear antibody (ANA) testing, stating, in part, "The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing". However, the immunofluorescent assay is labor intensive and requires trained, experienced individuals to properly interpret the fluorescent staining patterns. Thus there is a need for IFA automation, both for the preparation of the slides and for IFA pattern reading and interpretation. These needs may be addressed by the AESKU HELIOS instrument and software. The HELIOS is an automated system for immunofluorescence processing with image capturing. This instrument performs both the slide preparation and, with an integrated fluorescence microscope and software, can interpret HEp-2 staining patterns. This study evaluated the performance of the HELIOS Automated IFA System with AESKUSLIDES ANA HEp-2-Gamma from AESKU Diganostics and compared the HELIOS to AESKUSLIDES ANA HEp-2 slides processed and read manually. Specifically we:

1. Compared HELIOS results to manual results at 1:40 and 1:80 serum dilutions,
2. Compared the HELIOS pattern recognition interpretation to the interpretations of two trained laboratorians
3. Determined clinical sensitivity and specificity of the HELIOS based on the manual method.

**Methods:** We analyzed 556 clinical serum specimens from the clinical categories listed below at 1:40 and 1:80 dilutions using the AESKUSLIDES HEp-2 gamma kit. Slides were read by two independent,readers blinded to the identity of specimens in each well and one HELIOS.

- Normal Controls: 80
- Rheumatic Diseases associated with ANA positivity: 264
- Other diseases not associated with ANA positivity: 201
- Diverse samples (selected due to ANA positivity or rare patterns): 11

**Results:** Method comparison with manual assay % Total Agreement (95% CI) between HELIOS and manual method at 1:40 dilution

- Positive/negative total agreement = 96.0% (94.6-97)
- Pattern agreement = 94.2% (92.2-95.7)

at 1:80 dilution • Positive/negative total agreement = 93.6% (92.2-94.7)

- Pattern agreement = 93.6% (91.3-95.3)

Clinical studies: To determine clinical sensitivity and specificity, a cohort of 460 clinically characterized samples were tested.

- 264 Rheumatic Diseases associated with ANA positivity (CTD + AIL)
- 196 Other diseases not associated with ANA positivity
- 30 normal control samples

HELIOS vs. manual method at 1:40

- SLE: sensitivity = 88.9% vs. 87.8%
- CTD+AIL: sensitivity = 86.0% vs. 86.4%
- Specificity: 67% vs. 65.5%

HELIOS vs. manual method at 1:80

- SLE: sensitivity = 78.9% vs. 82.2%
- CTD+AIL: sensitivity = 78.8% vs. 86.4%
- Specificity: 77% vs. 78%

**Conclusion:** The HELIOS demonstrated excellent agreement with manual slide reading for both positive/negative interpretations and pattern recognition. The HELIOS also performed similarly to the manual method in terms of clinical sensitivity and specificity.

## A-348

### (K+L) Index: a new biomarker of disease activity in Rheumatoid Arthritis

J. Garcia de Veas Silva[1], M. López Vélez[1], A. Espuch Oliver[1], L. Pérez Albaladejo[1], N. Barbosa de Carvalho[2], J. García Lario[1], T. De Haro Muñoz[1]. *[1]Complejo Hospitalario Universitario de Granada, Granada, Spain, [2]The Binding Site, Barcelona, Spain*

**Background:** Rheumatoid Arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis and bone destruction. B cells are implicated in the inflammatory events of the RA by producing antibodies and a policlonal excess of serum free light chains kappa (K) and lambda (L). Thus, the sum of the serum levels of K and L could be associated with B cells activation and the inflammatory activity of the RA. The aim of the study is to evaluate the "(K+L) index" as new biomarker of activity disease in patients with RA based on the following studies: i) to compare the (K+L) index between healthy donors and RA patients, ii) to evaluate the diagnostic accuracy of (K+L) index in active disease and iii) to correlate (K+L) index with the disease activity index DAS28 and others biomarkers of RA (ACPA, PCR, FR and VSG).

**Methods:** study based on 69 healthy donors (12 male:57 female with a median age of 50 (43-57) years old) and 73 patients with RA (13 male:60 female with a median age of 58 (50-70) years old; 28 patients in remission and 45 patients with active disease). Disease activity was evaluated using DAS28 score. Pairwise comparison was carried out with Mann-Whitney U test, Receiver Operator Curve (ROC) was used to evaluate the efficacy of (K+L) index and Spearman correlation analysis was used for assessing the relationship between quantitative variables. A p value <0.05 was considered statistically significant. Statistical analysis was made with Prism 6.0.

**Results:**

**Objective 1:** Serum levels of (K+L) index in patients with RA were significantly higher than healthy controls: 37.86 (30.90-45.79) mg/L vs. 24.99 (19.14-29.20) mg/L, respectively (p<0.0001). Between RA patients; serum levels of (K+L) index in patients with active RA were significantly higher than those of patients in remission: 38.29 (34.23-53.05) mg/L vs. 33.73 (30.11-39.10) mg/L, respectively (p=0.018).

**Objective 2:** The area under curve in patients with active RA was 0.855 (95% CI 0.786-0.908) for (K+L) index. The optimal cut-off determined by ROC curve for (K+L) index was 32.98 mg/L. The maximum sensitivity and specificity were 80% and 85%; respectively.

**Objective 3:** A good linear correlation was found between (K+L) index and DAS28 score (r=0.503; p<0.0001) and VSG (r=0.270; p=0.02). No significantly correlation was found with PCR (r=0.09), FR (r=-0.03) and ACPA (r=-0.007).

**Conclusions:** The levels of serum "(K+L) index" in patients with active RA were significantly higher than those of patients in remission and of healthy controls. An optimal cut-off of 32.98 mg/L allows us to diagnose activity states of the disease. The (K+L) index could be used as biomarker of disease activity in RA.

## A-349

### Complement activation in systemic lupus erythematosus with antiphospholipid antibodies

R. V. Alexander, T. O'Malley, J. Conklin, C. Ibarra, T. Dervieux. *Exagen Diagnostics, Inc, Vista, CA*

**Background.** Complement activation is a hallmark of systemic lupus erythematosus (SLE) and leads to formation of cell-bound complement activation products (CBCAPs). We established that C4d split fragment bound to erythrocytes (EC4d) and B-cells (BC4d) is a sensitive and specific biomarker for SLE. Because subjects with SLE often develop auto-antibodies to phospholipid complexes (APL), we evaluated the relationship between complement activation in APL positive and negative subjects with SLE or other diseases.

**Methods.** Blood was collected in EDTA from subjects with SLE (n = 541, 91% females, mean age 41 years), other diseases (n = 615, 85% females, mean age 55 years, inclusive of 287 with rheumatoid arthritis) and normal healthy volunteers (NHV, n = 210, 65% females, mean age 41 years). EC4d and BC4d expression was determined by flow cytometry and reported as net mean fluorescent intensity (MFI). Positive CBCAPs consisted of positive EC4d (> 14 net MFI) and/or BC4d (> 60 net MFI). Anti-cardiolipin IgG, anti-beta-2-glycoprotein 1 IgG, or anti-phosphatidylserine/prothrombin complex IgG antibodies were determined using ELISA (INOVA Diagnostics, San Diego, CA). Presence of APL antibodies consisted of any of these 3 antibodies above manufacturer cutoff. Groups were compared by Fisher's exact test.

**Results.** CBCAPs yielded 61% sensitivity and 89% specificity in distinguishing SLE from other diseases (99% specific in NHV). APL positivity was higher in SLE than in other diseases (40% vs. 17%). APL positive SLE subjects had higher incidence of CBCAPs positivity than APL negative (77% vs. 51%, p<0.0001) (Table). Similar trends were observed in the group of other diseases and NHV.

**Conclusions**. APL occur at higher rate in SLE than other diseases. APL antibodies may contribute to complement activation and CBCAPs formation in SLE.

| | APL positivity (%) | CBCAPs positivity in APL positive (%) | CBCAPs positivity in APL negative (%) |
|---|---|---|---|
| SLE, n = 541 | 40% | 77% | 51% |
| Other diseases, n = 615 | 17% | 18% | 10% |
| NHV, n = 210 | 14% | 7% | 0% |

## A-350

### Evaluation of a novel Latex agglutination immunoturbidimetry assay kit, H. pylori-Latex "SEIKEN", for detection of anti-*H.pylori* antibody in serum and plasma.

A. Ishii. *Denka Seiken Co.,Ltd., Chuo-Ku, Tokyo, Japan*

**Background:***Helicobacter pylori* (*H. pylori*) is a helix-shaped Gram-negative microaerophilic bacterium existing in human gastric mucosa. It is well-known that *H. pylori* infection causes chronic gastritis and significantly increases risks of a gastric ulcer, a duodenal ulcer, and gastric cancer. *H.pylori* infection can be diagnosed by various methods such as endoscopic atrophy, rapid urease test (RUT), microscopy, culture, urease breath test (UBT), urine or serum/plasma antibody tests, and a stool antigen test. Among the choices, serum/plasma antibody test is widely used in Japan since it is comparatively less invasive and less expensive than the others and also allows analysis of a large number of samples with an auto-analyzer. We have developed H. pylori-Latex "SEIKEN" (Denka Kit), a novel latex agglutination immunoturbidimetry assay kit, for detecting anti-*H. pylori* antibody in human serum and plasma. The analysis by Denka kit is a simple procedure, which comprises measurement on an auto-analyzer capable of accommodating two-reagent assay, and is completed within 10 minutes. In this study, the basic performances and accuracy to clinical diagnosis of the Denka kit were evaluated.

**Methods:**The Denka kit was evaluated for the following basic performance: (1) within-run precision, (2) lower detection limit, (3) linearity, (4) prozone, (5) interferences, (6) comparison between serum and plasma collected from the same patients, and (7) accuracies to ELISA kits in market (IBL, Bio-Rad, Monobind). In addition, the results of 159 patients sera tested by the Denka kit were evaluated in comparison to clinical diagnosis, which was determined from the overall judgment of the endoscopic atrophy, the RUT, and the culture.

**Results:** (1) Samples with two different levels (5.1 U/mL and 21.1 U/mL) were measured in 20 replicates. The coefficients of variation (CVs) were within 1.0-2.0%. (2) The lower detection limit was 1.0 U/mL. (3) Linearity was observed up to 100 U / mL. (4) No prozone phenomenon was observed up to 400U/mL. (5) No interference effects were observed for hemoglobin up to 500 mg / dL, bilirubin up to 30 mg / dL, and chyle up to 2000 FTU. (6) Consistency was shown by 98.2% (55/56) between serum and EDTA plasma, and by 100.0% (39/39) between serum and heparin plasma. (7) The Denka kit had 98.8%, 93.3%, and 92.2% accuracies to the three ELISA kits. The sensitivity, specificity, and accuracy of the Denka kit compared with the clinical diagnoses were 93.1% (81/87), 97.2% (70/72), and 95.0% (151/159), respectively.

**Conclusion:**The Denka kit showed excellent basic performances and high diagnostic accuracies confirmed with clinical diagnoses. Compared to other diagnostic methods,

the Denka kit allows rapid measurements of a large number of specimens in a fully automated manner. The results of this study suggest that not only the Denka kit is highly efficient for diagnosis of *H. pylori* infection but also useful for mass screening in the preliminary stage such as endoscopic examination.

## A-351

### Adjusted serum free light chain reference ranges on the SPAPlus platform

T. Theparee, R. C. Benirschke, H. Lee. *NorthShore University Healthsystem, Evanston, IL*

**Background:** Serum free light chain measurements by nephelometry have been useful in the monitoring of disease progression or treatment success in patients with multiple myeloma. The original free light chain reference ranges measured on the Beckman IMMage 800 platform were the manufacturer's ranges. Our hospital recently switched to the Binding Site SPAPlus platform which did not have separate reference ranges as per the manufacturer. During validation with patient comparison studies, the SPAPlus platform demonstrated higher values when compared to the IMMage platform, establishing the need for new in-house reference ranges.

**Objective:** To establish and validate new kappa, lambda light chains, and K/L ratio reference ranges for our patient population on the Binding Site SPAPlus platform.

**Methods:** Patient sera were tested on the Binding Site SPAPlus Freelite Human Lambda and Kappa Free Kit according to the manufacturer's instructions. Forty-seven patient sera were tested on a Beckman Coulter IMMage 800 Immunochemistry platform concurrently with the Binding Site SPAPlus platform. Deming Regression for concentrations less than 5 mg/dL was used to determine theoretical reference ranges on the SPAPlus platform. An additional 82 normal patient sera with normal serum protein electrophoresis (SPE) results were used to validate these reference ranges. These 82 normal patients were without plasma cell neoplasms, hematologic malignancies, autoimmune diseases, active infections and had normal creatinine, calcium, liver enzymes, IgG, IgA, and IgM concentrations.

Statistical analysis was performed using R statistical software.

**Results:** The following table shows the old, new, and validated reference ranges.

**Conclusion:** Kappa, lambda light chains and K/L ratio reference ranges that were previously used on the Beckman IMMage 800 platform could not be translated to the Binding Site SPAPlus platform. We propose new reference ranges for the Binding Site SPAPlus platform based on our study.

| Old, new, and validated reference ranges | | | |
|---|---|---|---|
| | **Old Reference Ranges** | **New Reference Ranges** | **Validated Reference Ranges** |
| **Kappa Light Chains (mg/dL)** | 0.33-1.94 | 0.33-3.26 | 0.37-3.08 |
| **Lambda Light Chains (mg/dL)** | 0.57-2.63 | 0.46-2.71 | 0.51-1.99 |
| **K/L Ratio** | 0.26-1.65 | 0.49-2.54 | 0.60-2.26 |

## A-352

### Evaluation of the Stability of Centrifuged EDTA Specimens for HIV Serology Testing

N. Tran, A. Hardesty, P. Akl. *University of Oklahoma Health Sciences Center, Oklahoma city, OK*

**Background:** Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) is a major cause of illness and death in the United States. The diagnosis of HIV infection itself carries major personal, social and financial implications. It is therefore critical for both the patient and the physician to be certain of the diagnosis. In our laboratory, routine HIV serology testing is performed in a batched fashion, Monday through Friday. Whole blood specimens are centrifuged as soon as they are received in the laboratory and plasma is separated from the cells within 24hrs. This separation step however, increases the risk of specimen mislabeling, and thus may misclassify patients as either HIV reactive or non-reactive. To eliminate this separation step and thus prevent the risk of specimen mislabeling, we performed a stability study by testing HIV serology on centrifuged primary specimens, without plasma separation, at <24hrs and 4-5days post collection.

**Methods:** Forty-three patients were included in the study. EDTA whole blood specimens, recommended specimen type for HIV serology in our laboratory, were centrifuged, stored at 2-8°C, and tested at <24hrs and 4-5days. HIV serology testing was performed on both the Advia Centaur-XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) and Architect Plus-i2000 (Abbott Diagnostics, Abbott Park, IL).

**Results:** As shown in Table-1, 46.5% (20/43) of tested specimens were reactive for HIV, while 53.5% (23/43) were non-reactive. The concordance between the <24hrs and 4-5days stored specimens were 100% on both the Advia Centaur-XP and the Architect Plus-i2000. No discordant events were observed.

**Conclusion:** We conclude that HIV serology testing can be performed up to 4-5days on centrifuged primary EDTA specimens with no change in the results. Therefore, specimens received on the weekend could be tested on the following weekday without the need to separate plasma from the cells, decreasing the risk of specimen mislabeling.

| Table-1: HIV test results | | Centaur | | Architect | |
|---|---|---|---|---|---|
| | | 4-5 days | | 4-5 days | |
| | | Reactive | Non-reactive | Reactive | Non-reactive |
| <24 hours | Reactive | 20 | 0 | 20 | 0 |
| | Non-reactive | 0 | 23 | 0 | 23 |

## A-353

### High cut-off haemodialysis and serum free light chains: improving the treatment of patients with Multipl Myeloma and acute kidney injury

P. Menendez Valladares[1], J. M. Maesa[1], J. L. Garcia De Veas Silva[2], N. Barbosa de Carvalho[3], C. Bermudo Guitarte[1]. *[1]Virgen Macarena University Hospital, Seville, Spain, [2]Granada University Hospital Complex, Granada, Spain, [3]The Binding Site Group, Barcelona, Spain*

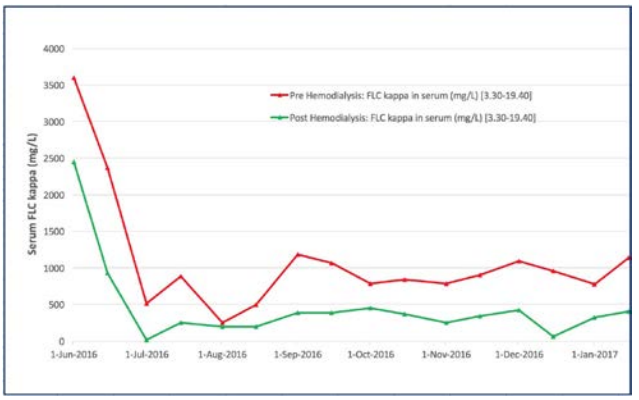
**Background:** Acute kidney injury (AKI) is present in 15-30% of patients with Multiple Myeloma (MM) and the survival of these patients is highly dependent on the recovery of the renal function. The effective elimination of serum free light chains (sFLC) with the application of haemodialysis with high cut-off membranes (HCO-HD) alongside with chemotherapy is associated with an improvement in the renal function.

**Methods:** A 60 year-old woman diagnosed of IgG Kappa MM ISS-II was admitted to the hospital due to relapse of the disease. During his stay, an AKI was detected with creatinine=5.35 mg/dL, urea=70 mg/dL, an EFGR=8.1 mL/min/1.73 $m^2$ and an altered sFLC ratio=373.8 (kappa sFLC=3595.95 mg/L). The patient underwent sixteen sessions of HCO-HD to remove sFLC in addition to Bortezomib and Dexamethasone (B/D) treatment. sFLC were measured by turbidimetry using the assay Freelite (The Binding Site, UK). Blood samples were collected pre- and post-HD to determine creatinine and sFLC.

**Results:** During therapy kappa sFLC levels decreased significantly (see graphic). After sixteen cycles of HCO-HD, kappa sFLC clearance was 89% from 3595.95 mg/L to 404.6 mg/L. This treatment produced an improvement in the patient´s renal function with a decrease of 51.78% in the creatinine serum levels, with an EFGR=19.5mL/min/1.73 $m^2$. After HCO-HD, the patient continued on conventional haemodialysis and finished the treatment with B/D achieving a partial response (PR) with negative immunofixation; kappa sFLC=404.6 mg/L, sFLC ratio=25.05. Bence Jones proteinuria of 0.2 g/24h and presence of 1.2% of plasmatic cells in bone marrow.

**Conclusion:** A combination of the efficient and direct removal of the nefrotoxic excess of sFLC using HCO-HD with effective chemotherapy with B/D allowed an efficient reduction of the sFLC levels. sFLC determination by Freelite allowed an accurate and rapid evaluation of the rate of sFLCs decrease, proving useful to monitor the efficiency of the therapy adopted.

adopted.




## A-354

### Quantitation of Serum Immunoglobulins by Capillary Electrophoresis

C. Wunsch. *University of Miami School of Medicine, Miami, FL*

OBJECTIVE: To enhance the utility of serum protein electrophoresis by developing a method for quantitating IgA, IgG and IgM immunoglobulins from the electrophoretogram (ELP). METHODS: Digital data from 700 clinical serum ELPs were downloaded from a CapillaryS™ analyzer. Values for IgA, IgG, IgM, total κ Ig, and total λ Ig measured on the same ELP specimen were obtained from a BNII analyzer. The adjusted sum of total κ + total λ Ig was required to be within 5% of the sum of G, A, M-Igs for further analysis. Specimens with ELP clonal characteristics were excluded. The majority of remaining 510 ELPs had one or more abnormalities. Seven features - curve heights or areas - of the ELP were chosen for regression studies. Each curve feature was regressed against IgA, IgG, and IgM. Regression yields an equation relating the feature to each Ig. β-Igs were calculated with graphical algorithms. For most ELPs, the β-γ boundary was defined as the valley between the zones. Simplex analysis was chosen to provide an approximate solution to the equations. Simplex analysis requires 4 equations to estimate the IgA, IgG and IgM of an ELP. Python programs and R statistical analysis were used. RESULTS: A simplex needs initialization with an estimate of the Igs for convergence. IgG and IgM were usually approximated from the γ peak height, and IgA from the β-Ig. The smallest R-square for any of the regressions used was 0.88. β-zone Ig, γ-zone Ig, γ-peak height, and γ-zone mid-point height all had R-square values of 0.96 or more. By itself, the γ-peak height is directly proportional to IgG with a correlation of 0.95, but the regressed peak correlation significantly improved to 0.98 due to inclusion of IgM - the IgA coefficient was not significant. Most sets of simplex equations included the β-γ boundary height. Three sets of simplex equations produced correlations better than 0.97 for the IgG and IgA of ELPs, but none produced an IgM correlation better than 0.50. DISCUSSION: The sum of β-Ig and λ-Ig should equal the sum of G, A, M-Igs, thus regression coefficients for β-Ig and λ-Ig should be complements of each other. Their coefficients, determined independently, however, are not exact complements of each other. The placement of the β-γ boundary plays a critical role in the regression of β- and γ-Ig and the height of the β-γ boundary. Large elevations of γ-Ig shift the valley between the β-γ zones upward and toward the anode. This small shift has a large effect on the least squares calculations of regression. The values used to initialize the simplex are also important. Seeding the best simplex with values within 5 to 10% of the target values produced G, A, M-Ig correlations above 0.95, but the IgM correlation dropped to 0.33 when simple approximations were used. CONCLUSION: Good estimates of IgG and IgA can be extracted from ELPs. With improvements to the above approach, one should also be able to obtain a good estimation of IgM.

## A-355

### Performance Evaluation of the ADVIA Centaur Lipopolysaccharide Binding Protein (LBP) Assay

V. Taylor[1], N. Smith[1], L. Buchan[1], J. King[1], P. Kelly[1], D. Hovanec-Burns[2]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics, Tarrytown, NY*

**Background:** Lipopolysaccharide binding protein (LBP) is a soluble acute-phase protein (65 kDa) that binds to bacterial lipopolysaccharide (LPS) to elicit immune responses and may be used in diagnosis and prognosis of diseases that are induced by exposure to endotoxin, such as sepsis and infectious complications of surgery and trauma. The ADVIA Centaur® LBP assay* for the quantitative measurement of LBP in human serum and plasma is under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics Inc. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), interference and cross-reactivity, and method comparison to the IMMULITE® 2000 LBP assay† (Siemens Healthcare Diagnostics Inc.).

**Method:** The ADVIA Centaur® LBP assay is a sandwich immunoassay that employs direct chemiluminescent technology. LBP is bound to mouse monoclonal anti-LBP antibody-coated particles and is then detected by an acridinium ester-labelled anti-LBP mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acidic and basic reagents are added to the reaction mixture, and the resulting chemiluminescence is measured. The method comparison study was performed per CLSI EP-09-A3 using 121 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively. In addition, following CLSI-EPO7-A2, performance of the assay was assessed against a list of potential interfering substances including hemoglobin, conjugated and unconjugated bilirubin, triglyceride, biotin, cholesterol, IgG, total protein, rheumatoid factor, lipemia, and HAAA. Furthermore, also per CLSI-EPO7-A2, the assay was tested with potential cross-reactants such as human serum amyloid A, IL-6, IL-8, TNFα, and CRP.

**Results:** Observed range of the assay was up to 120 µg/mL without dilution and up to 240 µg/mL with manual 1:2 dilution. The assay's linearity was observed up to 120 µg/mL. The limit of quantitation was observed at 1.5 µg/mL, with a total error of 19%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 3.3–4.1%. Passing-Bablok procedure comparison of the assay to the IMMULITE 2000 LBP assay gave a slope and intercept of 1.20 and 2.76 µg/mL, respectively. The Pearson's correlation r value was 0.95. The assay demonstrated no significant interference or cross-reactivity from the tested analytes.

**Conclusions:** The feasibility of an automated LBP assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of LBP.

*Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

†Not available for sale in the U.S.

## A-356

### Transcription Factor YY1 Induces Interleukin-8 Production in Rheumatoid Arthritis via PI3K/Akt Signaling Pathway

J. Lin, Y. He, Q. Ou. *Department of Laboratory Medicine, The First Affiliated Hospital of Fujian Medical University, Chin, Fuzhou, China*

**Background:** Previous studies have revealed a critical role of YY1 in cancer development and progression. However, whether YY1 has any role in rheumatoid arthritis (RA) remains unknown. This study aims to explore the potential role of YY1 in RA pathogenesis.

**Methods:** Expression of YY1 was detected by real-time PCR and western blotting. The signaling pathway was done by ingenuity pathway analysis (IPA). Vascular cell adhesion molecule 1 (VCAM-1) and interleukin-8 (IL-8) expression was detected by real-time PCR and ELISA. CIA mice were treated with YY1 related lentivirus to observe the role of YY1 *in vivo*. **Results:** We found that YY1 was over-expressed in RA patients and CIA mice. Blocking of YY1 action with YY1 shRNA lentivirus ameliorated disease progression in CIA mice. We further analyzed the signaling pathway involved by ingenuity pathway analysis (IPA), results showed IL-8 signaling and PI3K/Akt signaling pathway was significantly inhibited by LV-YY1-shRNA treatment. Moreover, we observed that blocking of YY1 reduced IL-8 and VCAM-1 production and neutrophil adhesion via PI3K/Akt signaling pathway. **Conclusion:** In conclusion, YY1 plays a critical role in promoting IL-8 production in RA which contribute to the inflammation of RA via stimulation of neutrophil adhesion. Thus, YY1 is likely a key molecule involved in the inflammation process of RA. Targeting of YY1 may be a novel therapeutic strategy for RA.

## A-357

### Performance Evaluation of the ADVIA® Centaur Erythropoietin (EPO) Assay*

V. Taylor[1], H. Laird[1], M. Chalmers[1], H. Lang[1], E. Ma[1], J. King[1], P. Kelly[1], J. Lei[2]. *[1]Axis-Shield Diagnostics Ltd, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

**Background:** Erythropoietin (EPO) is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. Measurement of EPO is used as an aid in the diagnosis of anemias and polycythemias. The ADVIA Centaur® EPO assay* (under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics Inc.) is intended for the quantitative measurement of EPO in human serum and plasma using the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), and method comparison to the ACCESS EPO assay (Beckman Coulter).

**Method:** The ADVIA Centaur EPO assay is a sandwich immunoassay that employs direct chemiluminescent technology. EPO is bound to mouse monoclonal anti-EPO antibody-coated particles and is then detected by an acridinium (NSP-DMAE)-labelled anti-EPO mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acidic and basic reagents are added to the reaction mixture and the resulting chemiluminescence is measured. In a preliminary study, performance of the ADVIA Centaur EPO assay was evaluated for precision, linearity, limit of quantitation (LoQ), and method comparison to Access EPO (Beckman Coulter). The method comparison study was performed per CLSI EP-09-A3 using 140 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A respectively.

**Results:** The reportable range of the prototype assay is up to 750 mIU/mL without dilution or up to 7500 mIU/mL with automated or manual 1:10 dilution. Linearity was demonstrated up to 750 mIU/mL. The limit of quantitation was 0.93 mIU/mL, with a total error of 29.1%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 1.55-5.64%. Method comparison of the ADVIA Centaur EPO assay to the ACCESS EPO assay using Passing-Bablok regression gave a slope and intercept of 1.00 and 0.23 mIU/mL, respectively. The Pearson's correlation r value was 1.00. Mean EPO values for the ADVIA Centaur and ACCESS assays were 41.02 and 41.45 mIU/mL, respectively, and sample doses ranged from 0.82 to 751.29 mIU/mL for the ADVIA Centaur EPO assay.

**Conclusions:** The feasibility of an automated EPO assay on the Siemens ADVIA Centaur XP Immunoassay Systems has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of EPO in human serum and plasma.

*Under development. The performance characteristics of this device have not been established. Not available for sale, and its future availability cannot be guaranteed.

## A-358

### Characterization and Engineering of Allergenic Materials Using Capillary Immunoblot and Application on BioCLIA® 4G Allergy Assay

S. W. P. Chan[1], L. Trondle[1], S. V. Wetering[1], L. Qian[2], C. Lee[2]. *[1]CRM Biotech, Irvine, CA, [2]HOB Biotech Group, Suzhou, China*

**Background:** *In vitro* allergen-specific IgE assays, integrated with component-resolved diagnostics (CRD), offer allergists an allergen-sIgE sensitization profile with insight into clinical relevance such asrisk of anaphylaxis versus benign local symptoms. CRD has been developed in various testing platforms, e.g.,BioCLIA® 4G Allergy (HOB Biotech Group, China). CRD uses allergenic molecules for reflex testing in response to a positive result from a traditional extract-based test. Extracts from different sources may contain varying allergenic component concentrations leading to discrepant results, and allergenic components are often used tosupplement raw allergen extracts to increase test sensitivity. An accurate quantitative method is required to determine and then adjust the component composition to ensure commutable results and lot-to-lot consistency. This study aims to quantitativelyprofile specific components present in several commercially-available allergen extract materials and strategize the engineering process using a recombinant component to adjust the responses of a patient panel to various materials, on the BioCLIA® 4G Allergy Assay.

**Methods:** Patient samples were purchased from plasma suppliers, and tested for peach (F95)-sIgE ImmunoCAP (Phadia, Sweden) reference values. Several commercially-available F95 materials and extractswere screened forrelative component contentby quantitative SDS-PAGE. Allergenic components, including Pru p 1, were identified using Western Blot on the WES platform (Protein Simple, San Jose, CA) with the patient panel. The patient panel was then tested for sIgE towards these raw materials and recombinant Pru p1 using the BioCLIA® 4G Allergy (HOB Biotech Group, China)Chemiluminescence Immuno Assay (CLIA) assays. Subsequently these extracts were supplemented with recombinant Pru p 1and the patient panel reactivity was re-assessed.**Results:** A panel of eighteen F95-positive sampleswasidentified. The sensitivity ofthis patient panel variedacross different F95 raw materials, ranging from 89% forVendor 1, 42% for Vendor 2and 53% for Vendor 3. WES positive signal resulted froman unidentified component of ~95 kDain Vendor 1 material, an unidentified component of ~66 kDain Vendor 2 material, and both ~66 kDa and ~95 kDa inVendor 3 material. All materials were essentially missing Pru p1.Eleven samples (61%) showed reactivity to rPrup1(rPru p 1-sIgE ≥ 0.35 IU/ml), with a response profile different from those of the three F95 materials. Adding defined ratios of rPru p1 increasedthe sensitivity of all three materials. **Conclusion:** This study has shown that raw material extracts are comprised of unique sIgE-binding components in varying concentrations leading to variable in vitro allergen-sIgE testing results, neither commutable across test methods nor consistent across lots of reagents. With modern analytical SDS-PAGEand capillary Western blot, raw materialcomponent ratioscan be identifiedin a patient-specific manner. Through guided adjustment of allergeniccomponentsin raw materials, test sensitivity can be greatly increased, particularly when important allergens are underrepresented or lacking.In summary, quantitative component composition information can be used to standardize allergen raw materials, engineer allergen-sIgE testing materials, and generate QC specifications to ensure lot-to-lot consistency.

## A-359

### A Sensitive Method for the Determination of Anti-CCP on the HOB 4G BioCLIA® 1200 Automated Immunoassay Analyzer

L. Cui, L. Liu, Z. Zhao, T. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting between 0.5-1 % ofthe adult population. Patients with RA has been found to develop endogenous auto-antibodies to cycliccitrullinated peptides (anti-CCP), and these are used as a marker in the diagnosis of early RA. Since the first report in 1998 that antibodies present in blood and reactive with synthetic peptides containing the amino acid citrulline are highly specific for RA, the measurement of anti-CCP in patient serum has become important in the early and accurate diagnosis of this disease. Recently, the innovative HOB 4G BioCLIA® anti-CCP kit, coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system, has been launched for detection anti-CCP antibody in serum .

**Methods:** In this study, the analytical characteristics including limit of detection (LOD), precision (intra-assay & inter-assay), dilution linearity, and interference were evaluated by BioCLIA® anti-CCP kit according to the CLSI guidelines. 235 clinical samples with RA collected from Beijing Xiehe Hospital Rheumatism Immunity Branch were analyzed by both BioCLIA® and EURODIAGNOSTICA(ELISA) kits. Sensitivity, specificity and total agreement were analyzed between two compared assays. Lastly, anti-CCP titers were measured and analyzed with sera from Chinese patients with RA(N=165), rheumatic diseases other than RA (non-RA) (N=100), Systemic Lupus Erythematosus (SLE) (N=98), osteoarthritis (OA) (N=45), chronic inflammatory diseases (CID) (N=120) and healthy donors (N=150). At the same time,the detection rate was studied by both HOB BioCLIA®RF and HOB BioCLIA® CCP kits in the RA group (N=165). **Results:** The BioCLIA®anti-CCP kit performed good linearity ranging from 2~400RU/mL, and the LOD was 0.064RU/mL. In the precision testing, the CV% was 5.77% for intra-assay and 8.14% for inter-assay, respectively. Bilirubin(up to 20 mg/dL), hemoglobin (up to 150 mg/dL), and lipid (up to 1000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 2000 mg/dL) did not affect the detection of anti-CCP in serum. In a clinical evaluation, using 235 clinical samples with RA patients, we found the BioCLIA®has similar sensitivity 92.8%(116/125)with EURODIAGNOSTICA assay (ELISA),and a similar specificity of 91.8%(101/110). The total agreement of BioCLIA®& ELISA were 92.8%(217/235), respectively. The clinical sensitivity of RA was 70%(116/165) and the specificity for non-RA,SLE,OA,CID, and healthy donors were 97%(97/100), 97%(95/98), 98%(44/45), 98% (117/120), 98% (147/150). By comparison, the prevalence rate of anti-CCP and anti-RF antibodies in the RA group was 70%(116/165) and 54%(89/165), respectively.
**Conclusion:**

BioCLIA®anti-CCP kit is an innovative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better

clinical relevance when compared with ELISA with a good total agreement of 92.8%. In conclusion, the BioCLIA®1200 anti-CCP kit is a sensitive and specific method in the detection of anti-CCP and valuable to aid other than anti-RF in the diagnostic process, treatment and monitoring of RA.

## A-360

### Comparison of the Agreement of 3 Serodiagnosis Algorithms for Syphilis in West China

D. Li, T. Wang, C. Tao. *West China Hosipital of SCU, Chengdu, China*

**Background**

The resurgence of syphilis in recent years has become a serious threat to the public health worldwide, and its diagnosis relies upon a combination of tests, including treponemal immunoassays (TT) and non-treponemal tests (NTT). The toluidine red unheated serum test (TRUST), T. *pallidum* particle agglutinationassay (TPPA) plus chemiluminescene immunoassay (CLIA) are employed in our hospital. There have not too many studies involving detailed analysis of the different algorithms for detecting syphilis.

**Methods**

Results of 4210 plasma sample simultaneously evaluated using the TRUST, TPPA, and CLIA were retrospectively collected study to analyze 3 syphilis testing algorithms: traditional algorithm, reverse algorithm, and the European Centre for Disease Prevention and Control (ECDC) algorithm (Fig 1). The kappa (κ) coefficient was used to compare the concordance between algorithms. The agreement of the results according to their κ values was categorized as near perfect (0.81-1.0), substantial (0.61-0.8), moderate (0.41-0.6), fair (0.21-0.4), slight (0-0.2), or poor (<0).

**Results**

Overall, 1477 subjects had TRUST+/TPPA+/CLIA+ results, and 527 subjects had TRUST-/TPPA-/CIA-results; 6 subjects were TRUST+/TPPA-. Among these 6 subjects, only 1 was CLIA+, which was discordant with the TPPA test result; These 5 TRUST+/TPPA-/CIA- cases were considered to have biological false positive reaction. 6 subjects were TRUST-/TPPA+/CLIA- without the presence of the prozone phenomenon may be considered to acute or early infection. 220 TRUST-/CLIA+/TPPA cases indicated the high sensitivity of CLIA assay. The overall percentage of agreement and κ value between the reverse algorithm started by TPPA and the ECDC algorithms were 99.0% and 0.968, respectively. The overall percentage of agreement and κ value between the reverse algorithm started by CLIA and the ECDC algorithms were 89.5% and 0.655.

**Conclusions**

It is recommended to use of the ECDC algorithm started by CLIA, complemented with a NTT test to determining serological activity and the effect of syphilis treatment.

Key words: syphilis; algorithm; diagnosis

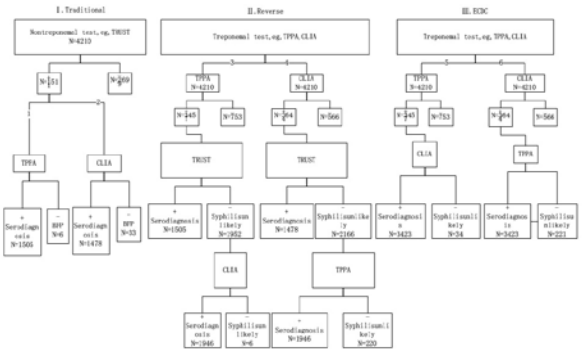



**Fig1 Syphilis testing algorithms.** Abbreviations: BFP, biological false positive; CIA, chemiluminescence immunoassay; ECDC, European Centre for Disease Prevention and Control; EIA, enzyme immunoassay; TRUST, toluidized red unheated serum test; TPPA, Treponema pallidum particle agglutination.

## A-361

### Performance Evaluation of the ADVIA Centaur Interleukin-6 (IL-6) Assay

S. Poynton[1], C. Grieve[1], N. McIntosh[1], A. Suttie[1], P. Kelly[1], D. Hovanec-Burns[2]. [1]*Axis-Shield Diagnostics, Dundee, United Kingdom,* [2]*Siemens Healthcare, Tarrytown, NY*

**Background:** Interleukin-6 (IL-6) is a pleiotropic cytokine with a broad range of biological activities. IL-6 production is rapidly induced in the course of acute inflammatory reactions associated with a number of clinical situations, including infection and may be used as an aid in the study of inflammatory diseases. The ADVIA Centaur Interleukin-6 (IL-6) assay* for the quantitative measurement of IL-6 in human serum and plasma on the ADVIA Centaur® Immunoassay Systems is under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), interference and cross-reactivity, and method comparison to the IMMULITE® 2000 IL-6 assay† (Siemens Healthcare Diagnostics Inc.).

**Method:** The ADVIA Centaur IL-6 assay is a sandwich immunoassay that employs direct chemiluminescent technology. IL-6 is bound to mouse monoclonal anti-IL-6 antibody-coated magnetic particles and is then detected by an acridinium ester-labelled anti-IL-6 mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acid and base reagents are added to the reaction mixture and the resulting chemiluminescence is measured. The method comparison study was performed per CLSI EP-09-A3 using 276 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, cholesterol, immunoglobulin G, total protein, rheumatoid factor, and lipemia. Also per CLSI EP07-A2, the assay was tested for cross-reactivity with soluble IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-8, TNF-α, TNF-β, IFY-γ, IFN-α, IFN-β, and IL-6 receptors.

**Results:** Observed reportable range of the assay was up to 5000 pg/mL without dilution and up to 50,000 pg/mL with automated or manual 1:10 dilution. The assay's observed linearity was up to 5000 pg/mL. The limit of quantitation was observed at 1.8 pg/mL, with a total error of 35%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 4.0–6.8%. Passing-Bablok method comparison of the assay to the IMMULITE 2000 IL-6 assay returned a slope of 1.04 and an intercept of -1.31pg/mL, with a Pearson correlation r value of 0.98. The assay demonstrated no interference and no cross-reactivity with the tested analytes.

**Conclusions:** The feasibility of an automated IL-6 assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of IL-6.

*Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

† Not available for sale in US

## A-362

### Evaluation of the Analytical Performance of Anti-Mitochondrial IgG Antibodies on the HOB BioCLIA® 1200 Automated Immunoassay Analyzer

L. Liu, Z. Zhao, L. Cui, T. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** Anti-mitochondrial IgG antibodies is one of the primary auto-antibodies present in patients with Primary Biliary Cirrhosis (PBC) and in vitro detection of Anti-Mitochondrial IgG antibody is the classic serological marker of PBC. Early studies described 9 subtypes of mitochondrial antigens, termed M1-M9. The major autoantigens targeted by PBC patient sera recognize the M2 antigen fraction. The primary components of the M2 antigen were found to be members of the dehydrogenase complex, the specific antigens were identified as the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), 2-oxo-glutarate dehydrogenase complex (OGDC-E2). branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2). Recently, the innovative HOB BioCLIA® AMA-M2 kit, coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system has been launched. **Methods:** In this study, the analytical characteristics including limit of detection (LOD), precision (intra-assay & inter-assay), dilution linearity, and interference were evaluated by HOB BioCLIA® AMA-M2 kit according to the CLSI guidelines. Furthermore, 300 clinical samples with indirect immunoflurescence assay (IFA) results, were analyzed by both BioCLIA® and ELISA (from an international

renowned manufacture). Sensitivity, specificity and total agreement of the compared assays were analyzed. Lastly, a total of 340 clinically characterized samples were used to study clinical sensitivity and specificity, specifically 100 patients for PBC, 50 patients for Systemic Lupus Erythematosus (SLE), 60 patients for Rheumatoid Arthritis (RA), 30 patients for virus hepatitis, 100 samples for healthy donors. **Results:** The BioCLIA® AMA-M2 kit performed good linearity ranging from 2-400 RU/mL, and the LOD was 0.15 RU/mL. In the precision testing, the CV% was 4.38% for intra-assay and 5.05% for inter-assay, respectively. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of anti-Mitochondrial antibody IgG in serum. In a clinical evaluation, using 300 clinical samples with IFA assay results, we found the BioCLIA® has higher sensitivity 97.0% (97/100) than ELISA results 90% (90/100), but with a similar specificity of 97%. The total agreement of BioCLIA® & ELISA compared to IFA assay were 97% (291/300) & 94% (284/300), respectively. From the clinical study in Chinese patients, the positive rate showed on HOB BioCLIA® AMA-M2 kit in PBC, SLE, RA, virus hepatitis and healthy donors were 92% (92/100), 8% (4/50), 1.6% (1/60), 0% (0/30) and 1% (1/100), respectively. **Conclusion:**

BioCLIA® AMA-M2 kit is an innovative semi-quantitative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better clinical relevance when compared with ELISA, and a good agreement (positive agreement=97%, negative agreement=97% and total agreement = 97%) with IFA assay, which is considered as the gold standard method. In conclusion, the BioCLIA® AMA-M2 assay is a sensitive and specific method, which could serve as a promising and fully automated alternative for IFA assay in the detection of anti-mitochondrial IgG antibodies and valuable to aid in the diagnosis of PBC.

## A-363

### Sensitive and Quantitative Methods of Anti-cardiolipin Antibodies Determinations with HOB 4G BioCLIA®Technologies

L. Liu, T. Zhao, L. Cui, Z. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** Anti-cardiolipin antibodies (aCL), against anionic phospholipids or protein phospholipid complexes, are strongly associated with venous, arterial thrombosis and obstetrical complications. These symptoms were defined as anti-phospholipid syndrome (APS) by Harris in 1987. High levels of aCL-IgG are known to be most diagnostically valuable for APS. aCL-IgA and aCL-IgM, relative to hemolytic anemia, also exist in APS patients. Four types of BioCLIA® aCL kits were launched, including cardiolipin IgG, IgA, IgM, and cardiolipin screening kits. The BioCLIA® aCL kits were more accurate, sensitive, quantitative and automated than traditional ELISA or LIA kits.

**Methods:** The analytical performancesincluding limit of detection (LOD), dilution linearity, precision (intra-assay & inter-assay), and interference were evaluated by HOB BioCLIA® aCL kits, according to CLSI guidelines. Method comparison was conducted using300clinical samples by both HOB BioCLIA® kits and ELISA kits (from international renowned manufactures). Clinical sensitivity and specificity were analyzed from various disease patients including 88 APS patient samples, 102 systemic lupus erythematosus (SLE) patient samples,105 rheumatoid arthritis (RA) patient samples,10 syphilis patient samples and 100 normal healthy donors.All samples mentioned were collected from major Chinese hospitals. **Results:** The LOD of the cardiolipin IgG, IgA, IgM, and cardiolipin screen kits were 0.41RU/mL, 0.13RU/mL, 0.06RU/mL, 0.06RU/mL, respectively. The linearity range of the four types of aCL kits were all ranging from 2 to 400RU/mL, and the relative coefficients were all above 0.99. In the precision testing of the four types of aCL kits, the CV% of intra-assay and inter-assay were all below 10%. Bilirubin, hemoglobin, and lipid, RF and HAMA proved to be no influence on the detection of aCL-IgG, aCL-IgA or aCL-IgM in serum. In the comparison with ELISA kits (EUROIMMUN or AESKULISA ELISA kits), the negative agreement of cardiolipin IgG kit, cardiolipin IgA kit, cardiolipin IgM kit, and cardiolipin screening kit were all above 90%, and their positive agreements were 77.0% (57/74), 86.1% (31/36), 80.8% (42/52), 90.9% (110/121), respectively. As for clinical sensitivity and specificity,it indicated 52 positive aCL-IgG (59.1%), 33 positive aCL-IgM (37.5%), 25 positive aCL-IgA (28.4%)and 72 positive aCL-IgA/IgG/IgM (81.8%) of 88 APS samples. 11 positive aCL-IgG (10.8%), 9 positive aCL-IgM (8.8%), 7 positive aCL-IgA (6.9%)and 13 positive aCL-IgA/IgG/IgM (12.7%) of 102 SLE samples were also detected. And 3 positive aCL-IgG (2.9%), 4 positive aCL-IgM (3.8%), 2 positive aCL-IgA (1.9%)and 5 positive aCL-IgA/IgG/IgM (4.8%) of 105 RA samples were detected.It showed all negative in syphilis samples or normalhealthy samples by HOB BioCLIA® 4G aCL kits. **Conclusion:** Coupled with the fullyautomated BioCLIA® 1200 ImmunoAssay Analyzer, the HOB BioCLIA®4G aCL kitsshowed faster and more accurate results with extended working ranges and good reproducibility. It consists of 3 individual Assay (aCL-IgA, aCL-IgG, aCL-IgM) and a screening assay (aCL-IgA/IgG/IgM) with comparable results with ELISA for detection of aCL-IgA, aCL-IgG, aCL-IgM&aCL-IgA/IgG/IgM. These clinical trials indicate that HOB BioCLIA®4GaCL kits will provide better diagnosis and treatment for patients with APS.

## A-364

### A Novel Magnetic Bead Chemiluminescence Immunoassay (CLIA) for Quantitative Determination of Autoantibodies to Glutamic Acid Decarboxylase (GAD65) and IA-2 in Serum

C. Zhou, F. Sun, W. Wu, S. Cao, L. Liu, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** The type 1 diabetes mellitus (T1DM) is characterized by insufficient production of insulin by the pancreas being called autoimmune destruction, affecting mainly children, adolescents and young adults. The autoantibodies to pancreatic beta cell antigens are important markers of T1DM. Presently there are 5 major autoantibodies that are used to define risk for T1DM: GADA, IA-2A, ICA, IAA and ZnT8A. The novel BioCLIA® GAD and IA-2 kits coupling with the fully automated, random-access BioCLIA® 1200 system aimed to measure the GAD and IA-2 antibodies in serum. **Methods:** The analytical performances including dilution linearity, limit of detection (LOD), precision (intra-assay & inter-assay), and interference were evaluated by BioCLIA® GAD & IA-2 kits according to CLSI guidelines. 100 clinical samples with Euroimmun ELISA results were also tested by BioCLIA® GAD and IA-2 kits. Meanwhile, total of 148 disease confirmed clinical samples (from a major hospital in China) were tested for GAD and IA-2 autoantibodies. Out of these samples, 38 were from the newly diagnosed T1DM patients, 50 were T2DM and the remaining 60 samples were confirmed negative from diabetes. **Results:** The BioCLIA® GAD and IA-2 kits both performed outstanding dilution linearity ranging from 2-2000 and 5-4000 IU/mL, respectively. The LOD was 0.0683 IU/mL for GAD kit and 0.045 IU/mL for IA-2 kit. In the precision study, the intra-assay CV were 2.59% and 2.2% and inter-assay CV were 6.30% and 5.20% for GAD and IA-2 kits respectively. Furthermore, the two kits showed no significant influence by bilirubin (up to 40 mg/dL), hemoglobin (up to 500 mg/dL), lipid (up to 3000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL). Compared to the ELISA, we found the sensitivity of the GAD and IA-2 kits were 84.0% (42/50) and 90.0% (45/50) and the specificity were both high upto 98% (49/50), respectively. Furthermore, in the evaluation result from 148 clinical samples, the sensitivity of GAD kits was to be 71% (27/38) and IA-2 kits was 76.3% (29/38). Notably the sensitivity was improved to 92.1% (35/38) when we simultaneously use these two kits for the measurement of GADA and IA-2A in the T1DM patient serum. In the same time the specificity of GAD and IA-2 kits were both high upto 98.3% (59/60) and 98% (49/50), respectively. Similarly, the specificity were 96.7% (58/60) and 98% (49/50) when we simultaneously use these two kits to measure the 60 healthy samples and 50 T2DM samples.

**Conclusion:** The BioCLIA® GAD and IA-2 kits exhibit fast and accurate analysis with an extended dynamic range as well as good reproducibility. It could serve as a promising and environmental-friendly alternative for IFA and ELISA assays in the detection of T1DM autoantibodies and would be a well-deserved choice in the diagnosis of T1DM.

## A-365

### Development and Validation of an IgE Captaure ELISA for Allergen-Specific IgE Antibody Detection on URANUS AE Analyzer System

Y. Li, J. Shi, H. Guo, Y. Gong, R. Wu, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** The detection of Allergen-specific IgE in vitro diagnostic (IVD) has better safety than the skin pick test and the result will not be affected by skin condition and medication history. HOB Allergen-specific IgE antibody detection on URANUS AE analyzer system which can run eight microtiter plates simultaneously is intended for the quantitative determination of sIgE in human serum using IgE capture enzyme-linked immunosorbent assay (ELISA) and Biotin-Avidin System. With automated and excellent throughput feature, URANUS AE analyzer system provides a great value for hospitals with large samples to be tested. In this assay, total IgE from patient sample is captured by anti-human IgE coated to the microtiter plate. After washing, the biotinylated allergen is added and incubated in the wells. Excess biotinylated allergen is removed by the wash step and streptavidin labeled peroxidase is added into the microwells to form the complexes consisting of specific IgE/ biotinylated allergen/

streptavidin conjugate. Blue color is developed by the addition of TMB substrate that reacts with the coupled peroxidase. After terminating the reaction,the optica density (OD) of the colured product is measured by spectrophotometrically at 450nm under refence wave length of 620nm.The quantitative determination of IgE antibodies in serum is detected.

**Methods:** In this study, the analytical performance of Allergen-specific IgE to six inhalant allergens including D1 (Dermatophagoides pteronyssinus), D2 (Dermatophagoides farina), H1(House dust ), T12(Willow), E1 (Cat epithelium), M6 (Alternaria alternate) and six food allergens including F2 (Milk), F3(Codfish), F4 (Wheat), F27(Beef), F88(Lamb), F245 (Egg) was evaluated including limit of detection (LoD), precision, sensitivity, linearity-range according to the CLSI guideline. Total of 3612 clinical samples collected from three major hospitals in China were evaluated by both of HOB Allergen-specific IgE system and ImmunoCAP.

**Results:** The CV%s of D1, D2, H1, T12, E1, M6, F2, F3, F4, F27, F28 and F245 were in a range from 3.17~ 7.17% for within-run, and4.53~8.64% for total-run. All LoDs were < 0. 15 kU/L. The linearity range was from 0.35 kU/L~90 kU/L. Bilirubin (up to 20 mg/dL), hemoglobin (up to 40 mg/dL), and lipid (up to 2000 mg/dL) did not affect the IgE qualitative detection in serum. It showed no cross-reactivity with other human classes of immunoglobulins (IgG, IgD, IgA and IgM) at physiological concentrations. Total of 3612 clinical samples collected from three major hospitals in China were compared between Phadia ImmunoCAP and HOB Allergen-Specific IgE Kits at the cut-off of 0.35 kU/L. The sensitivity, specificity and total concordance for the twelve allergens was 89.29% (1176/1317), 96.64% (2218/2295) and 93.96%(3394/3612), respectively. **Conclusion:** With a good precision and excellent agreements with ImmunoCAP, HOB Allergen-specific IgE antibody detection kits on URANUS AE analyzer system provides the fast & accurate detection in vitro diagnostic (IVD) for treatment of allergy patients.It runs eight microtiter plates simultaneously and can get 768 results within 4 hours. Now, our product has been accepted by Class A tertiary hospitals in China, the one of which having four instruments in the lab for the Allergen-specific IgE antibody detection and above 3000 tests are performed per day.

## A-366

### Performance Evaluation of Der p1 and Der p2 specific IgE in Chinese House Dust Mite Allergy Patients on the HOB BioCLIA® Analyzer

L. Qian, X. He, P. Qian, P. Qian, Y. Wang, Y. Liu, G. Hu, Y. Tao, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** The incidence of allergic diseases steadily increases year after year. Component-Resolved Diagnostics (CRD) utilize purified native or recombinant allergens to detect sensitization of patients to individual allergen molecules and have become of growing importance in clinical investigation of IgE-mediated allergies. The CRD could decrease the need for provocation testing and may also improve the specificity of allergen-specific immunotherapy. House dust mites (HDM) are among the most important allergen sources in the world.*Dermatophagoides pteronyssinus* and *Dermatophagpides farina* are the primary house dust mite species, while Der p 1 and Der p 2 represent the *D pteronyssinus* allergens with greatest clinical significance.

**Methods:** In this study, the analytical performance of BioCLIA® sIgE-Der p 1 and Der p 2Assays were evaluated on the fully automated, random-access BioCLIA® chemiluminescence analyzer, according to the CLSI I/LA20-A2 guideline. A total of 100 serum samples were collected from HDM allergy patients at local hospitals in China. Specific IgE to Der p 1 and Der p 2 were evaluated by BioCLIA® Allergy sIgE Assays (HOB Biotech, China) and ImmunoCAP (Phadia AB, Sweden).

**Results:** The BioCLIA® sIgE -Der p 1 and Der p 2 Assays showed exclusive reactivity to human IgE and performed with excellent linearity ranging from 0.1-100 IU/mL. The LoD of BioCLIA® sIgE - Der p 1 and Der p 2 Assays were 0.05 and 0.06 IU/mL. Good precision were observed with the intra-assay CV 3.1% and 2.2%, the inter-assay CV 6.2% and 5.6% for Der p 1 and Der p 2. Spiking recovery test showed the accuracy of Der p1 and Der p were 100.2%-103.8%, 97.9%-99.8%. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of the specific IgE in serum. Among all of the 100 HDM allergy patients, the sensitivity of HOB BioCLIA® Der p 1- and Der p 2performed against D1 samples were 69.8% and 72.3 %, respectively. HOB BioCLIA® Der p 1- and Der p 2-sIgE Assaysalso agree well with Phadia ImmunoCAP Der p1- and Der p 2-sIgE Assays, with positive agreements at95.2% and 97.2%, total agreementsat 96.8% and 98.4%, respectively. Regression analysis between HOB BioCLIA®and ImmunoCAP assays, the Der p1 was Y=0.7466X+3.6787, $R^2$=0.7082, and Der p 2 was Y=0.6405X+3.9165, $R^2$=0.6422.

**Conclusion:**

The innovative HOB BioCLIA® systemoffers well performing allergen-sIgE assays, including Der p 1- and Der p 2-sIgE assays on BioCLIA®, that are linear, precise, repeatable, reproducible,with extended dynamic ranges. Der p 1 and Der p 2 are the dominant allergens that elicitallergy in HDM allergic patients. BioCLIA®Der p 1- and Der p 2-sIgE Assays provide a promising diagnostic tool in the diagnosis of *D. pteronyssinus* sensitization and guidance for allergen-specific immunotherapy in HDM allergic patients.

## A-367

### Analytical and Clinical Performance of the Anti-TPO and Anti-TG Kits on the BioCLIA®1200 Automated Chemiluminescence Immunoassay Analyzer

Y. Li, S. Xu, C. Zhou, J. Gao, R. Wu, C. Lee. *HOB Biotech Group, Suzhou, China*

**Background:** The major thyroid autoimmune diseases are theHashimoto's thyroiditis and Graves' disease.Both thyroperoxidase (TPO) and Thyroglobulin (TG) are potentially autoantigenic. The anti-TPO is detected in most of Hashimoto's thyroiditis and Graves' disease samples. High levels antibodies of Anti-TPO and anti-TG, in the context of the clinical presentation of hypothyroidism deliver a much greater value to diagnosis of Hasimoto's disease. The BioCLIA®anti-TPO and anti-TG kits are designed for the specific, quantitative detection of anti-TPO or anti-TG in serum. BioCLIA®1200 chemiluminescent immunosassay system is a random-access, high-throughput and continuous automated platform.**Methods:** In our study, the analytical performances of BioCLIA® anti-TPO and anti-TG kits including the limit of detection(LOD), intra-assay,inter-assayand accuracystudies were evaluatedaccording to CLSI guidelines. Total of 625 clinical samplescollected from major Chinese hospitals were analyzed and compared with Beckman Coulter DxI 800 assays. The discordant samples were re-analyzed by the same kits from Roche Cobas® and Abbott Architect® Systems. **Results:** The LOD of anti-TPO & anti-TG were 0.037 IU/mL and 0.305 IU/mL. The linear range of the anti-TPO & anti-TG wereestablished as 0.25-1000 IU/mLand 1.5-2500 IU/mL. Assay precision studies demonstrated acceptable CV% of <5.1% &<8.9% for intra-assay and inter-assay for anti-TPO and anti-TG. The anti-TPO assay was traceable to the WHO International Standard; NIBSC Code: 66/387, while the anti-TG assay was traceable to the WHO International Standard; NIBSC Code: 65/093.Total of 625clinical samples were compared between Beckman Coulter DXI-800 assays and HOB BioCLIA® assays. The regression analysis for anti-TPO with Passing-Bablok regression fit of HOB BioCLIA = 0.9982 Beckman Coulter DXI800 +2.72 (r=0.994).The positive agreement for anti-TPO and anti-TG were 97.6% (359/368) and 98.1% (362/369),the negative agreement for anti-TPO and anti-TG were 99.2%(255/257) and 99.6%(255/256). The total agreement for anti-TPO and anti-TG were 98.2% (614/625) and 98.7% (617/625). The discordant samples were confirmed by Roche's Cobas® and Abbott's Architect® systems to resolve their differences.**Conclusion:** The BioCLIA ®anti-TPO & anti-TG kits perform an extended working range and good precision &reproducibility. Excellent agreements were observed between HOB BioCLIA®anti-TPO & anti-TG and Beckman Coulter DXI-800 assays. The HOB BioCLIA ®anti-TPO & anti-TG kits provide the fast & accurate detection of anti-TPO & anti-TG in serum and can be used as valuable aid in diagnostic process, treatment of autoimmune thyroid diseases.

## A-368

### Autoantibodies against CD74 - Associated with severe and fast progressive disease in Spondyloarthritis (SpA)

T. Matthias[1], E. Schweikhard[1], S. Reuter[2], M. Köhler[3], J. Georgi[3], N. Baerlecken[4], T. Witte[4]. *[1]AESKU. Diagnostics GmbH&Co KG, Wendelsheim, Germany, [2]AESKU. KIPP Institute, Wendelsheim, Germany, [3]Helios Ostseeklinik Damp, Damp, Germany, [4]Medical University Hannover, Hannover, Germany*

**Background:** The pathogenesis of axial spondyloarthritis (axSpA) including ankylosing spondylitis (AS) is still largely unclear. Establishing the diagnosis is difficult, since abnormalities in X-ray develop with a latency of several years and only HLA-B27 or radiographic sacroiliitis is used as a laboratory marker yet. To prevent destructive effects early diagnosis in SpA patients is indispensable. Therefore, further evaluation of the latest presented new diagnostic marker, antibodies to the HLA class II- antigen associated invariant chain (CD74), is necessary.

**Methods:** A total of 320 clinically defined sera from patients with different diseases and normal controls have been tested to investigate the clinical cross reactivity of *AESKULISA®* SpA detect. All donors provided informed consent for the study, which was approved by the local ethics committee (project number 4928).

**Results:** SpA patients were more often male and younger. HLA-B27 status was available in 109 patients. Anti-CD74- antibodies were detected in 91 % of SpA but in only 4 % of healthy controls. In other autoimmune diseases like Celiac Disease, Collagenosis, Vasculitis, Scleroderma, Polymyositis and mixed connective tissue diseases the anti-CD74- antibodies were detected in 7.7%, 0%, 10.5%, 6.3%, 0% and 12.5% of the patients, respectively ($p \leq 0.0001$). IgA autoantibodies against CD74 had a sensitivity of 91 % ($CI_{95\%}$; 84.2% - 95.3%) and a specificity of 97 %, ($CI_{95\%}$; 91.7% - 99.1%) and were even more frequent in SpA patients receiving TNF therapy (Pearson's chi-squared test ($\chi^2$), p=0.002).

**Conclusion:** Anti-CD74 IgA antibodies were strongly associated with SpA and can be used as a new marker. Moreover, they were found to be strongly associated in patients receiving TNF-inhibitor therapy, which is more frequent in patients with a more severe and fast progressive disease.

## A-369

### Measurement Limits on BioTek ELx800 ELISA Plate Reader for Biomarker Analysis

S. Narla[1], I. Patzer[1], J. Bailey[1], J. Emerson[1], R. Philippe[2], D. Cavard[2], F. Martine[2], P. Oefinger[1]. *[1]Covance Central Laboratories, Indianapolis, IN, [2]Covance Central Laboratories, Geneva, Switzerland*

**Background:** The BioTek ELx800 is a single-channel reader-assay system, designed to automatically perform endpoint analysis for ELISA-based applications. The current acceptance criteria for Optical Density (OD) is up to 2.75 at 450 nm which was determined by performing an absorbance plate test, but the readers are capable of reading up to OD 4.00. This test uses BioTek's Absorbance Test Plate (Part: 7260522, BioTeK) to confirm the Mechanical Alignment, Accuracy, Linearity, and Repeatability of the ELx800 at 450 nm. The Absorbance Plate Test compares the reader's optical density measurements and mechanical alignment to NIST-traceable values. However, often the OD's encountered are higher than 2.75 for many newly developed biomarker ELISA kits, leading to more failed runs and/or increasing more repeat runs with sample dilutions.

**Methods:** The linearity and % CV are considered as acceptable assessments of accuracy. A liquid test using a dye solution was performed to evaluate the accuracy of the plate reader at OD > 2.75. To perform the liquid test at 450 nm, a stock liquid test solution (QC Check #1, PN 7120782, BioTek Instruments, Inc.) was diluted to obtain an OD reading as close as possible to 4.00. This solution was taken as 100% solution and was diluted in 5% increments up to 5% with DI water. These samples (200µL volume) were added to an ELISA plate in duplicate wells; whereupon the plate was read at 450 nm for 5 times to assess repeatability. The mean OD of duplicate wells was calculated for each dilution. Regression analysis was performed to identify the OD at which the results are linear. An $R^2$ value greater $\geq 0.990$ is considered acceptable for linearity; while the %CV $\leq 1.5\%$ is considered acceptable for repeatability. Reading of the experimental plate was repeated on three different plate readers across our two laboratory institutions.

**Results:** All readers yielded linear results with ODs $\geq 3.30$ for a linear and log-log linear curve fit with an $R^2$ >0.99. Additionally, a 4-parametic logistic (4-PL), 5-parametic logistic (5-PL), spline and polynomial$^2$ curves were assessed by the ELx800 software (Gen 5 Secure 2.00.18) on one reader at each laboratory. The readers yielded linear results with ODs $\geq 3.50$ for all these curves with R2 of >0.99. Also, all readers yielded acceptable repeatability with %CV $\leq 1.5\%$.

**Conclusion:** At an absorbance of 450 nm, ELx800 plate readers yielded linear and accurate results with OD of 3.30 for linear and log-log linear curves and up to 3.50 OD for 4PL, 5PL, spline and polynomial$^2$ curves. Therefore, the achieved ODs can be used as upper limit for the respective curves. This extension of ODs is beneficial by reducing failed and/or repeat runs, thus saving samples, labor time and expenses.

## A-370

### Validation of EUROIMMUN ELISA Kit for Neurogranin TruncP75 Measurement in Cerebrospinal Fluid (CSF)

S. Narla[1], J. Emerson[1], T. Allen[1], P. Oefinger[1], H. Vanderstichele[2]. *[1]Covance Central Laboratories, Indianapolis, IN, [2]ADx NeuroSceinces, Gent, Belgium*

**Background:** Alzheimer's disease (AD) is the most common neurodegenerative disorder and is a major health problem among older people. AD can be characterized by pathological hallmarks such as Amyloid-β containing neuritic plaques and neurofibrillary tangles composed of hyper-phosphorylated tau proteins in CSF. Synaptic dysfunction and loss are directly linked to memory disturbances and other cognitive symptoms that are present at the early stages of AD. The synaptic loss occurs early and it correlates with cognitive deficits in patients with AD. Therefore, the measurement of synapse proteins in CSF could be useful for studies of disease mechanism, to improve tools for early diagnosis and prognosis, and to monitor drug effects on synaptic degeneration in clinical trials of disease modifying therapies for AD. The postsynaptic protein Neurogranin in CSF has been reported to increase in AD, including pre-dementia stage of disease, making it a promising CSF biomarker.

**Methods:** The EUROIMMUN ELISA test kit (EQ6551-9601-L) is designed for the quantitative determination of Neurogranin truncated at P75 in CSF samples. In the first analysis step, samples are incubated with biotinylated monoclonal anti-Neurogranin antibody, followed by addition to microplate wells coated with monoclonal antibodies specific for human Neurogranin truncated at P75. In this process, truncated Neurogranin is bound in a complex. In a second incubation, the biotin binds to streptavidin peroxidase conjugate. Incubation of the complex with substrate and chromogen promotes a color reaction. The color intensity is proportional to the truncated Neurogranin concentration in the sample.

**Results:** Linearity was established by using CSF spiked with 8 concentration levels of Neurogranin, each level run 3 times. The method was linear up to 1000.0 pg/mL. The Lower Limit of Quantification (LLOQ) was established using CSF spiked with 5 concentration levels of Neurogranin tested in replicates of 40 over 5 days, LLOQ was set at 50.0 pg/mL with a %CV of 17.0. The within-run precision was performed using 2 levels of QC that were run 20 times on single plate; the average %CV is $\leq 5\%$. For between-run precision the 2 levels of QC were run over 10 different runs over 10 days, the average CV is $\leq 6.6\%$. Accuracy was accessed based on the recovery of CSF spiked with 6 concentration levels of Neurogranin, with an average recovery of 94%. The maximum manual dilution was verified up to 1:8 with a recovery of 97% at 1:8 times dilution, extending the upper limit of quantification (ULOQ) to 8000.0 pg/mL. To analyze the Length of Run, 2 kit QC samples were assayed across the plate over three runs, yielded no significant difference observed with results generated across the plate.

**Conclusion:** The EUROIMMUN Neurogranin ELISA kit was successfully validated as per the CLSI guidelines. The assay kit is suitable to perform testing on CSF samples to assess the concentration of Neurogranin.

## A-371

### Determination of Infliximab Drug Levels by the ALPCO Infliximab Drug Level ELISA and Method Comparison to LC/MS-MS

B. Bell[1], R. Pollok[1], M. A. Willrich[2], M. Snyder[2], L. A. Brunelle[1]. *[1]ALPCO, Salem, NH, [2]Mayo Clinic, Rochester, MN*

**Background:** Infliximab is a biologic therapy widely used to treat inflammatory bowel disease (IBD). Efficacy of this therapy is highly dependent upon maintaining the appropriate levels of drug between treatment sessions. Several infliximab immunoassays exist which allow physicians to monitor drug level status in their patients and tailor treatment regimens accordingly, however method standardization across the industry has not been established to date. This study is aimed to investigate method comparability of the ALPCO® Infliximab Drug Level ELISA to tandem mass spectrometry (LC/MS-MS).

**Methods:** Serum samples (n=30) from patients undergoing infliximab treatment were classified as containing low (n=10), medium (n=10) or high (n=10) levels of infliximab determined using an LC/MS-MS method (API 5000, AB SCIEX) at the Mayo Clinic, Rochester, MN. Frozen aliquots (-80°C) were blinded then subsequently analyzed by the ALPCO Infliximab Drug Level ELISA according to the instructions for use. Experiments were performed manually for the ALPCO assay; results were read on the VERSAmax plate reader (Molecular Devices) and data analysis was performed using SoftMax® Pro GxP software. For the ALPCO assay, intra- (n=24) and inter-assay (n=10) precision was performed according to CLSI Guidelines. Limit of quantitation (LOQ) of the ALPCO assay was determined according to CLSI Guideline EP17-A2 with a specified accuracy goal of a coefficient of variation (CV) less than or equal to 20%. Dilutional linearity was also assessed.

**Results:** A quantitative comparison between the ALPCO assay and the LC/MS-MS assay focused on values in the clinically relevant range (up to 20 µg/mL) with values below the LOQ (ALPCO = 0.7 µg/mL and LC-MS/MS = 1.0 µg/mL) normalized to their respective absolute LOQ values. The comparison demonstrated a good correlation between the two methods with a slope of 0.81 and $R^2$ of 0.947. The ALPCO assay demonstrated precision values with CVs less than or equal to 11% and dilutional linearity studies showed a slope of 1.00 and $R^2$ of 0.989.

**Conclusion:** This study demonstrates that the ALPCO immunoassay, currently available in this field, provides a reliable means by which to quantitate serum levels

of infliximab as an alternative to mass spectrometry. Although some level of bias exists between the ELISA when compared to an established LC/MS-MS method, the clinical information provided by both methodologies are similar. While these methods provide comparable results, industry wide standardization would benefit all methods in the field.

## A-372

### Assessing the Laboratory Protocol for the Hevylite® Assay on a Multiple Myeloma Patient Cohort

S. M. Kenyon, M. C. Kohlhagen, L. Tostrud, M. R. Snyder, J. A. Katzmann, D. L. Murray, M. A. Willrich. *Mayo Clinic, Rochester, MN*

Background: The Hevylite® assay (HLC) quantifies intact immunoglobulin (Ig) heavy and light chain pairs and is complementary to serum protein electrophoresis (SPEP) for monitoring patients with multiple myeloma (MM). An abnormal Igκ/Igλ ratio suggests a monoclonal immunoglobulin. The HLC assay may overcome some of the known limitations of SPEP, including difficulty detecting monoclonal proteins co-migrating with other serum components. At our institution, HLC is performed in conjunction with nephelometric total Ig measurements to evaluate antigen excess. An expected recovery (sum of Igκ + Igλ to total Ig) of 80-120% was established, analogous to the one used for IgG subclasses measurement. However, empirical observations suggest HLC recoveries from MM patient samples often fall outside this range, resulting in additional dilution steps/repeats to confirm results.

Objective: The objective of this study was to assess the laboratory protocol for measuring HLC that compares percent recoveries between HLC and total IgG measurements on a MM patient cohort.

Methods: Diagnostic serum samples from patients with untreated IgGκ (n=241), IgGλ (n=124), IgAκ (n=99), and IgAλ (n=54) MM were analyzed for SPEP (Helena, Beaumont, TX), immunofixation (Sebia, Lisses, FR), and total Ig (Siemens, Munich, DE) and HLC (Binding Site, Birmingham, UK) on the BNII System (Siemens). Involved HLC (iHLC) concentrations and Igκ/Igλ ratios were calculated. The clinical sensitivity of HLC, as determined by elevated Igκ/Igλ ratios for IgGκ and IgAκ and low Igκ/Igλ for IgGλ and IgAλ, was reviewed for each HLC pair. Percent recoveries (median and range) and the number of measurements outside the 80-120% recovery range were calculated. For all samples outside the recovery range, iHLC concentrations and Igκ/Igλ ratios were assessed to determine if they were outside the published reference intervals. Potential antigen excess misses were defined as <50% recovery, combined with assessment of Igκ/Igλ ratio and iHLC.

Results: Of the 518 MM patient samples, 509 (98%) had a measurable M-protein on SPEP. The clinical sensitivity was determined to be 97% for IgGκ, 98% for IgGλ, 97% for IgAκ, and 96% for IgAλ, with 503 samples (97%) having an abnormal Igκ/Igλ ratio, and 494 samples with elevated iHLC concentrations. The median recovery was 82% for IgGκ (range: 35-136%), 80% for IgGλ (50-140%), 130% for IgAκ (4-246%), and 110% for IgAλ (41-164%). A total of 254 measurements (49%) were outside the 80-120% recovery range (104 IgGκ, 64 IgGλ, 67 IgAκ, 19 IgAλ). 170 measurements were assessed for antigen excess misses, with only one, the IgAκ measurement with 4% recovery, showing evidence of true antigen excess. For measurements with recoveries >120% (84), all samples had elevated iHLC concentrations and/or abnormal Igκ/Igλ ratios.

Conclusions: Of the 518 MM samples analyzed, nearly half fell outside the 80-120% recovery range, resulting in additional dilutions and replicate measurements. Despite repeating 254 measurements, only one demonstrated evidence of antigen excess miss, indicating repeating all measurements outside the 80-120% recovery range is not justified for HLC. While discontinuing the practice of %recovery comparison entirely seems daring, an option to improve lab workflow could include only repeating measurements that recover <10%, which would reduce the repeats to 2%.

## A-373

### Validation of a Non-FDA Approved Specific IgE Allergen (RF345 Macadamia Nut) by Fluoroenzymeimmunoassay.

J. C. Harrington, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Quantitative detection of circulating IgE antibodies can be used to evaluate patient allergies and determine sensitization to a specific allergen. There is a direct relationship between the concentration of IgE antibodies and the probability of allergic symptoms which include itchy eyes, eczema, rhinitis, bronchoconstriction, vomiting, diarrhea, and anaphylaxis. Here we report the performance characterization of an allergy assay for macadamia nut by fluoroenzymeimmunoassay.

Method: Validation testing was performed on the Phadia Thermo Scientific ImmunoCAP 1000 (Phadia US Inc., Portage, MI, USA) using leftover patient serum samples in our laboratory. The performance validation included linearity, precision, method comparison, interference, sample stability, and reference range. The data was analyzed using EP Evaluator Version 10 (Data Innovations LLC, Burlington, VT, USA).

Results: Assay linearity was assessed by serial diluting a high patient specimen with the sample diluent included in the reagent package and assaying the resulting specimens in triplicate. The validated linear range was 0.35-100.00 kUA/L with a maximum dilution factor of 200. Within-day precision was evaluated by assaying a low patient pool and a high patient pool 10 times in a batch, and was found to be 4.5% and 4.6%, respectively. The between-day precision was assessed by analyzing the same pools twice a day for 10 days, and found to be 5.6% CV (low pool) and 5.3% CV (high pool). The method was compared to a previously vetted Phadia Thermo Scientific ImmunoCAP 1000 using leftover patient specimens (n=40). The Deming regression showed an R of 0.9999, an intercept of 0.034, a slope of 1.040, and a mean difference of 4.4%. There was no significant interference by lipemia, hemolysis, icterus, and uremia. The analyte was found to be stable in serum for 48 hours ambient (18-32°C), 7 days refrigerated (2-8°C), and 14 days frozen (-20°C). The reference range was verified at <0.35 kUA/L using 43 patient samples with normal total IgE and no indication of macadamia nut in the patient charts.

Conclusion: The method performance characteristics of the non-FDA approved specific IgE allergen RF345 Macadamia Nut assay were consistent with Phadia specific IgE allergen assays that are approved for in vitro diagnostics by FDA.

## A-374

### Performance Evaluation of the ADVIA Centaur aCCP Assay

G. Campbell[1], E. Bull[1], D. Hovanec-Burns[2]. [1]*Axis-Shield Diagnostics, Dundee, United Kingdom,* [2]*Siemens Healthcare, Tarrytown, NY*

**Background:** Anti-cyclic citrullinated peptide IgG (aCCP) is a specific marker for rheumatoid arthritis (RA), a disease that affects 1% of the population worldwide. Studies show that the presence of anti-CCP IgG can be detected in the early stages of RA and is indicative of a more-progressive form of the disease. Measurement of aCCP, in conjunction with other laboratory findings and clinical assessments, may be used as an aid in the diagnosis of RA. Autoantibody levels represent one parameter in a multi-criteria diagnostic process encompassing both clinical and laboratory-based assessments. The ADVIA Centaur® aCCP assay (Siemens Healthcare Diagnostics Inc.) is intended for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide in human serum or plasma (K2-EDTA and lithium heparin) using the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate assay performance for precision, linearity, limit of detection (LoD), interference and cross-reactivity, and method comparison to the ARCHITECT anti-CCP assay (Abbott).

**Method:** The ADVIA Centaur aCCP assay is a fully automated, two-step immunoassay using chemiluminescent technology. The assay utilizes an acridinium ester-labelled anti-human IgG as the Lite Reagent. The solid phase consists of biotinylated CCP coupled to streptavidin, which is then coated onto magnetic latex microparticles. The method comparison study was performed per CLSI EP-09-A3 using 253 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A2. Linearity and LoD studies followed CLSI EP17-A2. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, total protein, rheumatoid factor, lipemia, and caprine IgG. Also per CLSI EP07-A2, the assay was tested for cross-reactivity on 22 subgroups of non-RA subjects (n = 460) with potentially cross-reacting conditions and disease states where other autoantibodies may be present in the subject samples.

**Results:** The ADVIA Centaur aCCP assay measured anti-CCP IgG concentrations from 0.40 to 200.00 U/mL, with LoD determined to be 0.40 U/mL. The assay was determined to be linear from 0.40 to 200.00 U/mL. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 3.0–4.3%. The diagnostic concordance between the ADVIA Centaur aCCP and Abbott ARCHITECT anti-CCP assays was 96.84% (confidence interval 93.89–98.39%). The assay demonstrated no sample-tube type bias and no significant interference with the tested analytes or cross-reactivity with potentially cross-reacting conditions.

**Conclusions:** The results of these studies show good performance of the fully automated ADVIA Centaur aCCP assay and good agreement with the ARCHITECT anti-CCP assay.

## A-375

### Importance of Sealing the Samples during Incubation for an Aspergillus Galactomannan Antigen Assay

L. C. Reed, A. McShane, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: The galactomannan antigen is released in the circulation during active invasive aspergillosis. The Platelia Aspergillus Antigen assay is an FDA-cleared one-stage immunoenzymatic sandwich microplate assay which detects galactomannan in human serum or bronchoalveolar lavage (BAL) fluid (Bio-Rad Laboratories in Hercules, California). The test takes four major steps: 1) Serum or BAL fluid samples are heat-treated in the presence of EDTA to dissociate immune complexes and to precipitate proteins that could interfere with the test; 2) The treated samples and conjugate reagent are added to the microplate wells coated with monoclonal antibodies, and incubated at 37°C for 90 min. A monoclonal antibody-galactomannan-monoclonal antibody/peroxidase complex is formed in the presence of galactomannan antigen; 3) After wash to remove any unbound material, the chromogen solution is added, which reacts with the complex enzyme to form a blue color product; 4) After stopping the reaction with addition of an acid reagent, the absorbance (optical density or O.D.) was measured by a spectrophotometer set at 450 and 620/630 nm. When we first brought up the assay using an automated ELISA platform, we had to incubate the plate at step 2 without having the samples sealed. It was noted that the Cut-off controls were continuously outside the acceptable O.D. range of ≥ 0.300 and ≤ 0.800 as indicated in the package insert. We hypothesized that incubation without a plate sealer on the automated ELISA platform allowed the fluid in the plate wells to evaporate and caused elevated control results and failure to meet testing validity criteria. The aim of this work was to revalidate the assay with plate wells sealed during the 90 min incubation (step 2).

Method: Validation testing was performed using leftover patient BAL and serum samples in the laboratory and specimens obtained from a reference laboratory. Precision, method comparison, stability, and reference interval studies were performed and O.D. was closely monitored.

Results: Within-day and between-day reproducibility produced a 100% concordance with QC materials intended for negative and positive results. Qualitative method comparison resulted in a 97.5% concordance for serum specimens and 100% for BAL. Reference interval studies using culture Aspergillus free samples verified an index cutoff of <0.50 for negative results. The O.D. of each cut-off control was consistently ≥ 0.300 and ≤ 0.800 for all re-validation experiments.

Conclusion: A plate sealer was proven an important requirement during incubation at 37 °C for 90 min. This newly validated assay was validated for patient testing.

## A-376

### Development of a Novel Carrier Protein for Polyclonal Antibody Production

J. L. Miller[1], B. Bucher[1], R. Harney[2], G. Lundell[1], R. C. Bayer[3], I. Baburina[2], G. F. Krug[1]. *[1]Lampire Biological Laboratories, Pipersville, PA, [2]Saladax Biomedical Inc., Bethlehem, PA, [3]Lobster Unlimited LLC, Brewer, ME*

**Background**: Carrier proteins are critical in the antibody production process as they confer immunogenicity to poorly immunogenic compounds such as small molecules or peptides. Hemocyanins are copper containing proteins used for oxygen transport in arthropods and mollusks and are routinely utilized as carrier molecules due to their size, potent immunogenicity, and phylogenic distance from mammalian hosts/antigens. The most commonly used carrier protein is keyhole limpet hemocyanin (KLH). However, current immunization protocols often require alternating carrier molecules to prevent immunodominance of the carrier to the detriment of the antibody response to the hapten. Thus, there is a need for an alternative highly immunogenic carrier protein. Lobster hemocyanin (LH), is readily available as a byproduct from the food industry. Here, we evaluate the utility of lobster hemocyanin as a carrier protein of hapten antigens in comparison with KLH. **Methods**: Hemocyanin was purified from American lobster (*Homarus americanus*) serum. Peptide and protein antigens were covalently conjugated to LH and KLH using MBS. New Zealand white rabbits (N = 3 per immunogen) were immunized with either the KLH-antigen conjugate or the LH-antigen conjugate. Serum was collected at designated time points following immunizations and antigen specific antibody titers were measured by indirect ELISA. Antibody responses directed against the carrier molecule (LH or KLH), as well as cross-reactivity of anti-LH and anti-KLH antibodies, were also measured via indirect ELISA. **Results**: Pre-existing antibodies against LH were undetectable by ELISA in sera isolated from naïve rabbits (N = 9). Immunization with both LH and KLH conjugated antigens resulted in the production of antigen specific antibodies. There was no significant difference in the antibody titers generated against the peptide or protein antigens conjugated to LH or KLH. Antibody titers generated against LH were consistently less than that generated against KLH, however these differences were not significant at all time points. Anti-LH antibodies did not cross react with KLH, however sera from one animal immunized with KLH had low cross-reactivity to LH (titer <100). **Conclusions**: LH is an immunogenic and effective carrier protein that promotes the generation of rabbit polyclonal antibodies to conjugated haptens as effectively as KLH. Anti-LH antibodies do not cross-react with KLH, signifying that the LH epitopes are unique from those on KLH. Thus, LH is novel carrier protein that can be used as an alternative to or in conjunction with KLH.

## A-377

### CSF Free Light Chain Identification of Demyelinating Disease: Overcoming Challenges of Oligoclonal Banding

K. Gurtner, D. Murray, E. Shosha, D. Barnidge, S. Pittock, M. V. Willrich. *Mayo Clinic, Rochester, MN*

**Background:** Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). Cerebrospinal fluid (CSF)-index and oligoclonal bands (OCBs) are common laboratory tests used in MS identification, employing isoelectric focusing (IEF) and nephelometry, each requiring paired CSF and serum. IEF is not only costly and labor-intensive, but also involves subjective interpretation and little consensus defining the amount of OCBs required for positivity. Our laboratory compared both free light chain (FLC) presence and various published calculations/indexes that suggest representation of humoral immune response, to determine a more sensitive, specific, and cost-efficient means of diagnosing MS.

**Methods:** 325 residual CSF and serum specimens were obtained after physician-ordered OCB testing (211 OCB negative, 114 OCB positive). Paired CSF and serum specimens were characterized using IEF on the SPIFE 3000 (Helena) platform, followed by band pattern interpretation requiring 4 or more CSF exclusive bands. FLC (Freelite® Human Kappa/Lambda, The Binding Site), albumin and total IgG (Siemens) were measured on paired specimens using a BNII nephelometer (Siemens). Calculations were performed based on combinations of analytes: CSF sum of Kappa and Lambda [(KFLC+LFLC)], kappa-index (K-index) [(CSF KFLC/serum KFLC)/(CSF albumin/serum albumin)], kappa intrathecal fraction (K-IF) {[(CSF KFLC/serum KFLC)-(0.9358 x CSF albumin/serum albumin)^0.6687)x serum KFLC)]/CSF KFLC)}, and CSF-index [(CSF IgG/CSF albumin)/(serum IgG/serum albumin)]. Positivity with each calculation/index was correlated to medical decision points after ROC curve analysis and chart-review. Precision, accuracy, measuring and reportable ranges, and carry-over studies for CSF KFLC were performed on an additional cohort of residual CSF specimens (n=360).

**Results:** Patients were categorized by clinical condition: Demyelination (n=67), Autoimmunity (n=53), Non-Inflammatory (n=50), Inflammation (n=38), Degeneration (n=28), Peripheral Neuropathy (n=24), Infection (n=13), Cancer (n=11), Neuromyelitis Optica (n=10), and Other (n=31). Each FLC measurement or calculation/index exhibited ≥90% agreement with OCB, with the exception of CSF LFLC and CSF-index, which only demonstrated 80 and 83% agreement, respectively. CSF KFLC measurement used alone exhibited 90% agreement with OCB, subsequently reducing the number of analytes measured and variables associated with calculations. When cases of demyelinating disease were reviewed, KFLC measurements showed 85% clinical sensitivity/69% specificity, whereas OCB exhibited 78% clinical sensitivity/76% specificity. BNII analytical imprecision studies produced an intra-assay precision of <10% (n=20) and an inter-assay precision of <20% (n=20), along with demonstrating the absence of carryover.

**Conclusion:** Results have shown that KFLC alone demonstrates comparable performance to OCB along with increased sensitivity for demyelinating diseases. Replacing OCB with KFLC for MS investigation would alleviate the need for serum IgG and albumin, CSF IgG and albumin, and calculated conversions. KFLC measurement would allow the laboratory to decrease technologist bench time from 4 hours to 20 min/specimen, creating an automated set-up with reduced turnaround time, subsequently reducing the overall testing-related costs by 75%. CSF KFLC can overcome challenges associated with performance, interpretation, and cost of traditional CSF diagnostics, in turn, reducing costs to the patient while maintaining sensitivity and specificity supporting MS diagnosis.

## A-378

### Detection of antiphosphatidylethanolamine among cardiovascular patients with ELISA using synthetic antigens

Y. Zhang[1], S. Hou[2], X. Wang[1], G. Chen[1], Z. Zhou[1], M. Zhao[2]. *[1]National Center for Cardiovascular Diseases & Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, [2]Northwestern University, Chicago, IL*

**Background:** Antiphosphatidylethanolamine (aPE) is a form of "non-criteria"antiphospholipid syndrome (APS), where the presence of antiphospholipid antibodies has clinical symptoms consistent with APS but fall outside current standard tests. A major limiting factor in diagnosing aPE is a lack of standardized assays. This makes it a challenge for positively identifying patients and for investigating the prevalence, pathogenesis and impact of aPE. The goal of the current study was to determine the presence of aPE antibodies among cardiovascular patients with ELISA-based assays using synthetic phosphatidylethanolamine (PE) antigens.

**Methods:** A total of 400 continuous cardiovascular patients and 160 normal controls were enrolled. The presence of aPE antibodies, including cofactor-dependent IgG, IgM and IgA and cofactor-independent IgG and IgM, were examined using ELISA. The assays involved the use of synthetic PE species as antigens, including 18:1 PE for the detection of cofactor-dependent aPE isotypes and 20:4 PE as a major component for detecting cofactor-independent aPE isotypes. The lipid antigens were coated onto microtiter plates. The wells were rehydrated and blocked with BSA. Human plasma samples were added to the plates in duplicates at 1:100 dilution. Visualization was achieved using alkaline phosphate-conjugated anti-human immunoglobulin antibodies which were isotype-specific, and then a colorimetric reaction with p-nitrophenyl phosphate. The outcome of the aPE assays, based on a threshold of control mean plus 6 standard deviations, were used to calculate the percentage of aPE positive cases among cardiovascular patients, and the association with different clinical diagnoses and symptoms. **Validation:** The outcome of the aPE assays was compared with ELISA tests using conditions reported in the literature involving natural (egg yolk) PE as antigen. The remaining ELISA protocols were identical as described above. The percentages of aPE-positive cases and the coefficients of correlation between the two sets of aPE assays were determined. **Results:** Different PE species gave rise to distinct physical properties, which allowed the differentiation among sub-types of aPE antibodies with specificity. The use of synthetic PE species led to the identification of cofactor-dependent or cofactor-independent aPE cases, whereas when egg PE was used as antigen such differences could not be clearly resolved. Notwithstanding these differences, the overall ELISA results between the current and prior methods were significantly correlated, with Pearson correlation coefficients being 0.80, 0.79, 0.93, 0.82 and 0.93 for cofactor-independent IgG and IgM, and cofactor-dependent IgG, IgM and IgA, respectively. The data indicated that there were significantly higher incidences of aPE among cardiovascular patients (6.75%) compared to controls (1.25%). Cofactor-dependent aPE cases accounted for the majority of aPE cases among cardiovascular patients (6.0%) versus controls (0.625%). Overlap was identified between aPE and lupus erythematous, anticardiolipin and anti-beta2 glycoprotein I. However, the majority of aPE cases (88.37%) were not associated with lupus, anticardiolipin or anti-beta2 glycoprotein I, suggesting aPE may be an independent etiological entity.

**Conclusion:** The present data demonstrated the feasibility of using synthetic lipid antigens for aPE ELISA, which minimizes uncertainties associated with natural antigens. The aPE antibodies has a significant presence among cardiovascular patients.

## A-379

### Evaluation of Customizable Allergen Mixes on a New Immunoassay Technology Platform

E. Sinson, S. Ortega, S. Neath, R. Hockins, D. Canfield, T. Reid, E. Whitters. *Hycor Biomedical, Garden Grove, CA*

**Background:** Multi-allergen tests (or allergen "mixes") are readily available and sold by various diagnostic manufacturers. Often these mixes comprise a group of similar allergens such as trees, grasses, or foods that will be tested together. However, "one-size-fits-all" pre-manufactured mixes may not be best for initial evaluation of allergic disease given regional variations in allergenic sensitization. We are developing a novel *in vitro* IgE testing platform that enables a clinician to design a custom mix of 3-6 allergens for each patient, providing flexibility across a menu of allergens.

**Methods:** The new system is a fully-automated chemiluminescent immunoassay platform to quantify specific IgE (sIgE) concentrations in human serum. The system utilizes magnetic microparticles to which allergens are coupled by a process called 'on-board kitting'. The assay then adds 4uL of serum to the coated beads to quantify sIgE concentrations for that allergen. By the same on-board kitting process, multiple allergens can be coupled to these beads to create a mix test. This study was designed to determine sensitivity and specificity of samples across individual allergens and multi-allergen mixes. Using 36 known low-reactivity samples (0.3 - 0.5 kU/L), sensitivity was calculated on a 6-allergen mix compared to the results of the same 6 individual allergens. Additionally, mono-sensitized samples were evaluated for 3-, 4-, 5-, and 6- allergen mixes to determine whether sensitivity is affected for larger mixes. 30 negative samples were evaluated across 6 allergens on both individual and multi-allergen tests.

**Results:** Sensitivity for a 6-allergen mix was calculated at 97.2% with 35/36 samples recovering positive. One sample reported a 0.37 kU/L sIgE concentration for Bermuda Grass when tested individually but negative in the mix test. Specificity for a 6-allergen mix was calculated to be 96.7% with 29/30 samples negative. Across 3-, 4-, 5-, and 6- allergen custom mixes, 9 mono-sensitized positive samples and 5 negative samples were tested. Positive sample recovery was consistent across each mix; however, in the 3-allergen mix, 1 sample tested negative. This sample reported 0.44 kU/L sIgE concentration for House Dust Mite when tested individually but was negative in the 3-allergen mix test. Other mono-sensitized samples with lower kU/L values did successfully report positive, indicating that the multi-allergen test can recover low sIgE concentrations. Sensitivity for 3-, 4-, 5-, and 6- allergens mixes was 89%, 100%, 100%, and 100%, respectively. Specificity for 5 negatives across each allergen mix number was 100%.

**Conclusion:** The ability for a clinician to select the most appropriate allergens that are geographically relevant and/or optimized based on the patients' medical history and physical work-up is a step toward personalized diagnostics. The study findings demonstrate that multi-allergen mixes up to 6-allergens have good sensitivity for a selection of low-reactive samples and across different mixes.

## A-380

### Characterization of B- and T-cell Immune Repertoires Using Anchored Multiplex PCR and Next-Generation Sequencing

J. Eberlein[1], T. Harrison[1], J. Simms[2], M. Wemmer[1], L. Griffin[1], B. P. Culver[1], L. Johnson[1], B. A. Kudlow[1]. *[1]ArcherDX, Boulder, CO, [2]Q2 Solutions, EA Genomics, Morrisville, NC*

**Introduction:** NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP™) uses molecular barcoded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single side. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clone dropout due to somatic hypermutation. Here, we describe AMP-based NGS assays for IR characterization, Immunoverse™ IGH and TCR, which utilize a minimal set of unidirectional GSPs and MBC adapters that reduce amplification bias.

**Methods:** The quantitative reproducibility and sensitivity of our assays was validated using mRNA isolated from PBMCs of healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.

**Results:** Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CDR3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.

**Conclusions:** AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

## A-381

### TSG-6 Neutralizing Monoclonal Antibodies (MAb) As A Potential Therapeutics For Asthma Treatment

N. Albtoush[1], A. Abbadi[1], S. Merkoulov[1], M. Lauer[1], T. Glant[2], K. Mikecz[2], V. Hascall[1]. *[1]Cleveland Clinic, Cleveland, OH, [2]Rush University Medical Center, Chicago, IL*

**Background:** Asthma, a long-term respiratory disease, is characterized by increased hyaluronan (HA) extracellular matrix (ECM) and infiltration of inflammatory cells. The HA ECM has been modified by formation of covalently bound heavy chain proteins (HCs) to HA. This modification is mediated by a unique enzyme called tumor necrosis factor-stimulated gene 6 (TSG-6) that is upregulated in inflammations. TSG-6 transfers HCs from inter-α-inhibitor (IαI), a serum proteoglycan with 2 HCs on the bikunin chondroitin chain, onto HA forming the HC-HA matrix. The resulted HC-HA matrix extensively recruits Th2 lymphocytes, eosinophils and macrophages that accumulate in the airway mucosa and submucosa, which increases asthmatic symptoms and disease progression. In contrast, a milder form of asthma with significantly less inflammation and less airway hyperactivity has been shown in our TSG-6 knockout mouse model that lacks the ability to form HC-HA matrix. Accordingly, we propose to lower the levels of HC-HA crosslinking by inhibiting the action of TSG-6 through a novel immunotherapy approach to manage and suppress the Th2 cell-mediated inflammatory response in asthmatic airways. **Methods:** We have tested a panel of murine TSG-6 monoclonal antibodies (mAb) referred to as NGs (NG1-NG7) in a TSG-6/HC transfer assay. Recombinant TSG-6 is mixed with HC donor IαI to form the TSG-6-HC intermediate. In the presence of HA14, a short HA oligosaccharide, TSG-6-HC irreversibly transfers the HC to HA14 to form HC-HA14 with MW of about 75 kDa. The absence of the HC-HA14 and the continued presence of IαI will indicate that TSG-6 HC transfer was inhibited by NG mAb. Additionally, time course studies were performed to determine reaction kinetics from 0 to 6 hours in the presence and absence of TSG-6:NG4 and TSG-6:NG5 at equimolar ratios. All samples were probed with the Dako IαI Ab and analyzed by Western blot. Moreover, the binding affinities of NGs to TSG-6 were measured by surface plasmon resonance (SPR) technique. **Results:** Our initial results show, a 75 kDa band for NG1, NG2, NG3, NG5, NG6 and NG7 indicating that they did not prevent HC transfer to HA14. In contrast, the band is not present for NG4 indicating that NG4 binding to TSG-6 is a very effective inhibitor for HC transfer. In a time course, the NG4 mAb showed nearly complete inhibition of HC-HA14 within the first 4 hours. The SPR results revealed a tight and almost irreversible binding of TSG-6 to NG4 with (Kd ~ 50 nM). NG5 bound to TSG-6 weakly (Kd ~300 nM) and was reversible. **Conclusion:** We identified NG4 as the most active neutralizing mAb that inhibits HC transfer to HA by TSG-6, which will be used in subsequent experiments to determine if it can prevent or greatly inhibit formation of a HC-HA matrix in asthmatic airway smooth muscle cell culture models.

## A-382

### Comparison of fecal lactoferrin and calprotectin as screening markers of inflammatory bowel disease

K. W. Cradic, M. Willrich, M. R. Snyder. *Mayo Clinic, Rochester, MN*

**Background:** Inflammatory response in the intestines results in activation of neutrophils, leading to release of the cytoplasmic granular proteins calprotectin and lactoferrin. Screening assays for these biomarkers in feces can be used to distinguish between inflammatory and non-inflammatory conditions. Previous reports suggest that lactoferrin and calprotectin perform equally as markers of intestinal inflammation. However, these studies used differentiated populations of patients with and without inflammatory diseases. These populations do not reflect the majority of patients who present with GI symptoms, making it necessary to evaluate the utility of lactoferrin and calprotectin as screening tests in a cohort of patients for whom disease status is uncertain. Therefore, the aim of this study was to determine the concordance of calprotectin and lactoferrin by various assays in a routine clinical setting.

**Methods:** Residual stool samples (n=33) were collected from specimens submitted for routine calprotectin testing. In addition to the clinical lab's calprotectin assay (Inova Diagnostics; San Diego, CA), specimens were tested using a second calprotectin ELISA and a point-of-care kit (both from Immundiagnostik; Bensheim, Germany). Each sample was also tested for lactoferrin using a commercially-available ELISA (ALPCO; Salem, NH). All testing was performed according to manufacturer's instructions. Results were compared qualitatively using 50 mcg/g as the cut-off for all calprotectin assays and 7 mcg/g as the cut-off for lactoferrin. Results between calprotectin assays were also compared quantitatively by linear regression.

**Results:** Qualitatively, the calprotectin ELISA and point-of-care device, from the same manufacturer, showed the highest agreement at 97%. The current clinical calprotectin assay demonstrated concordance with the second ELISA and the point-of-care device at 88% and 84%, respectively. However, quantitative analysis showed that the clinical assay correlated best with the second ELISA (slope = 3.3 [95% CI 2.6 - 5.9], intercept = -18.7 [95% CI -56.7 - 1.0], r = 0.761) and least with the point-of-care device (slope = 3.99 [95% CI 2.7 - 6.6], intercept = -29.9 [95% CI -61.3 - -8.5], r = 0.390). Correlation between the second ELISA and the point-of-care device was also calculated (slope = 1.07 [95% CI 0.5 - 1.7], intercept = -1.4 [95% CI -21.2 - 75.4], r = 0.604). Lastly, qualitative concordance of lactoferrin with the clinical calprotectin assay was 82%. Similar agreement with the second calprotectin ELISA and the point-of-care device, at 76% and 73% respectively, was observed. When compared to calprotectin, of the 6 discrepant results, 5 (83%) were negative for lactoferrin, despite being abnormal by all 3 calprotectin assays.

**Conclusion:** Various calprotectin assays, including a point-of-care device, showed qualitative concordances >80%. However, quantitative agreement was poor, suggesting that the assays do not have standardized calibrations and cannot be used interchangeably. Lactoferrin showed lower concordance with calprotectin, regardless of methodology. The majority of discordant samples were abnormal by calprotectin and negative for lactoferrin, suggesting that calprotectin is a more sensitive marker for identifying patients with a higher likelihood of active intestinal inflammation. For these reasons, calprotectin should be considered first for screening patients suspected of having IBD.

## A-383

### Evaluation of the impact of choosing wisely campaign recommendations on ANA and subserology testing in current practice

A. Muthukumar, A. Alambo, B. Crook. *UT Southwestern Medical Center, Dallas, TX*

**Background:** American college of Rheumatology recommended the following guidelines for ANA and subserology testing as a part of choosing wisely campaign: A stepwise approach to ANAS testing in which ANAS to be tested only if ANA is positive and there is clinical suspicion of immune disease. The exception to this rule when there is suspicion of myositis in which case anti-Jo-1 can be tested or occasionally, anti-SSA can be tested in the setting of lupus or Sjogren's syndrome. Since, ANA is a screening test and not for monitoring, serial measurement of ANA is not recommended, especially in those with an existing diagnosis of immune disease. Given this background, the purpose of the study was to review how frequently ANA and ANAS testing were ordered together and how many were repeats, especially those orders from rheumatologists. **Methods:** All patients from our institution who underwent ANA and ANAS testing from January 2011 to February, 2017 were evaluated through retrospective chart review. ANA and dsDNA testing were performed by indirect immunofluorescence assay. ENA panel testing (Smith, SSA, SSB, RNP, Scl-70, and Jo-1) and anti-centromere testing were performed by multiplex flow immunoassay. ANA and ANAS tests were available for routine ordering by all our physicians.

**Results:** A total of 25319 patients had about 26992 test orders for ANA during the study period. The mean age of the study population was 54 and the women to men ratio was 2:1. The majority of the test requests came from specialties like Neurology and Rheumatology. Year wise orders for ANA test remained almost the same throughout the study period (4393). A total of 2910 ANA test orders (11%) were repeats, with 2170 being repeated twice and 611 orders being tested thrice. 6 patients were tested more than 10 times during the study period. Total requests for dsDNA and ENA testing during the 6 year study period were 12323 and 9964 respectively. About 25% of test orders had either dsDNA or ENA ordered simultaneously with ANA and about 14% of test requests had all 3 tests ordered together. Nearly 3328 orders for ENA were placed in patients who had previously negative result for ANA (12%). Likewise, dsDNA orders were placed in 3055 encounters when ANA was negative (11%). Rheumatologists placed as many orders as non-specialists for dsDNA (15%) and ENA testing (13%) in patients with previous negative ANA results. The percentage requests from rheumatogists for simultaneous testing of ANA and ENA, ANA and dsDNA as well as all 3 tests together was more than the twice the requests from non-specialists. The number of repeat orders for ANA were significantly lower (<1%) for the rheumatologists group. **Conclusion:** ANA repeat orders as well as simultaneous orders for ANA and ANAS testing remain significantly high in our hospital. The percentage of simultaneous orders for ANA and ANAS testing was even substantially higher in the rheumatologists group. Further education and placing restrictions on ordering of these tests in the electronic medical record system may be the most effective way to reduce unnecessary test orders.

## A-384

### Determining the optimal antinuclear antibody screening strategy using a computer-aided immunofluorescence microscopy system

A. Chin, J. Buse. *Calgary Laboratory Services, Calgary, AB, Canada*

**Objective:** To determine the optimal antinuclear antibody screening strategy using a computer-aided immunofluorescence microscopy system. **Relevance:** Investigations of systemic autoimmune disease include measurement of autoantibodies with immunofluorescence microscopy. Recent advances in technology have allowed traditional manual microscopic methods to be automated and facilitated through computer-aided recognition of ANA patterns and prediction of ANA titers without extensive manual serial dilutions. Although most laboratories utilize a 1:80 screening dilution, it is unclear whether ANA screening strategies involve a 1:80 single screening titer, which require further titers on positive samples, or an initial full titer screen. A single screening titer run will allow for more samples to be processed but will require repeat analysis for the determination of the actual titer. Conversely, an initial full titer screen would allow the operator to promptly sign out cases and reduce the prozone effect associated with methods based on antigen-antibody interactions and avoid underestimation of laboratory results, however this requires more consumption of reagents **Methodology:** All initial screens were performed at 1:80 by computer-aided indirect immunofluorescence (Euroimmun EuroPattern). For the first part of the study, 738 patient serum samples were analyzed for autoantibodies by a full titer screen. For the second part of the study, 534 1:80 ANA positive patient samples were further titered to 1:640. Results were judged according to whether the decision to titer was correct when the titer changed from the initial 1:80 dilution or incorrect when the titer did not change from 1:80. **Results:** For the first part of the study, 8.1% of samples required further dilution and no prozone effect was detected. For the second part of the study, 54.1% of samples were correctly judged to be titered further whereby 6.1% would have been falsely low or 26.0% to be falsely high if the initial titer at 1:80 was only used. However, running a full titer screen consumed more reagents and medical laboratory technologist time. **Conclusions:** Given the small number of samples requiring further dilution and low incidence of the prozone effect, extensive screening titers are not needed. It is recommended that all positive ANA screens at 1:80 be further titered to reduce falsely low or high results.

## A-385

### Adipose Tissue-derived Mesenchymal Stem Cells Have A Heterogenic Cytokine Secretion Profile

Y. Wu[1], M. Hoogduijn[2], C. Baan[2], S. Korevaar[2], P. de Kuiper[2], L. Yan[1], L. Wang[1], N. van Besouw[2]. *[1]West China Hospital of SiChuan University, Chengdu, China, [2]Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands*

**Background:** Mesenchymal stem cells (MSC) derived from adipose tissue (ASC) have immune regulatory properties, which make them interesting candidates for cellular immune therapy. ASC cultures are, however, heterogeneous and it is unclear whether all ASC contribute to immunomodulatory processes.

**Methods:** In the present proof of concept study we determined the frequency of ASC secreting the immune signaling molecules IL-6 and IFN-γ by ELISPOT assay, performed IL-6 and IFN-γ ELISA and measured IL-6 receptor (CD126 and CD130 subunits) and IFN-γ receptor (CD119) expression by ASC by flow cytometry.

**Results:** The results showed that nearly/ about 100% of ASC produced IL-6, whereas around 1% of ASC produced IFN-γ. IL-6 receptor was not expressed by ASC, whereas a subpopulation of ASC expressed the IFN-γ receptor. Treatment of ASC with IFN-γ (50ng/ml) had no effect on the frequency of cytokine secretion, while only more IFN-γ producing cells and more IFN-γ secretion was found in the presence of TGF-β (10ng/ml).

**Conclusion:** These results demonstrate that ASC cultures are heterogeneous in their cytokine secretion and cytokine receptor profiles. This knowledge can be employed for selection of desired ASC subsets for immunotherapy.

# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Mass Spectrometry Applications

## A-386

### Paper-spray Ionization Analysis of Endogenous Glucose and Cholesterol in Human Serum

L. Li[1], H. Hsieh[2], W. Kao[3], C. Hsu[2]. *[1]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital and School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [2]Department of Chemistry, National Taiwan University, Taipei, Taiwan, [3]Department of Emergency Medicine, School of Medicine, Taipei Medical University, Taipei, Taiwan., Taipei, Taiwan*

**Background:** Paper spray ionization mass spectrometry (PSI-MS) is a soft ionization, high-throughput analysis method that can be operated in atmospheric pressure. This technique allows direct detection of chemical compounds in complex mixtures such as metabolites in serum.

**Method:** Serum volume as low as 2 μL is required for paper spray analysis. The fragmentation pattern of endogenous glucose match well with that of glucose standard**.** To improve the sensitivity and specificity of targeted compounds in serum, betaine aldehyde (BA) is used as a highly selective reagent to conduct nucleophilic addition with hydroxyl group of cholesterol and glucose. After reacting with betaine aldehyde, higher sensitivity is achieved by forming ion products with permanent positive charge. Isotope labeled compounds can be spiked into the serum samples as internal standards for quantitative measurement of targeted metabolites through this PSI-MS platform.

**Results:** A calibration curve was obtained by spiking plasma samples containing different amounts of cholesterol (Chol) with known amounts of deuterated cholesterol (d6-Chol) as internal standard, with molar ratios [Chol]/[d6-Chol] in the range from 0 to 2. A quite good linearly was obtained ($R^2$=0.995) and some experiments performed on spiked plasmas samples with known amounts of cholesterol confirmed the validity of this method.

**Conclusion**: A simple protocol of PS-MS described above successfully identify endogenous glucose and cholesterol in human serum. The application of betaine aldehyde reagent improves sensitivity of cholesterol. In the future, this rapid and high-throughput platform can further be applied to the analysis of diverse biomolecules in fluid biopsy.

## A-387

### Validation of Serum Voriconazole Assay Using Liquid Chromatography and Tandem Mass Spectrometry for Therapeutic Drug Monitoring in Cancer Patients in a Tertiary Cancer Hospital in India.

S. Chakraborty[1], S. N. Arumugam[1], V. Goel[2], M. Chandy[1]. *[1]Tata Medical Center, Kolkata, India, [2]Centre Of Excellence, Agilent Technologies India Pvt Ltd., Manesar, India*

**Background:** Voriconazole is currently the antifungal of choice for treatment of invasive aspergillosis (IA) in hematological malignancies. A high degree of inter and intra-patient variability has been noted during the administration of this drug. Inter-patient variations result mostly from genetic polymorphisms in cytochrome p450 enzymes whereas intra-patient variations may occur due to disturbances in absorption, distribution, and elimination of the drug in the patient during the course of cancer therapy. This variability may be responsible for toxicity or therapeutic failure which carry a high degree of morbidity and mortality in IA in the backdrop of cancer. In our clinics, in the absence of TDM, toxicity or a breakthrough infection could be perceived as a failure of voriconazole therapy, even though only a dose correction of voriconazole could have rectified the problem. Voriconazole assays based on high-performance liquid chromatography are available only in a handful of centers across India. Here we describe a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the purpose of TDM and pharmacokinetics at our hospital. This study has received ethical approval from the Institutional Review Board.

**Methods:** To prepare the samples, 30 microliters of serum, 600 microliters of posaconazole (as internal standard (IS)) in methanol, and 120 microliters of zinc sulfate (0.1 M) were sequentially added to microfuge tubes. Serum proteins, the principal interferent, was precipitated by adding a combination of methanol (containing IS) and zinc sulfate. The tubes were shaken vigorously and centrifuged at 15,000 rpm for 10 minutes. 300 microliters of the resulting supernatant were then transferred to glass vials for estimation by LC-MS/MS. The same preparation was applied to the serum calibrators and internal quality control (IQC) samples. An ultrahigh-pressure liquid chromatography system was used to perform separation on a diphenyl 50 x 2.0 mm, 2.8-micron column with a step-up gradient of 30% to 95% methanol containing 0.1% formic acid (v/v). The column was held at a constant temperature of 45 degrees Celsius. The run time per sample was 7 minutes. The analytes were monitored on an Agilent 6420 Triple Quadrupole Mass Spectrometer using the following transitions in Multiple Reaction Monitoring mode: Voriconazole (m/z 349.9>280.9) and Posaconazole (m/z 701.7> 683.3).

**Results:** The retention times for voriconazole and posaconazole were 2.7 and 3.7 min respectively. The lower limit of quantification in serum was 0.3 microgram/mL, well below the therapeutic value of Voriconazole (1 - 5.7 micrograms/mL). The method was found to be linear over a range of 0.3 - 10 micrograms/mL with a correlation coefficient better than 0.999. Reproducibility experiments showed good inter-batch and intra-batch coefficient of variations <15% and <5% at 0.3 micrograms/mL and 10.0 micrograms/mL in serum, respectively. The ruggedness of the assay was shown by its stability which was < 10% when calibrator and IQC materials were run over a period of 4 months. A recovery between 87.5% to 102.5% at 0.3, 5.0 and 10.0 micrograms/mL indicated good accuracy for the assays.

**Conclusion:** Following satisfactory validation of the voriconazole, the method has been made available for TDM service and pharmacokinetic measurements in our Institute.

## A-388

### State-of-the-Art thyroid hormone (Total Thyroxine, Free Thyroxine) Assays: A Comparison of Automated Immunoassays with Liquid Chromatography-Tandem Mass Spectrometry Method

S. Yu[1], W. Zhou[2], X. Cheng[1], C. Zhang[2], L. Qiu[1]. *[1]Peking Union Medical College Hospital, Beijing, China, [2]National Center for Clinical Laboratory, Beijing, China*

**Background:** Accurate serum thyroid hormone (Total Thyroxine,TT4; Free thyroxine, FT4) is useful in the diagnosis and management of various thyroid disorders. Presently, the testing is mainly performed on automated clinical chemistry instruments in clinical laboratories with proficiency schemes demonstrating a wide dispersion of result. Here, we compared the performance of 6 automated immunoassays and one liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, and investigated the effects of different diseases, TSH and thyroglobulin on these methods.

**Methods:** Aliquots of 154 randomly selected patient samples were collected and the following cohorts were created: hyperthyroidism patients, hypothyroidism patients, patients with postoperative thyroid carcinoma, pregnant women. TT4 and FT4 was measured by automated immunoassays from Abbott(Architect), Siemens(ADVIA Centaur XP), Roche(E601), Beckman(Dxi 800), Autobio(Autolumo A2000), and Mindray(CL-1000i), TT4 also was measured by a candidate reference measurement procedure LC-MS/MS method of National Center of Clinical Laboratory. Within-run and between-run imprecision were evaluated by measurement of 4 replicates of three serum pools on 5 consecutive days.

**Results:** Among the automated immunoassays detecting TT4, the concordance correlation coefficient (CCC) of the performance comparable to LC-MS/MS varied between 0.94 (Siemens, Beckman) to 0.97(Roche, Mindray). The absolute mean bias ranged between -1.3(Abbott) to 1.6(Siemens). For FT4, the CCC of the performance comparable to mean value of the immunoassays varied between 0.69 (Abbott) to 0.99(Beckman). The mean bias ranged between -0.61(Abbott) to 0.55(Roche).All immunoassays demonstrated good intra- and inter-assay precision, with CV% <10% for both TT4 and FT4.

**Conclusion:** All the automated immunoassays for TT4 showed excellent comparable performance, while not all assays had comparable results for FT4, the standardization of FT4 in China is urgent.

## A-389

### Development of an LC-MS/MS method for biomarkers of alcohol ingestion for use with post-mortem samples

E. Dewar, I. Clunie, L. McKay, J. Allison, D. Stephen. *NHS Grampian, Aberdeen, United Kingdom*

**Background:** Numerous factors can affect post-mortem ethanol concentrations generating uncertainty relating them accurately to ante-mortem levels. There has been increased interest in the quantification of direct ethanol metabolites, such as ethyl glucuronide (EtG) and ethyl sulfate (EtS), in order to better corroborate post-mortem levels to ante-mortem ethanol consumption.

**Objective:** To develop and compare 3 co-extraction methods for EtG and EtS with quantification by LC-MS/MS to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

**Methods:** Drug free whole blood was spiked with various concentrations of EtG and EtS to create IQC material and calibrators to cover the expect post-mortem range of 0-20 mg/L (EtG) and 1-6 mg/L (EtS). EtG-d5 and EtS-d5 were used as internal standards. IQC material and calibrators were subjected to liquid extraction, extraction from dried blood spots (DBS) or solid phase extraction prior to quantification with LC-MS/MS. Each method was assessed to SWGTOX standards including linearity, precision, stability, carry over, matrix effects, recovery, stability.

**Results:** All extraction methods were linear across the analytical range ($r^2$ >0.95) and had minimal carryover. The most promising method was liquid extraction as it met all criteria outlined by SWGTOX. However, the DBS method was imprecise at low EtG concentrations and demonstrated poor stability while the SPE method failed the majority of the criteria.

**Conclusions:** Liquid extraction combined with LC-MS/MS was the optimal method for quantification of EtG and EtS.

| | | EtG | | | EtS | | |
|---|---|---|---|---|---|---|---|
| | | Liquid | DBS | SPE | Liquid | DBS | SPE |
| Linearity ($r^2$) | | 0.999 | 0.997 | 0.997 | 0.998 | 0.997 | 0.993 |
| Sensitivity (mg/L) | LLOD | 0.007 | 0.023 | 0.257 | 0.003 | 0.002 | 0.025 |
| | LLOQ | 0.014 | 0.055 | 0.642 | 0.007 | 0.004 | 0.057 |
| Inter-assay Precision (% CV) | Low IQC | 6.93 | 9.39 | 39.51 | 5.27 | 7.37 | 17.72 |
| | Mid IQC | 5.46 | 8.21 | 22.59 | 9.09 | 7.71 | 31.78 |
| | High IQC | 5.73 | 7.38 | 17.12 | 9.03 | 6.4 | 20.21 |
| Intra-assay Precision (% CV) | Low IQC | 6.15 | 5.69 | 21.99 | 2.96 | 3.47 | 13.07 |
| | Mid IQC | 2.66 | 5.37 | 15.79 | 1.99 | 1.67 | 22.25 |
| | High IQC | 2.53 | 3.97 | 10.35 | 1.43 | 2.6 | 11.72 |
| Recovery (%) | Low IQC | 96.78 | 95.05 | 238.3 | 97.39 | 75.85 | 81.21 |
| | Mid IQC | 108.76 | 106.91 | 252.3 | 110.08 | 107.85 | 147.46 |
| Matrix Effects (%) | | 3.22 | -1.61 | 3.8 | -2.3 | -0.75 | -1.34 |
| Stability (% Remaining) | Low IQC | 96.98 | 116.59 | 74.91 | 95.24 | 90 | 85.36 |
| | Mid IQC | 99.8 | 106.54 | 89.79 | 99.79 | 90.71 | 85.19 |
| | High IQC | 94.54 | 108.13 | 96.81 | 94.53 | 107.73 | 91.16 |
| Carry Over (%) | | 0.01 | 0.027 | 0.171 | 0.003 | 0.009 | 0.094 |

## A-390

### Quantitation of 5-Hydroxyindoleacetic Acid (5-HIAA) and Vanillylmandelic Acid (VMA) in Urine using Liquid Chromatography Electrospray Tandem Mass Spectrometry

T. D. Hawley, D. M. Goertz, K. Yaraei. *Pathology Associates Medical Laboratories, Spokane, WA*

**Background:** 5-Hydroxyindoleacetic Acid (5-HIAA) and Vanillylmandelic Acid (VMA) are metabolites of serotonin and catecholamines, respectively. Urinary 5-HIAA is used to detect, diagnose, and monitor carcinoid tumors. While there are many factors that can cause 5-HIAA concentrations to increase in urine including medications and various foods, patients with a carcinoid tumor will show highly elevated levels of 5-HIAA. Moreover, urinary VMA is used to detect and monitor pheochromocytomas and catecholamine producing tumors. In the case of these tumors, urine VMA levels will be significantly elevated. For both 5-HIAA and VMA, a 24-hour urine collection is the preferred method as levels can vary throughout the day.

**Methods:** Aliquots of 50 µL of patient sample, calibrators, and controls and 50 µL of internal standard (20 mg/L 5-HIAA-D6 and 30 mg/L VMA-D3) were added together. The samples were vortexed and then diluted with 400 µL of 0.05% formic acid. Once the samples were mixed, they were centrifuged at 15,000 g for 10 minutes. The supernatant was removed and placed into a 96 deep well plate. The samples were analyzed on an API 3200 LC-MS/MS (AB Sciex) using negative electrospray and Multiple Reaction Monitoring (MRM). A Phenomenex Gemini® 3 µm C18 110 Å, LC Column 100 x 3 mm column was used and 10 µL of supernatant was injected at a flow rate of 0.6 ml/min. Total run time was 6 minutes.

**Results:** For VMA, validation was done by running 59 samples previously run by HPLC and 25 samples previously run by LC-MS/MS from an independent clinical lab. Correlation for the HPLC and LC-MS/MS samples showed high agreement with a slope of 0.995 and 1.033 and an R value of 0.9967 and 0.9993, respectively. For 5-HIAA, the validation was performed by analyzing 91 samples previously run by LC-MS/MS from an independent clinical laboratory and showed good agreement with a slope of 1.012 and an R value of 0.9968. The overall CV for VMA and 5-HIAA was less than 6% and 8%, respectively. The linearity for both analytes was 0.5 mg/L to 60 mg/L. Matrix effect was observed, but the deuterated internal standard was shown to compensate. No interfering substances were observed.

**Conclusion:** We have developed and validated a simple and effective method for quantitating 5-HIAA and VMA levels in urine.

## A-391

### Multiomics Analysis of a Drop of Blood for Clinical Diagnosis

D. Wang, P. Mao, G. Gil, M. Li. *Newomics Inc., Emeryville, CA*

**Background:** Rapid, sensitive, and specific, biofluids-based clinical diagnosis will greatly advance precision medicine. However, there is no current technology that could analyze thousands of molecules including proteins, metabolites, lipids, and exogenous environmental toxins using small volumes of blood samples. We here report the development and proof-of-principle applications of a silicon-microfluidic-chip platform that can perform highly-sensitive, multiplex, and multiomics analysis of a drop of blood for biomonitoring and disease diagnosis.

**Methods:** We developed various types of silicon microfluidic chips for LC-MS analysis of a drop of blood. For analysis of full-length proteins including their posttranslational modifications, we incorporated C4 columns on the chip. For analysis of small molecules, we monolithically integrated on-chip solid-phase extraction (SPE) with online nanoflow liquid chromatography-electrospray ionization-mass spectrometry (nanoLC-ESI-MS) detection. The chip contains several key functional modules including a SPE column, a herringbone mixer, a trap column, a LC column, an emitter, and multiple inlet/outlet holes for sample and solvent input and output. We next developed multiomics assays of a drop of blood. We used Waters Q-TOF MS interfaced with a Waters CapLC system, and Thermo Fisher Q-Exactive plus interfaced with Dionex LC system, for top-down proteomics, bottom-up proteomics, and metabolomics. We used Thermo TSQ Quantiva Triple Quadrupole MS, interfaced with an UltiMate 3000 nanoUPLC system for selected reaction monitoring (SRM) assay of peptides and small molecules such as perfluorinated compounds (PFCs) and pesticides, as well as for shotgun lipidomics. We then analyzed the molecules in small volumes of blood for diabetes monitoring and biomonitoring of PFCs.

**Results:** We demonstrated proof-of-principle applications using our platform. We have developed a new assay that is able to measure glucose, HbA1c, glycated HSA, and glycated apolipoprotein AI (apoA-I) for monitoring of individual blood glycemia, as well as cysteinylated HSA, S-nitrosylated HbA, and methionine oxidized apoA-I for gauging oxidative stress and cardiovascular risks, all in 5 microliters of blood. We have also developed a new microassay for analyzing PFCs in small volumes (less than five microliters) of blood. We demonstrated high sample recovery, excellent interday and intraday accuracy and precision, and a limit of detection down to 50 femtogram of PFCs, in one microliter of human plasma. We validated our assay performance using pooled human plasma and NIST SRM 1950 samples. Our ongoing efforts are towards integrating the multiplex assays for multiple classes of molecules on a single chip, as well as targeting clinical diagnosis of other diseases including Alzheimer's disease and cancer.

**Conclusion:** Our silicon microfluidic chip platform may enable ultrahigh-sensitivity LC-MS analysis of thousands of endogenous and exogenous molecules in small volumes of biological fluids for clinical diagnosis.

## A-392

### Renal metabolomic LC-MS/MS analysis for 7 metabolites

B. Pang[1], L. R. Moss[1], M. Rocco[2], A. K. Bryant[3], W. E. Nell[4], M. E. Hinsdale[4], J. Yurco[1], R. K. McCall[1], P. Persad[1], S. H. Wong[1]. *[1]Department of Pathology, Wake Forest School of Medicine, Winston-Salem, NC, [2]Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, NC, [3]Department of Physical Medicine and Rehabilitation, Pain Management, Wake Forest School of Medicine, Winston-Salem, NC, [4]Department of Pathology, Wake Forest Baptist Health, Winston-Salem, NC*

**Background:** Targeted metabolomic analysis allows different biochemical pathways to be analyzed simultaneously in a single assay to monitor organ function, damage, and/or prognostic markers. We developed and validated a multiplexed LC-MS/MS assay of urine to monitor 7 renal metabolites including kidney function marker (creatinine), Krebs cycle intermediates (citrate, succinate, and oxoglutarate), oxidative stress (trimethylamine oxide, TMAO), reabsorption (sorbitol), and active kidney secretion and amino acylase activity (hippurate). **Methods:** De-identified urine samples from 24 pain management patients were used to detect seven metabolites. Six stable isotopes labeled metabolites as internal standards were added, followed by scheduled multiple reaction monitoring by ABSciex 6500 Triple Quadrapole mass spectrometer with electrospray ionization in a positive and negative ion switching mode. A Shimadzu Nexeral LC with a Fusion-RP (4x2 mm) guard column and a Synergi 4 µm Fusion-RP 80Å (100 x 2mm) column were used for the analyte separation. Mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Starting mobile phase consisted of 20% B at 0.7mL/min flow rate, transitioned to 60% B from 0.3 to 0.6 min, 100% B at 0.7 min, and reverted to 20% B at 5.1 min. For sample preparation, 1: 50 diluted urines, calibrators, and quality controls (QC) were centrifuged at 20000 rcf for 15 min. Then 480 µl aliquots of supernatants were mixed well with 20 µl internal standards solution. Aliquots of 5 µl samples were analyzed LC-MS/MS. **Results:** Calibration was linear except oxoglutarate which was established with the quadratic fit. Correlation coefficients ($R^2$) were greater than 0.99 with a range of 0.1 - 100 µm for hippurate, oxoglutarate, sorbitol, succinate, and TMAO, 0.2 - 200 µm for citric acid, and 1 - 1000 µm for creatinine. Coefficients of variation for low, middle, and high QCs were less than 8% for intra-day assays and less than 10% for inter-day assay. Method validation also did not show significant matrix effects and carry-over. Correlation of urinary creatinine concentrations by the LC-MS/MS method and AU5800 in our clinical lab was acceptable with $R^2 = 0.9947$ ($y = 0.8925x + 8.1852$). The other 6 metabolites level in the urines were also comparable with the published references from Human Metabolome Database. **Conclusions:** The developed multiplexed LC-MS/MS method may be used to quantify 7 renal metabolites in urine with minimal sample preparation and accepted precision and accuracy.

## A-393

### Urine Purine and Pyrimidine metabolite determination by LC-MS/MS for research use

R. M. Doyle. *Thermo Scientific, Inc, Somerset, NJ*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC-MS/MS) is suited for rapid analysis of multiple analytes. Inborn errors of purine and pyrimidine metabolism lead to a wide spectrum of biological presentations either due to deficiencies of synthesis, degradation, salvage and metabolic pathways. The purine and pyrimidine metabolites analyzed included succinyladenosine, succinyladenosine monophosphate, beta-alanine, ureidopropionate, beta-aminoisobutyric acid, AICAR, uric acid, orotic acid, cytosine, dihydrouracil, uracil, 5-hydroxy-methyluracil, pseudouridine, cytidine, xanthine, hypoxanthine, guanine, uridine, AICar, dihydrothymine, thymine, deoxyuridine, inosine, deoxyinosine, guanosine, deoxyguanosine, adenine, thymidine, adenosine, and deoxyadenosine. A highly sensitive and specific LC-MS/MS analytical method has been developed for the determination of purine and pyrimidine nucleoside and base concentrations in urine. A simple sample preparation technique that involved sample dilution was utilized along with a one (1D) dimensional liquid chromatographic configuration. The described method achieves the required sensitivity and is capable of determining the purine and pyrimidine metabolites over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed for the quantitatively measurement of Purine and Pyrimidine metabolites in urine.

Method: A Thermo Scientific™ Endura™ tandem mass spectrometer in positive and negative Electrospray mode and a Thermo Scientific™ Dionex™ Vanquish™ Horizon HPLC system were utilized for this analysis. 100 µl of urine were used for the analysis of the purine and pyrimidine metabolites. Various columns were evaluated and an Thermo Scientific™ Accucore™ C18 100 x 2.1 mm, 3 µm with a water:methanol mixture containing 2 mM Ammonium Acetate and 0.1% Formic Acid achieved baseline chromatographic separation for all the purine and pyrimidine metabolites in less than 8 minute run time. Quantitative analysis was performed using selective reaction monitoring (SRM) with transition pairs for each analyte and internal standard in negative mode and the precision and accuracy of the method was verified using pooled quality control materials and urine samples.

Result: Good linearity and reproducibility were obtained with the concentration range of 0.5 to 100 µmol/L for the respective purine and pyrimidine metabolites with a coefficient of determination >0.95 for the sample preparation technique and the LC-MS/MS configuration used. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.25 to 1 µmol/L and excellent reproducibility was observed for all compounds (CV < 10%).

Conclusion: A sensitive, simple, specific and accurate liquid chromatography tandem mass spectrometry method was developed and verified for the simultaneous determination and screen of purine and pyrimidine metabolites in urine. The sample preparation technique is quick and easily applied for high throughput analysis.

## A-394

### Analysis of Immunosuppressive Drugs from Whole Blood by LC/MS/MS

S. Liang. *Restek Corporation, Bellefonte, PA*

**Background:**Immunosuppressive drugs are used to suppress the body's immune response and are typically administered to prevent the rejection of transplanted organs or tissues. Cyclosporin A, tacrolimus, sirolimus, and everolimus are four of the most commonly used drugs in the therapy of organ transplantation. Cyclosporin A and tacrolimus are classified as calcineurin inhibitors, and sirolimus and everolimus are grouped as mTOR inhibitors. These two classes of drugs can be used in combination for synergistic blocking of T cell activation and proliferation. Due to their pharmacokinetic variabilities and narrow therapeutic indexes, time-sensitive and highly accurate therapeutic drug monitoring is necessary, not only to prevent rejection but also minimize toxic side effects. Therefore, a fast and accurate measurement of drug concentration is critical to assist the clinicians for timely and proper treatment of the patients. By combining a simple sample preparation step and a fast chromatographic elution with a Raptor™ Biphenyl column, a high-throughput analysis was established for simultaneous measurement of these four drugs in human whole blood.**Methods:**Human whole blood was fortified with 4 analytes to prepare the calibration standards and QC samples. For quantitation, cyclosporin D was used as the internal standard for cyclosporin A and ascomycin was used as the internal standard for tacrolimus, sirolimus, and everolimus. The blood sample (100 µl) was mixed with 200 µl of precipitation solution (1:4 v/v 0.2M $ZnSO_4$:methanol) containing 50 ng/mL of cyclosporin D and 5ng/mL of ascomycin. After centrifugation, the supernatant was directly injected (5 µl) onto the Raptor™ Biphenyl 2.7 µm, 50 mm x 2.1 mm column for analysis using Waters Acquity UPLC® System coupled with Xevo® TQ-S mass spectrometer.

**Results:** Linearity was evaluated in the range of 10-1000 ng/mL for cyclosporin A and 1-100 ng/mL for tacrolimus, sirolimus, and everolimus. Using $1/x^2$ weighted linear regression for cyclosporin A and 1/x weighted linear regression for tacrolimus, sirolimus, and everolimus, all 4 compounds showed good linearity with $r^2$ values of 0.999 or greater, and the % deviations were less than 10%. The signal-to-noise ratios of the lowest standard samples were from 100 to 300 indicating that this method could be used for the detection of much lower concentrations if necessary. Three QC levels were prepared at 15, 150, and 800 ng/mL for cyclosporin A; 5, 15, and 80 ng/mL for tacrolimus, sirolimus, and everolimus. Precision and accuracy analyses were performed on three different days. The method accuracy was demonstrated with %recovery of less than 10% of the nominal concentration for all QC levels. The %RSD was 0.2-4.0% and 1.2-5.4% for intra-day and inter-day, respectively, indicating good method precision. No chromatographic interferences were observed from the analysis of blank blood samples.

**Conclusion:**It was demonstrated that the Raptor™ Biphenyl column is excellent for rapid and accurate analysis of cyclosporin A, tacrolimus, sirolimus, and everolimus in human whole blood. With a fast and simple sample preparation procedure and 3 minutes of chromatographic analysis time, the established method provides high-throughput therapeutic drug monitoring for these commonly used immunosuppressive drugs.

## A-395

### Targeted Benzodiazepines Screening Assay for Pain Management Using High Resolution Mass Spectrometry

D. Danso. *Mayo Clinic, Rochester, MN*

**Background:** Benzodiazepines represent a large family of medications used to treat a wide range of disorders from anxiety to seizures and they are also used in pain management. With a high risk for abuse/diversion, professional practice guidelines recommend compliance monitoring for these medications using urine drug tests. However, traditional benzodiazepine immunoassays suffer from a lack of cross-reactivity with all the benzodiazepines, so many compliant patients taking clonazepam (Klonopin®) or lorazepam (Ativan®) may screen negative by immunoassay but are positive when confirmatory testing is done. This new targeted benzodiazepine screening test provides a more sensitive and specific test to check for compliance to all the commonly prescribed benzodiazepines and looks for both parent and metabolites in the urine.

**Aims:** The aim of this study was to validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method using high-resolution, accurate-mass Orbitrap detection for the qualitative identification of twenty-six benzodiazepines and metabolites (aprazolam, alpha-hydroxyalprazolam, chlordiazepoxide,clonazepam , 7-amino clonazepam,diazepam, nordiazepam, oxazepam, oxazepam glucuronide, temazepam , temazepam glucuronide,flunitrazepam , 7-amino flunitrazepam, flurazepam, a-hydroxyethylflurazepam, lorazepam, lorazepam glucuronide, midazolam, alpha-hydroxymidazolam, triazolam, alpha-hydroxytriazolam, zolpidem, zolpidem phenyl-4-carboxylic acid, clobazam, norclobazam, and prazepam.

**Methods:** A simple dilute and shoot method was developed in which urine samples (100 µL) were diluted (1:10) with internal standard in clinical laboratory reagent water (CLRW) and analyzed by LC-MS/MS using high-resolution, accurate-mass Orbitrap detection with heated electrospray ionization in positive mode. Mass spectrometer method was Full MS/ddMS$^2$. A step gradient elution off of an Ultra Biphenyl (3 µM 50 x 3.0 mm) column achieved complete chromatographic separation of isobaric compounds with a total run time of 8 minutes, a flow rate of 0.5 mL/min, and the ability to multiplex samples. Mobile phase A contained 10mM ammonium formate with 0.1% formic acid in CLRW, while mobile phase B contained 0.1% formic acid in acetonitrile. Compounds were identified by retention time; exact masses (m/z) at (<5ppm), and spectra library match.

**Results:** A LC-MS/MS targeted screening method was validated. The cutoffs for the twenty-six benzodiazepines ranged from 10 ng/mL for (alprazolam, lorazepam, temazepam, clonazepam, etc), 50 ng/mL for the glucuronides (lorazepam, temazepam, and oxazepam), and 200 ng/ mL for norclobazam. The intra- and inter-assay precision coefficients of variation for all compounds were <10% at concentrations 50% below the cutoff, at the cutoff, and at 50% above the cutoff concentrations for each analyte. Analytical accuracy was determined by comparing patient and proficiency testing samples for

each analyte against quantitative confirmatory LC-MS/MS tests and spiked recovery experiments. The Orbitrap method showed 100% concordance with the confirmatory method for all analytes based on the individual drugs' cutoff concentrations. Absence of interferences from the common prescribed drugs, over-the-counter drugs, therapeutic drugs, and common drugs of abuse tested at ≥ 100µg/mL was observed. No significant carryover was seen at concentrations up to 100µg/mL, and no matrix effect observed.

**Conclusions:** The laboratory was able to successfully validate a highly specific and sensitive LC-MS/MS targeted screening assay using a high-resolution, accurate-mass method for the comprehensive detection of benzodiazepines in urine.

**Keywords:** Benzodiazepines, Pain Management, Liquid Chromatography Tandem Mass Spectrometry, High-resolution, Accurate-mass

## A-396

### Development and validation of a quantitative method for plasma folates

J. A. Maggiore, Z. Wu, K. Urek, E. Roth, A. Gruszecki, D. Quig. *Doctor's Data, Inc., Saint Charles, IL*

**Background:** An increased interest in folate analysis has arisen from an enhanced ability to detect single nucleotide polymorphisms (SNPs) in the genes that transcribe the enzymes of folate metabolism. Defects in folate metabolism affect the remethylation of homocysteine to methionine. The C677T and T677T methyltetrahydrofolate reductase (MTHFR) SNPs occur at a frequency of 32.4% and 7.4%, respectively, in the general US population [(1)]. When consuming folate-rich foods or supplements, the majority of individuals may experience excessive plasma levels of unmodified folic acid (UMFA), which has been associated with cellular hyperplasia and neoplasia[(2)]. Folinic acid and 5-methyltetrahydrofolate (5-MTHF) are synthetic folate supplements recommended by clinicians for those with specific MTHFR C677T heterozygous (C/T) and homozygous (T/T) genotypes to provide a source of folates while minimizing undesired levels of UMFA in circulation. Immunoassays that determine total folates in serum or red blood cells cannot differentiate between UMFA and the other folates. To aid in the monitoring of the folate status, we developed a method for measuring UMFA and the folate metabolites folinic acid, 5-MTHF, and tetrahydrofolate (THF) using liquid chromatography with tandem mass-spectrometry (LC-MS/MS).

**Methods:** Plasma samples prepared in 100 mM ascorbic acid were mixed with internal standards and extracted by polymeric reversed phase solid phase extraction cartridges. Certified standards traceable to the National Institute of Standards and Technology (NIST) were acquired to derive a 5-point calibration curve for each analyte. Calibrators, NIST standard reference materials (SRM 1955), and plasma extracts were analyzed on an Agilent 6490 LC-MS/MS with chromatographic separation achieved on a C18 analytical column, permitting identification and quantitation of the folate compounds of interest. Analytical performance characteristics evaluated include precision, linearity, recovery, and stability.

**Results:** The intra-assay and total imprecision coefficients of variation ($CV_W$ and $CV_T$) (n=42) in plasma samples was determined for UMFA: 4.0% and 7.0% at 6.5 nmol/L, and 3.7% and 7.7% at 9.6 nmol/L; folinic acid: 4.5% and 10.9% at 45.2 nmol/L, and 4.1% and 11.6% at 51.7 nmol/L; 5-MTHF: 1.0% and 4.9% at 46.7 nmol/L, and 1.1% and 5.6% and at 57.2 nmol/L; and THF: 5.3% and 14.6% at 2.7 nmol/L, and 3.9% and 12.9% at 5.0 nmol/L. Linearity range (n=11) and percent recovery was confirmed in spiked plasma samples for UMFA: 1.0-425.0 nmol/L, 98.3%-107.6%; folinic acid: 0.15- 402.4 nmol/L, 95.7%-108.6%; 5-MTHF: 1.2-632.8 nmol/L, 99.7%-104.1%; and THF: 0.25-405.2 nmol/L, 98.6%-109.0%. Stability of all analytes in acidified plasma samples was demonstrated for 8-days stored at -20ºC, and 4-days stored at 2-8ºC.

**Conclusion:** This LC-MS/MS method developed for assessing plasma folate status has been validated to be analytically precise, accurate, and sensitive for the measurement of UMFA, folinic acid, 5-MTHF, and THF. Sample suitability has been established for the expedited temperature-controlled transportation of acidified plasma aliquots from remote locations to a central laboratory for analysis. Studies are underway to use this method to assess reference intervals for the folate analytes in those with previously determined MTHFR C677T genotypes.

**References**: 1) *J Med Genet.* 2003;40:601-605. 2) *J Nutr*. 2015**;** 145(3):520-531.

## A-397

### Simultaneous LC/MS/MS Quantitation of 20 Antiepileptic Drugs in Human Serum

L. E. Frick[1], C. J. Adler[2], V. P. Miller[1]. *[1]Agilent Technologies, Lexington, MA, [2]Agilent Technologies, Santa Clara, CA*

**Background:** One major strength of liquid chromatography-mass spectrometry (LC/MS/MS) as a detection method is that it allows the concurrent monitoring of multiple analytes in a single injection. Here, a method has been developed to quantify a panel of 20 antiepileptic drugs in human serum. Compounds included were: Acetylretigabine, Carbamazepine-10,11-Epoxide, Carbamazepine, 10,11-Dihydro-10-Hydroxy-Carbamazepine, Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Oxcarbazepine, Phenobarbital, Phenytoin, Pregabalin, Retigabine, Rufinamide, Tiagabine, Topiramate, Valproic Acid, Vigabatrin, Zonisamide. The method further utilized the ability of LC/MS/MS to detect compounds over a wide range of concentrations simultaneously, as the calibration concentrations ranged from 12 ng/mL to 200,000 ng/mL for the various analytes. Top concentrations ranged from 1.5 to 200 ug/mL.

**Methods:** MS/MS transitions were obtained using MassHunter Acquisition's Optimizer software to determine optimal parent and fragment ions, fragmentor voltages, and collision energies upon injection of a neat solution of each individual compound or internal standard. Samples were prepared by spiking compounds into clean human serum to create an 8-point serially-diluted calibration curve. Each calibrator or blank control was then combined with an internal standard solution and extracted through a protein precipitation using methanol before vortexing and centrifugation. An aliquot of supernatant was then diluted into water and injected onto the LC system. Compounds were separated from each other and from regions of phospholipid suppression on an Agilent Poroshell 120 EC-C18 analytical column (2.1 x 100 mm, 2.7 um) paired with an Agilent Poroshell 120 EC-C18 guard column (2.1 x 5 mm, 2.7 um). Water supplemented with 2 mM ammonium acetate was employed as mobile phase A and methanol with 2 mM ammonium acetate as mobile phase B. A

6460 triple quad mass spectrometer was used to detect the 20 compounds in dynamic MRM mode. Positive/negative switching was utilized to monitor compounds of both polarities in a single injection. Compounds were separated over 7.5 minutes, followed by a 1.5 minute column reequilibration at starting mobile phase conditions, resulting in a total cycle time of ~9.5 minutes injection to injection. Data were analyzed using MassHunter Quantitative Analysis B.07.01 and Qualitative Analysis B.07.00.

**Results:** Calibration curve accuracies were within 20% of the expected concentration at the lowest level, and well within 15% at all higher levels. Reproducibility was good, with all CVs <15% and most well under 10%. $R^2$ values were all >0.995, with some compounds displaying a linear response across their concentration range, and others requiring a quadratic fit.

**Conclusion**: An accurate, reproducible, and robust LC/MS/MS analytical method has been developed to quantitate 20 antiepileptic drugs simultaneously in human serum. Future work will include testing multiple sources of human serum for interferences that would impact the quantitation of any of the members of the panel. Additionally, different sources of mobile phase and samples from an alternate source will be analyzed. For Research Use Only. Not for use in diagnostic procedures.

## A-398

### Urinary Biomarkers of Idiopathic Membranous Nephropathy Identified by High Resolution Mass Spectrometry Coupled with Liquid Chromatography

L. Pang, H. Li, N. Duan, Y. Liu, D. Yu. *Peking University First Hospital, Beijing, China*

**Objectives**: Idiopathic membranous nephropathy (iMN) is an important cause of nephrotic syndrome in adults. Recently, M-type phospholipase A2 receptor (PLA2R) and thrombospondin type 1 domain containing 7A (THSD7A) have been identified as target antigens in iMN. However, it is likely that these are not the only proteins involved in the pathogenesis of iMN. Urine may provide important clues of pathophysiological mechanisms in iMN because it directly reflects the physiological state of the kidney. In the current study, we analyzed and compared the proteome of urine from patients with iMN and normal controls.

**Methods:** Second morning urine samples were collected from patients with biopsy proven iMN with serum anti-PLA2R antibody (group A, n = 5), iMN without serum anti-PLA2R antibody (group B, n = 5) and healthy volunteers (n = 5). Trichloroacetic acid (TCA) precipitation and enzymatic digestion were performed to prepare peptides. Peptides were processed strictly according to the manufacturer's protocol for 6-plex Tandem Mass Tags (TMT). Nanoscale liquid chromatography tandem mass spectrometry analyses were performed for protein identification. Gene ontology (GO) enrichment analysis was performed to analyse general characterization of the proteins. The proteins were also matched against the database of well-known metabolic pathways (KEGG). The increase of candidates was validated by Western blot.

**Results**: In this study, we identified 509 proteins in the iMN urinary proteome. There are 187 and 177 proteins up- or down-regulated in group A and group B with a fold change of 2, respectively. GO enrichment analysis revealed immune response (16 proteins) and complement activation (13 proteins) as the dominant biological process in group A and group B, respectively. Immune system is the major classification in the pathway analysis using the KEGG database in both groups. Intersecting the set of up- or down-regulated proteins in the group A and group B proteomes with a fold change of 10, we identified 5 proteins may provide targets for iMN. Western blot analysis confirmed our proteomics findings that alpha-1-antitrypsin (A1AT) and crk-like (CRKL) were up-regulated in the patients with iMN but not in normal controls.

**Conclusions**: In conclusion, our data show the important role of immunologic mechanism in the development of iMN, and the value of urinary A1AT and CRKL in biomarker discovery of patients with iMN. The discovery of the up-regulation of A1AT and CRKL in the urine can help to further elucidate pathogenetic mechanisms involved in this disease.

## A-399

### Comparison of human serum 17β-estradiol quantification using the ID-LC-MSMS assay with the chemiluminescent immunoassays

Q. Zhang, J. Wang, H. Lin, L. Han, P. Ke, J. Zhuang, X. Huang. *the Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China*

**Background:** 17*β*-Estradiol is routinely analyzed in clinical laboratories of the assessment of female reproductive function and has expanding roles in other fields. However, due to low concentration levels as well as the presence of the metabolites or structural analogues which having molecular masses close to 17*β*-Estradiol that can cross react with the immunoassay, measurements of 17*β*-Estradiol in human serum are complicated.

**Methods:** [2,3,4-$^{13}C_3$] 17*β*-estradiol was used as an internal standard. The estradiol and its internal standard were extracted from serum matrix using liquid-liquid extraction prior to reversed-phase LC-MS/MS and require no derivatization. The analysis was carried out with electrospray ionization in the negative ion mode monitoring the m/z 271→145 m/z as the quantifier, 271→183 m/z as the qualifier, and 274→148 m/z for [2,3,4-$^{13}C_3$] 17*β*-estradiol. Bracketing calibrators was used for quantification. The accuracy of the measurement was evaluated by a comparison of results of this reference method on lyophilized human serum reference materials for estradiol [2015 IFCC external quality assessment scheme for Reference Laboratories in Laboratory Medicine] with the certified values determined by six reference laboratory from different countries and by a recovery study for the added E2. We evaluated intra-assay and inter-assay imprecision. The method procedure was validated against the JCTLM-certified reference method and used in measuring 17*β*-estradiol of 60 patient serum samples for evaluating 3 immunoassays, that are commonly used in China, i.e., Siemens IMMULITE 1000 (Siemens), ARCHITECT i2000$_{SR}$ (Abbott), and Cobas 6000 (Roche).

**Results:** The LC-MS/MS method was validated and showed limit of detection 5 pg/mL; limit of quantification 10 pg/mL; linearity of response to 14.82 ng/mL; The intra-assay precision CVs (n = 15) were 3.69%, 1.92%, and 1.84%; and the inter-assay precision CVs (9 runs/day, over 5 days) were 4.21%, 2.54%, and 2.74%, respectively. And analytical recoveries were from 98.73 to 100.77%. The linear regression equation showed $r^2$=0.9395 (Siemens IMMULITE 1000 = 0.9429 LC-MS/MS+28.1300, 95% CI for the slope 0.8800 to 1.0060, 95% CI for the intercept: -1.8790 to 58.1400 pg/mL, $S_{y.x}$=88.64, P < 0.0001). $r^2$=0.9797 (Cobas 6000 = 1.0390 LC-MS/MS+16.5300, 95% CI for the slope 1.0000 to 1.0790, 95% CI for the intercept:-2.2490 to 35.3000 pg/mL, $S_{y.x}$=55.46, P < 0.0001). $r^2$=0.9962 (ARCHITECT i2000$_{SR}$ = 1.0630 LC-MS/MS-12.9200, 95% CI for the slope 1.0460 to 1.0810, 95% CI for the intercept: -21.1700 to -4.6740 pg/mL, $S_{y.x}$=24.36, P < 0.0001). Bland-Altman plots were achieved by MedCalc and showed: By Siemens IMMULITE 1000, there are 16.7% of samples showed exceed ±30% biases from the mean of difference (2.4%). And there are only 5.0% and 6.7% of samples showed exceed ±30% biases in Cobas 6000 and ARCHITECT i2000$_{SR}$ systems.

**Conclusions:** We report a direct comparison of the ID-LC-MS/MS assay with the chemiluminescent immunoassays for human serum 17*β*-estradiol. Linear regression revealed good overall correlation with the LC-MS/MS and chemiluminescent immunoassays, and Bland-Altman plots showed that the differences were concentration dependent.

## A-400

### Improvement of DNA methylation quantitation method with LC-MS/MS by optimization reaching a breakthrough

T. Nakagawa, M. Wakui, M. Murata. *1)Department of Laboratory Medicine, Keio University School of Medicine, Tokyo, Japan*

**Background:** DNA methylation is one of the typical phenomenons of epigenetics. Many methods for detecting global DNA methylation have been developed, among which the method using LC-MS/MS is an excellent method from the viewpoint of sensitivity, reproducibility, and cost. Several studies have proved that the LC-MS/MS method can detect a slight change of global DNA methylation. However, the potential problems seem to lie in the absence of studies on the stability and standardization of measurement methods. The aim of the present study is to establish a robust assay that guarantees high accuracy.

**Methods:** For the standard materials, ribonucleoside (Adenosine, Guanosine, 5-Methyluridine, Uridine, and Cytidine), deoxynucleoside (2'-Deoxyadenosine, 2'-Deoxyguanosine, 2'-Deoxythymidine, 2'-Deoxyuridine, 2'-Deoxycytidine, 2'-Deoxy-5-methylcytidine and 2'-Deoxy-5-(hydroxymethyl) cytidine), 5-Azacytidine, and 5-Aza-2-deoxycytidine were used. The LC-MS/MS apparatus, Acquity UPLC and TQD (Waters) was used. To separate the substances shown above, we utilized a column distinct from the C18 column reported in previous studies. To degrade DNA, DNA Degradase Plus Kit (Zymo Research) was used, together with some degrading enzymes. For preparing a calibration curve, a mixture of cytosine DNA standard and 5-methylcytosine DNA standard (Zymo Research), which are linear dsDNA (897bp), were used. The ratio of

methylated cytosine (%mdC) of cytosine DNA standard and 5-methylcytosine DNA standard were 0% and 100%, respectively. The typical linear regression equations with the use of no weighting factor of the calibration curves were: y = ax + b with

y as %mdC and x (%) as $\%mdC_{nominal}$. As a result, the DNA methylation rate was determined. For the QC samples, Human genomic DNA (Clontech Laboratory) (Middle QC), EpiScope Unmethylated HCT116 DKO gDNA (Low QC) and methylated HCT116 DKO gDNA (Takara Bio) (HighQC) were used.

**Results & Discussion:** By improving the eluent, it was possible to remove sodium ion adducts observed by nucleoside ionization. In addition, the chromatograms showed good separation by selection of column. Intra assay precision CV = 2.6%, 2.3% and 2.3% (Low, Middle, HighQC) (n=10), inter assay CV = 4.7, 4.2, 4.1% (Low, Middle, HighQC) (n=15). The accuracy (CV) were 94.9% (3.7%), 99.9% (3.6%), 101.9% (4.5%) (n=4) ($\%mdC_{nominal}$ were 0.96%, 4.80% and 9.60%), respectively.

We developed a new method with favorable conditions for the measurement of very small amount of methylated nucleoside. Three points should be discussed. First, our ionization conditions resulted in improvement of detectable sensitivity limited by sodium adduct ions. It has been found that ionization of nucleosides was different depending on the composition of the eluent. Second, the advantageous column selection

made proper separation of various nucleosides without unsuitable overlapping of chromatography peaks. Third, the reduction of errors by the improved calibration method solved the problematic limitations given by the matrix effects on assays and the errors due to two-step quantification for individual nucleosides. These advantageous points are essential to the successful solution of the problems of LC-MS/MS-based assays reported previously.

**Conclusion:** Our developed method is robust and easy to standardize for quantitative assays of DNA methylation based on LC-MS/MS.

## A-401

### Primary Q1/Q3 ion pair positive interference for internal standard in a 25-OH-Vitamin D assay by LC-MS/MS among patients receiving the antiemetic ondansetron (Zofran)

D. Fortuna[1], W. R. Korn[1], M. J. Brune[1], X. He[2], A. Y. Wang[1], J. M. Hevko[2], D. F. Stickle[1]. *[1]Jefferson University Hospitals, Philadelphia, PA, [2]AB Sciex LLC, Redwood City, CA*

**BACKGROUND**: Monitoring of the consistency of counts of internal standard (IS) across samples (often referred to as the metric plot) is an essential aspect of quality assurance in MS/MS assays. Aberrant IS cps for an individual sample can flag improper processing, or the presence of an interferent affecting ionization by enhancement or suppression, or, rarely, the presence of interference by an isobaric compound. Soon after adoption of LC-MS/MS for measurement of 25OH-vitamin D (25-OH-D) at our institution, we noted regular occurrences of samples having distinct apparent elevations of IS (d6-25OH-D3). We describe our investigation to determine that this interference was associated with patients receiving the antiemetic ondansetron (Zofran). **METHODS**: Our method for measurement of 25OH-D by LC-MS/MS is based on that of Garg et al. [PMID: 23001980] using positive-ion APCI and performed on an AB Sciex 3200MD instrument, with IS = d6-25OH-D3. In our first encounter with this interference, there was an approximately 2-fold elevation of IS (m/z Q1/Q3 = 389.3/211.3). It was determined immediately that this primary Q1/Q3 signal was not derived from d6-25OH-D3, as the interference lacked qualifier Q1/Q3 ion pairs characteristic of d6-25OH-D3 (389.3/263.3, 389.3/229.2). Among the first 6 patients encountered, there were no known medications in common. For the 7th patient, an inpatient, records review of medications and analysis of samples predating and postdating the original interference sample indicated onset of interference immediately after administration of the antiemetic ondansetron, with a rise and fall of interference occurring over an interval of 4 days. Ondansetron itself was tested in samples and produced no interference, indicating that interference was likely due to a metabolite of ondansetron. We then identified in-house patients with known time of administration of ondansetron, and tested existing specimens from these patients for the presence of the interference. **RESULTS**: Using a database of a four-day interval of inpatient pharmacy records for administration of ondansetron, we were able to retrieve existing post-administration plasma or serum specimens for 20 patients from this interval. Samples for 3 patients (15%) exhibited IS interference, being positive for the primary Q1/Q3 signal for IS and negative for the qualifier Q1/Q3 signals for IS. Alteration of LC parameters (e.g., lengthening of column) could shift the retention time of the interferent away from coelution with d6-25OH-D3. **CONCLUSIONS**: Results indicated that a low-incidence metabolite of ondansetron was the likely cause of a positive interferent in the primary Q1/Q3 measurement of d6-25OH-D3 in our 25-OH vitamin D assay. Low incidence may very plausibly reflect conditions of inherited or induced variations in CYP2D activity known to affect ondansetron metabolism. As yet, however, we are unable to deduce a plausible structural identification of the interference derived from ondansetron or its known metabolites. Given longstanding and widespread use of LC-MS/MS for measurement of 25-OH-D using this IS, discovery of an IS interferent was surprising. The findings are a case study in the importance of a review of the metric plot (monitoring of IS across samples) as quality assurance for MS/MS assays.

## A-402

### Dried urine and blood spot analysis of essential and toxic elements by ICP-DRC-MS with an emphasis on inter-assay stability of samples kept at room temperature

T. T. Zava. *ZRT Laboratory, Beaverton, OR*

**Background**:

Elemental analysis plays a key role in monitoring the heath of individuals and populations around the world. The sensitivity and specificity of inductively coupled plasma mass spectrometry (ICP-MS) has allowed precise quantification of a broad range of elements using various sample types. The collection, storage, and transport of samples without preservatives at room temperature while maintaining accuracy would be advantageous for health surveys, specifically in remote areas without refrigeration. We aimed to validate an assay for, and show the stability of, essential and toxic elements in urine and whole blood collected on filter paper.

**Materials and Methods:**

Our laboratory developed dried urine and blood spot elemental assays using Whatman 903 filter paper for sample collection and a Perkin Elmer NexION 300D ICP-MS with Dynamic Reaction Cell Technology for analysis. We focused on elements that have shown clinical utility in population health surveys and wellness assessments. Analytes selected were iodine, bromine, selenium, arsenic, cadmium, and mercury in dried urine, and zinc, copper, magnesium, selenium, cadmium, lead, and mercury in blood spot.

Dried urine and blood spot samples were extracted in 96-well fritted filter blocks using dilute ammonium hydroxide, L-cysteine, ethylenediaminetetraacetic acid (EDTA), triton-X, and internal standards. Six 6-mm punches and 1.2 mL of extraction solution of were used for dried urine analysis while two 6-mm punches and 0.55 mL of extraction solution were used for blood spot analysis. Dried urine and blood spot assays were run separately on the ICP-MS in kinetic gas mode using helium, and employing a micro-flow pump and nebulizer to make effective use of the small sample volume.

**Results:**

Accuracy of the method was assessed by spotting onto filter paper available proficiency samples from the Centers for Disease Control and Prevention (CDC) and College of American Pathologists (CAP), which demonstrated excellent agreement with expected concentrations ($R^2$>0.96). Elements for which CDC or CAP samples were not available were validated against Seronorm and ClinChek Trace Elements Urine and Whole Blood controls. Recovery was demonstrated by spiking blood and urine samples with a known concentration of analyte; acceptable recoveries of 80-120% were obtained. Linearity was assessed by diluting samples and comparing results to expected concentrations, and found to be acceptable. Limits of quantification were based on analysis of blank and low level samples, and were found to be acceptable. Intra-assay precision was based on 20 sample replicates, and the coeffient of variation was <8.3% for all analytes. Inter-assay precision was tested during 14 sample runs over 1 month keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The coeffient of variation for inter-assay precision was <15.1% for all analytes.

**Conclusions:**

Dried urine and blood spot element analysis using ICP-MS was successfully validated. Demonstrated stability of elements in samples dried on filter paper allows accurate elemental analysis in population studies in remote areas without access to refrigeration, as samples can easily be collected, transported, and stored without the use of preservatives for at least a month.

## A-403

### Adapting High-Resolution Mass Spectrometry for Clinical Toxicology: Comparison and Optimization of SWATH to Data-Dependent Acquisition for Drug Screening

J. D. Whitman, K. L. Lynch. *University of California, San Francisco, San Francisco, CA*

**Background:**

The field of clinical toxicology is overrun with ever-changing synthetic drugs, adulterants, and unregulated supplements. Tandem mass spectrometry (LC-MS/MS) is not readily adaptable to these changes considering it relies on targeting expected ion fragments within a sample. High-resolution mass spectrometry (HRMS) has shown great success in unknown screening by allowing for compound detection through database comparison. The current standard for HRMS scanning is data-dependent acquisition (DDA), which acquires fragment ion spectra from the 10-20 most abundant precursor ions. This has come to show weaknesses in clinical and forensic toxicology where compounds of interest may be in low abundance compared to polypharmacy background or physiologic matrix.

Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH) is an emerging method of data acquisition that partitions all ions within a small mass window (e.g. 20 Da) for fragmentation so that no spectral data is lost. Additionally the size of these windows can be fixed (fSWATH) or varied (vSWATH) in order to separate similar compounds (e.g. amphetamine and methamphetamine). This study seeks to optimize and compare SWATH to DDA in clinical drug screening to assess and validate the fidelity of this emerging method and provide a model for adoption in the clinical laboratory.

**Methods:**

Urine samples were diluted 1:10 and separated with a Kinetex C18 column (50x3mm, 2.6µm) (Phenomenex). Data was acquired on a TripleTOF®5600 (SCIEX) in positive-ion mode. Analsyt TF® software (SCIEX) was used to create three acquisition modalities; DDA: TOF-MS survey scan (100-650 Da) with triggered-collection of 20 product ion scans; fSWATH: SWATH acquisition (100-650 Da) with 30 fixed 18 Da windows; vSWATH: SWATH acquisition (100-650 Da) with 30 variable mass windows (6-59 Da) created to specifically separate similar drugs.

These methods were compared by limit of detection of 88 drug-spiked urine samples (5ng/mL-100ng/mL; duplicate runs) and drug/metabolite detection in 50 clinical samples previously characterized by LC-MS/MS.

**Results:**

Drug/metabolite limit of detection (LOD) was the lowest in vSWATH compared to fSWATH and DDA. vSWATH had the lowest LOD for 37(43%) of the drugs/metabolites as compared to 20(22%) for DDA; however in many cases the difference in LOD was 5-15 ng/mL. Thirty-five(31%) drugs/metabolites had equal LODs between vSWATH and DDA.

Detection of drugs/metabolites in clinical urine samples was similar for DDA and vSWATH (275 and 274, respectively). vSWATH detected 92% of compounds previously found where DDA and targeted LC-MS/MS confirmed 90%.

Additionally, 5 low-concentration compounds were confirmed by vSWATH that were negative by DDA (not triggered as abundant fragments), but could be observed in the extracted ion chromatogram.

**Conclusion:**

This study demonstrates that HRMS acquisition by vSWATH was optimal to DDA and fSWATH for clinically-relevant drug detection in both spiked urine and clinical samples. Furthermore, we demonstrated that vSWATH was able to accurately detect a number of low-abundance compounds that DDA missed due to biased acquisition of abundant ions. These findings are novel and clinically relevant considering they are the first to compare these four acquisition methods (LC-MS/MS, DDA, vSWATH, and fSWATH) and provide a model for optimizing HRMS platforms already in use.

## A-404

### Analytical and Clinical Validation of a Novel Metabolite-based Serum Test to Precisely Determine the Glomerular Filtration Rate (GFR)

L. Ford[1], Q. Zhang[1], T. Freed[1], J. Conner[1], J. Coresh[2], L. Inker[3], A. Levy[3], D. Toal[1]. *[1]Metabolon, Inc., Research Triangle Park, NC, [2]Johns Hopkins Medical Institutions, Baltimore, MD, [3]Tufts Medical Center, Boston, MA*

**Background: :** Traditionally, glomerular filtration rate (GFR), an indicator of kidney function, is estimated using equations based on creatinine combined with age, sex, and race (eGFR) because the gold standard methodology, measured GFR (mGFR), is complex, time-consuming, invasive, and expensive. The commonly used eGFR equations show limited precision and accuracy against mGFR. We have developed and validated a method to estimate GFR from a single serum sample using LC-MS/MS, using no demographics. The test measures four analytes: pseudouridine, acetylthreonine, phenylacetylglutamine, and tryptophan. The clinical performance of the assay has been validated against mGFR and eGFR.

**Methods:** The four analytes were measured using LC-MS/MS on an Agilent 1290-Sciex Qtrap 5500 UPLC/MS/MS system. Creatinine was also included and validated to use for clinical comparisons. All analytes were assayed in one run, with a run time of less than 4 minutes. The precision in serum was validated at the low, mid, and high points of the calibration range for each analyte. Linearity, precision, accuracy, LLOQ, recovery, specificity, interference, matrix effect, carryover, method comparison, ruggedness, and stability validation experiments were performed. Analyte results were used in a logistic regression analysis against mGFR data to generate a 4-term equation to calculate GFR. The clinical performance of the metabolite-based equation was compared to that of eGFR estimated by the CKD-EPI equation. Clinical performance of the method was evaluated against mGFR in1,618 patient samples, equations developed, then validated in an additional 811 samples drawn randomly from four different cohorts (AASK, MDRD, CRISP, AGES-kidney). The sample cohorts included normal or reduced GFR patients with and without known kidney disease, of varying age and race; average mGFR result was 55 (26 SD) and ranged from 6-169 in the overall cohort.

**Results:** The assay for pseudouridine, acetylthreonine, phenylacetylglutamine, tryptophan, and creatinine was validated according to CLSI guidelines in a 20-day validation with two runs per day. All five of the analytes performed robustly over the 20-days with %CVs ranging from 3.7% to 6.9%. All other validation tests met acceptance criteria. Clinically, concordance with mGFR was better for the four metabolite-based test vs. for CKD-EPI and MDRD Study eGFR (0.68 vs. 0.55 and 0.54 in development, and 0.68 vs. 0.58 and 0.56 in validation, $p<0.001$). The rate of large errors expressed as $1\text{-}P_{30}$ was 16. 3% for CKD-EPI, 16. 6% for MDRD but only 9.4% for our four metabolite-based test in development (14.3% and 14.2% vs. 10.0% in validation), equivalent to a 30-40% reduction in the rate of large errors ($p<0.001$). Added precision translates into more accurate diagnosis at the threshold of 60 ml/min/1.73m2 , the cutpoint used to diagnose kidney disease. At 60 ml/min/1.73m2 , the four metabolite-based test led to a reduction in the rate of misclassification of more than 30%.

**Conclusion:** In summary, we developed and validated a robust metabolite assay which substantially improves precision and accuracy of GFR estimation, thus having the potential to improve clinical care when greater GFR accuracy is needed.

## A-405

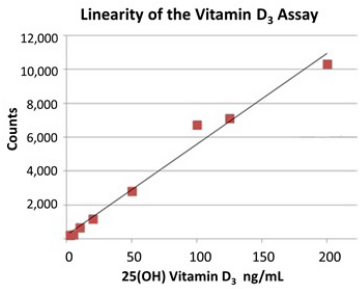
### Fast, New Derivatization Method for Vitamin D Analysis by LCMS

T. Schlabach, J. Im. *Novilytic LLC, West Lafayette, IN*

**Background:** Vitamin D analysis has become a major clinical test with millions of tests performed annually. Liquid Chromatography/Mass Spectrometry (LCMS) is the gold standard for the determination of vitamin D. Native vitamin D does not produce a strong signal in LCMS because its lacks a positive charge. Derivatization agents for vitamin D have addressed this problem but these reactions are slow. This new derivatization agent forms a vitamin D derivative in just 2 minutes.

**Methods:** Vitamin D plasma reference standards were obtained from NIST (Gaithersburg, MD) from 2014 to 2016. Human blood was collected in accordance with our IRB protocol. A portion was used to generate plasma by centrifugation. An internal standard (d6-vitamin D) from Medical Isotopes (Pelham, NH) was added and then plasma was extracted using methyl tert-butyl ether. The ether layer containing vitamin D was separated and evaporated to dryness. Reagents were obtained from a vitamin D kit developed by Novilytic. An aliquot was injected onto a C18 column followed by gradient elution. Vitamin D was determined with an AB Sciex 4000 mass spectrometer.

**Results:** The Vitamin D derivatization method was evaluated with plasma reference standards obtained from the NIST Quality Assurance Program (VitDQAP) from 2014 to 2016. The vitamin D results agreed within 5% of the specified target levels for these NIST samples. The within-run precision was 6% or better and the average CV was 3.1%. These results were in the top 20% of all LCMS submissions. The linearity of the Vitamin D assay was measured by spiking plasma with varying amounts of the heavy isotope form of the vitamin $D_3$ from 5 to 200 ng/mL. The coefficient of determination ($R^2$) was 0.982 over the assay range. **Conclusion:** A new derivatization reagent reduces sample preparation time and improves the analysis of Vitamin D by LCMS.




## A-406

### Clinical validation of an LC-MS/MS method for tenofovir and emtricitabine in urine test

C. Lu. *Global Molecular Labs, Plano, TX*

**Background:** Tenofovir and emtricitabine (brand name, Truvada) are widely-prescribed antiretroviral drugs used for the treatment and prophylaxis of HIV (Human immunodeficiency virus) infection. They can also be used against Hepatitis B virus infection. High risk groups for HIV infection of people are recommended for pre-exposure prophylactic (PrEP) consideration by FDA. As Truvada only works when it is taken regularly in order to maintain the reduction of the incidence of HIV infection, it is suitable for clinical or research studies to monitor patients in special populations. Another consideration for testing in urine would be the less invasive sample collection as compared to that of blood testing. **Methods:** Urine samples are processed by using acetonitrile for protein precipitation. Tenofovir and emtricitabine were separatedby Poroshell 120 EC-C18 column and dected with with positive electrospray ionization mode by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).**Results:** A LC-MS/MS method is developed and validated for testing tenofovir and emtricitabine in urine. The assay was validated over the range of 2 ng/mL to 1000 ng/mL for both analytes. The accuracy of both tenofovir and emtricitabine fell in the acceptance range 90-110% with a recovery range 100±10%. The assay was linear for both analytes with linear regression coefficient ranging 0.995-1.000. Good precision was observed in this assay and the percentages of coefficient of variation were less than 15% (intra-assay precision was between 2.3 and 5.5%; inter-assay precision was between 4.9 to 6.4%). No carryover was observed. No significant interference with other common over-the-counter drugs or elevated levels of proteins in urine is observed and individual internal standard for each analyte is used to compensate for any possible matrix effect. LLOQs of tenofovir and emtricitabine are 1.92 ng/mL and 1.88 ng/mL respectively. **Conclusion:** This method can be applied for the determination of tenofovir and emtricitabine use of any patients treated with these drugs.

## A-407

### Implementation and Validation of Liquid Chromatography Mass Spectrometry Method for the Quantitation of Immunosuppressive Drugs

S. H. Sobki, A. L. Almotiri, T. A. Alahmari, A. T. Dalupang, A. Y. Algharawi. *PSMMC, Riyadh, Saudi Arabia*

**Background**: Measurement of immunosuppressive drug concentration in blood is an important application of the Therapeutic Drug Monitoring (TDM) concept. Therapeutic drug monitoring of immunosuppressive drugs in organ-transplanted patients is crucial to prevent intoxication or transplant rejection due to inadequate dosage. After transplantation, there is a need for immunosuppressive treatment with individualized drug dosing and continuous life-long monitoring. Recently, LC MS/MS was installed in the laboratory for analysis of tacrolimus, cyclosporine and sirolimus as LC MS/MS is the preferred technique for the assessment of immunosuppressive drugs. The commonly used immunoassays have been gradually undergoing replacement by mass spectrometry, since this physical method offers both a higher sensitivity and specificity and low cost of analysis as well. The objective of this study is to evaluate the performance of Shimadzu LC MS/MS using Recipe® reagents for the analysis of tacrolimus, cyclosporine, and sirolimus.

**Methods**: The validation was performed using the on-line SPE LCMS/MS method for immunosuppressant therapeutic drug monitoring (Recipe GmbH, Germany) on analytical platform Shimadzu HPLC NEXERA X2 – LCMS-8050. A total of 100 samples were evaluated in this study. Samples were collected in 5 ml EDTA tubes from adult renal transplant, bone marrow transplant and pediatric liver transplant patients. Precision study was verified according to CLSI EP05-A2 using Recipe Clincheck controls in three concentration levels: 48.3 nmol/L, 98.1 nmol/L and 189 nmol/L for Cyclosporine A ; 4.37 ng/ml, 13.2 ng/ml and 21.5 ng/ml for Sirolimus ; 3.93 ng/ml, 8.13 ng/ml and 15.9 ng/ml for Tacrolimus and coefficient of variations (CV's) were calculated. Verification of Linearity was verified according to CLSI EP06-A using ClinCal®-Calibrator with six (6) different concentration spanning the analytical range of each assay.

**Results**: All % CVs were consistent with those claimed by the manufacturer for all tests . The method was found to be linear over the range of 0.017 - 1455 nmol/L for cyclosporine; 0.014 - 65 ng/mL for tacrolimus and 0.24 - 72 ng/mL for sirolimus. Analytical precision revealed a coefficient of variation of less than 10% on all assay. Analytical sensitivity was 0.017 nmol/L, 0.014 ng/mL. 0.24 ng/mL for cyclosporine, tacrolimus and sirolimus respectively. Recovery ranged from 82.5 to 92.0% for Cysclosporine A; 70.8 to 106.0 % for Sirolimus and 89.1 to 115.5 % for Tacrolimus. Matrix effects were well compensated by deuterated internal standards. Furthermore, the obtained results from the proficiency testing program (College of American Pathologists) were within the target value.

**Conclusion**: Overall performance of the immunosuppressive drugs on Shimadzu LC MS/MS using Recipe Reagents was acceptable. It provides reliable results for the required tests for all transplant services. Verification of the method has confirmed that all set criteria are achieved and suitable for routine use in clinical laboratory as the LC MS/MS technique provides a viable platform for the analytical routine service for Therapeutic Drug Monitoring of immunosuppressive drugs that meet clinical needs of reporting timely and reliable results.

## A-408

### Determination of Voriconazole in human plasma by ultra-liquid chromatography-tandem mass spectrometry

Z. Yuangao, S. Zhenzhen, B. Yangjuan, T. Jiangtao, W. Lanlan. *China Hospital of Sichuan University, Chengdu, China*

**Background:**

Voriconazole(VCZ), a triazole antifungal agent, was approved for the treatment of invasive fungal infection with a broad spectrum, including Aspergillus Cryptococcus and Candida species. However, a high incidence of adverse reactions may occur during the treatment, such as liver dysfunction and neurological toxicity. Because of the above findings, it has been suggested that the blood concentration of VCZ should be maintained between 1.0 and 5.5μg/mL and the measurement of blood levels could assist with decisions about dose adjustment. So we develop a sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method to determine VCZ concentration in human plasma.

**Methods:**

We built a simple UPLC-MS-MS method for quantifying VCZ concentration in human plasma, using Cyproheptadine as an internal standard (IS). VCZ and IS were extracted from plasma samples by liquid-liquid extraction with 1 ml of Methyl Tertiary Butyl Ether. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column (2.1*50mm, 1.7um) using an isocratic mobile phase system composed of acetonitrile and 0.02mol/L NH4Ac containing 0.1% formic acid (40:60, v/v) at a low rate of 0.30 mL/min. Mass spectrometric analysis was performed using a TQ-S mass spectrometer coupled with an electrospray ionization source in the positiveion mode. The multiple reaction monitoring (MRM) mode was used, and the transitions selected for quantification were m/z 350.4→m/z 127.2 and m/z 288.4→ m/z 96.2for VCZ and IS, respectively.

**Results:**

Good linearity (R2= 0.9991) was observed throughout the range of 0.0005-10ug/ml in 0.1 ml plasma. The overall accuracy of this method was 99.2-109.5%, and the lower limit of detection was 0.25ng/ml. The intra- and inter-day variations were lower than 3.84% and 6.72%, respectively. This method was used to examine the VCZ concentrations of 83 patients, the blood concentration levels of VCZ were between 0.32 and 7.75ug/ml.

**Conclusion:**

A UPLC-MS/MS method for the determination of VCZ in human plasma was developed and validated. This method was rapid, sensitive, specific, selective, reproducible, and successfully applied in therapeutic drug monitoring of VCZ.

## A-409

### Clinical validation and comparison of serotonin analysis in various blood fractions for the follow-up of neuroendocrine tumor patients

H. van Rossum[1], A. C. Heijboer[2], D. van den Broek[1], M. E. T. Tesselaar[1], O. van Tellingen[1], C. M. Korse[1]. *[1]NKI-AvL, Amsterdam, Netherlands, [2]VUMC, Amsterdam, Netherlands*

**Background:** Serotonin, an endogenous neurotransmitter and paracrine agent, is used in the diagnosis and follow-up of neuroendocrine tumors (NET). We recently developed a LC-MSMS based method for serotonin analysis in serum and platelet-rich plasma (PRP). Here, we study the clinical application of various blood fractions for serotonin analysis used for the follow-up of NET patients. **Methods:** 94 patient samples obtained from 78 patients visiting our NET outpatient clinic were collected. Furthermore blood samples of 112 healthy volunteers were used for determination of the upper limits of normal for serum and PRP serotonin. Serum and PRP serotonin concentrations were determined using the LC-MSMS method and whole blood serotonin analysis was performed using the Chromsystems HPLC-ECD method. Method comparisons were performed using Passing-Bablok regression and Spearman's correlation analysis. Furthermore serotonin concentrations of the healthy volunteers, 14 NET patients without evidence of disease and 51 NET patients with evidence of disease were compared. **Results:** All obtained correlation coefficients were 0.98 and the slope of the whole blood versus serum regression was not significantly different from 1. The slopes obtained when comparing whole blood and serum serotonin with PRP serotonin were 0.74 and 0.71 respectively. NET patients with confirmed evidence of disease had significantly higher whole blood, serum and PRP serotonin concentrations when compared to NET patients without evidence of disease and healthy volunteers. **Conclusion:** Our results suggest that as long as serotonin is expressed per platelet, serotonin results obtained from whole blood, serum and PRP seem to be interchangeable and a similar clinical performance can be expected.

## A-410

### A Clinical Research UPLC-MS/MS Method for the Quantitative Analysis of Urinary Free Cortisol

R. Sanig, H. Brown, G. Hammond, L. Calton. *Waters Corporation, Wilmslow, United Kingdom*

**Background:** Cortisol is a glucocorticoid (steroid hormone) produced in the adrenal glands and is connected with stress response in humans. A clinical research method has been developed to aid hypercortisolism research using UPLC-MS/MS.

**Methods:** Samples (50 µL) were prepared with cortisol-$^2H_3$ internal standard in methanol, diluted with aqueous trichloroacetic acid (0.5 %), vortex mixed, centrifuged and analysed directly (10µL). Chromatographic separation was achieved, in less than 3 minutes, using a Waters® ACQUITY UPLC® CSH C18 column (2.1 x 30 mm, 1.7 µm) with a water/methanol/ammonium acetate/formic acid gradient on the Waters ACQUITY UPLC I-Class system. Cortisol was detected using electrospray positive ionization with multiple reaction monitoring on the XEVO® TQD mass spectrometer. The precursor to product ion transitions used for the detection of cortisol were *m/z* 363.2 > 121.0 (quantifier ion) and 97.0 (qualifier ion) and *m/z* 366.2 > 124.0 for the detection of cortisol-$^2H_3$. In-house calibrators (13.8-1103.0 nmol/L) and quality controls (20.7, 41.4, 206.9, and 827.7 nmol/L) were prepared using cortisol reference material from Cerilliant (Round Rock, TX) in phosphate-buffered saline.

**Results:** The method was shown to be linear from 4.0 to 1340.0 nmol/L, with total precision and repeatability coefficients of variation (CV) for the four quality control levels all ≤6.20 % (n = 5, days = 5). Analytical sensitivity of the method allows for cortisol quantification at 1.38 nmol/L (≤20 %, inter-day CV). The method demonstrated no interferences from analogous steroids. Carryover following a 1655.4 nmol/L extracted sample was below the limit of quantification. Minimal matrix effects were observed and recovery was unaffected (mean 100.5 %, range 93.9-105.6 %) when six urine pools were spiked with 20.7, 41.4, 206.9, and 827.7 nmol/L cortisol. The accuracy of the method was determined by analysing samples (years 2015-2016, n=48) from the UK NEQAS Scheme (Birmingham, UK); good agreement was obtained with a mean method bias of ≤4.1 % for all samples (Altman-Bland).

**Conclusions:** A rapid, simple and cost-effective UPLC-MS/MS procedure for the determination of urinary free cortisol concentrations has been developed for clinical research. The method has shown good analytical sensitivity, selectivity, linearity, precision and accuracy.

For Research Use Only, Not for use in diagnostic procedures.

Keywords: Cortisol, Urine, Mass Spectrometry, LC-MS/MS

## A-411

### Mucopolysaccharides quantitation in dried blood spots by liquid chromatography-tandem mass spectrometry

J. Lacey, K. Raymond, D. Gavrilov, D. Oglesbee, P. Rinaldo, D. Matern, S. Tortorelli. *Mayo Clinic, Rochester, MN*

**Background:** Mucopolysaccharides (MPS) are sulfated polysaccharides that contain repetitive disaccharide units attached to a protein core. The various mucopolysaccharidoses exhibit different patterns of excretion of four mucopolysaccharides: dermatan (DS), heparan (HS), keratan (KS S1 and KS S2) and chondroitin sulfate (CS). We describe a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for routine determination of DS, HS, KS S1, KS S2 and CS in dried blood spots (DBS), as an efficient and effective screening test for MPS-I/MPS-II, MPS-III, MPS-IV and MPS-VI.

**Method:** Four 1/8" disks from a DBS are utilized for DS, HS and KS determination (two disks) and CS determination (two disks). DS, HS and KS are enzymatically digested to disaccharides by the addition of heparinase I, II, III and chondroitinase B, while CS is enzymatically digested to disaccharides by chondroitinase AC. Elution mixture rotates at 30°C for 120 minutes. The reaction is stopped by the addition of EDTA buffer including internal standard GlCNCoEt-6S. The reaction mixture is then centrifuged and subjected to LC-MS/MS analysis. The MS/MS is operated in the multiple reaction monitoring negative mode to follow the precursor to product species transitions for DS (*m/z* 458.0 to *m/z* 300.0), HS (*m/z* 378.0 to *m/z* 175.0), KS S1 (*m/z* 462.0 to *m/z* 361.0), KS S2 (*m/z* 542.0 to *m/z* 462.0), CS (*m/z* 458.0 to *m/z* 282.0) and the internal standard GlCNCoEt-6S (*m/z* 472.0 to *m/z* 97.0).

**Results:** Inter-assay calibration curves (N=3) were linear and reproducible over the concentration range 0-600 nmol/L for DS, HS and KS S2, 0-1200 nmol/L for KS S1 and 0-1800 nmol/L for CS. Intra- and inter-assay precision were assessed using three DBS samples of varying concentrations (DS = 118, 219 and 318 nmol/L; HS = 96, 217 and 290 nmol/L; KS S1 = 411, 603 and 640 nmol/L; KS S2 = 140, 227 and 265 nmol/L; CS = 408, 862 and 760 nmol/L). Intra-assay precision CVs were 13.0, 10.4 and 11.5% for DS; 16.9, 13.5 and 8.1% for HS; 11.1, 16.3 and 9.2% for KS S1; 12.0, 14.0 and 11.3% for KS S2; 14.1, 20.6 and 17.2% for CS, respectively (N=20). Inter-assay precision CVs were 14.4, 10.5 and 17.1% for DS; 20.9, 18.7 and 18.1% for HS; 16.9, 13.0 and 16.7% for KS S1; 16.3, 13.8 and 14.3% for KS S2; 35.2, 19.5 and 20.6% for CS, respectively in the same specimens (N=20). Two DBS specimens spiked with DS, HS and KS S2 (100 and 400 nmol/L), KS S1 (200 and 800 nmol/L) and CS (300 and 1200 nmol/L) standard solutions exhibited recoveries ranging from 87% - 113%. Newborn DBS (N=208), pediatric DBS (age > 2 week - 18 years) (N=97) and adult DBS controls (N=125) were analyzed for reference range determination. Clinical sensitivity was 100% for MPS-I (N=10), MPS-II (N=6), MPS-III (N=5), MPS-IVA (N=4) and MPS-VI (N=3). Low molecular weight heparin and sodium or lithium heparin interfere with HS determination.

**Conclusions:** Preliminary data show that our test is a rapid and specific method for timely identification of patients with MPS-I, MPS-II, MPS-III, MPS-IV and MPS-VI.

## A-412

### Serum Vitamin E, metabolite and derivatives determination by LCMSMS for research use

R. M. Doyle. *Thermo Scientific, Inc, Somerset, NJ*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC-MS/MS) is suited for rapid analysis of multiple analytes. Vitamin E contributes to the normal maintenance of biomembranes, the vascular system, and the nervous system, and provides antioxidant protection. The Vitamin E, derivatives and metabolites analyzed included Tocopherol (Alpha, Beta, Gamma and Delta), Tocotrienol (Alpha, Beta, Gamma and Delta), Tocofersolan, derivatives such as acetate and palmitate and metabolites. A highly sensitive and specific LC-MS/MS analytical method has been developed for the determination of vitamin E, it's derivatives and metabolites in serum. A simple sample preparation technique that involved a simple liquid-liquid extraction was utilized along with a one (1D) dimensional liquid chromatographic configuration. The described method achieves the required sensitivity and is capable of determining the vitamin E, it's derivatives and metabolites over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed for the quantitatively measurement of vitamin E, it's derivatives and metabolites in serum.

Method: A Thermo Scientific™ Endura™ tandem mass spectrometer in positive Electrospray mode and a Thermo Scientific™ Dionex™ Vanquish™ Horizon HPLC system were utilized for this analysis. 200 µl of serum were used for the analysis

of the vitamin E, it's derivatives and metabolites in serum. Various columns were evaluated and an Thermo Scientific™ Accucore™ C18 100 x 2.1 mm, 1.5 µm with a water:methanol mixture containing 5 mM Ammonium Formate and 0.1% Formic Acid achieved baseline chromatographic separation for all the vitamin E, it's derivatives and metabolites in serum in less than 8 minute run time. Quantitative analysis was performed using selective reaction monitoring (SRM) with transition pairs for each analyte and internal standard in positive mode and the precision and accuracy of the method was verified using pooled quality control materials and serum samples.

Result: Good linearity and reproducibility were obtained with the concentration range of 1 to 50000 ng/ml for the respective vitamin E, it's derivatives and metabolites in serum with a coefficient of determination >0.95 for the sample preparation technique and the LC-MS/MS configuration used. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.25 to 1 ng/ml and excellent reproducibility was observed for all compounds (CV < 10%).

Conclusion: A sensitive, simple, specific and accurate liquid chromatography tandem mass spectrometry method was developed and verified for the simultaneous determination of vitamin E, it's derivatives and metabolites in serum. The sample preparation technique is quick and easily applied for high throughput analysis.

## A-413

### Simple sample preparation technique for the determination of Metanephines, Catecholamines and their metabolites in Urine by LCMSMS for research use

R. M. Doyle. *Thermo Scientific, Inc, Somerset, NJ*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC-MS/MS) is suited for rapid analysis of multiple analytes. The metanephrines and catecholamines are compounds involved neuromodulation and act as hormones. The Metanephines, Catecholamines and their metabolites analyzed included metanephrine, normetanephrine, dopamine, epinephrine, noepinephrine, 3-methoxytyramine, vanillylmandelic acid, homovanillic acid and 5-Hydroxyindole Acetic Acid. A highly sensitive and specific LC-MS/MS analytical method has been developed for the determination of Metanephines, Catecholamines and their metabolites in urine. A simple sample preparation technique that involved liquid-liquid extraction and diphenyl-boronate complexing was utilized along with a one (1D) dimensional liquid chromatographic configuration. The described method achieves the required sensitivity and is capable of determining the purine and pyrimidine metabolites over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed for the quantitatively measurement of Metanephines, Catecholamines and their metabolites in urine.

Method: A Thermo Scientific™ Endura™ tandem mass spectrometer in positive and negative Electrospray mode and a Thermo Scientific™ Dionex™ Vanquish™ Horizon HPLC system were utilized for this analysis. 500 µl of urine were used for the analysis of the Metanephines, Catecholamines and their metabolites in urine. Various columns were evaluated and an Thermo Scientific™ Accucore™ PFP 100 x 2.1 mm, 3 µm with a water:methanol mixture containing 0.2% Formic Acid achieved baseline chromatographic separation for all the Metanephines, Catecholamines and their metabolites in urine in less than 8 minute run time. Quantitative analysis was performed using selective reaction monitoring (SRM) with transition pairs for each analyte and internal standard in positive and negative mode and the precision and accuracy of the method was verified using pooled quality control materials and urine samples.

Result: Good linearity and reproducibility were obtained with the concentration range of 5 to 1000 ng/ml for the respective Metanephines, Catecholamines and their metabolites in urine with a coefficient of determination >0.95 for the sample preparation technique and the LC-MS/MS configuration used. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 2 to 5 ng/ml and excellent reproducibility was observed for all compounds (CV < 10%).

Conclusion: A sensitive, simple, specific and accurate liquid chromatography tandem mass spectrometry method was developed and verified for the simultaneous determination and screen of Metanephines, Catecholamines and their metabolites in urine. The sample preparation technique is quick and easily applied for high throughput analysis.

## A-414

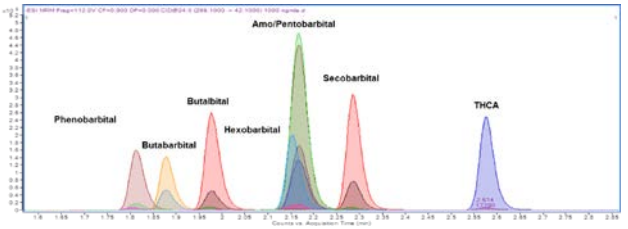
### A Fast LC/MS/MS Method for the Simultaneous Analysis of Barbiturates and 11-nor-9-Carboxy-$\Delta^9$-Tetrahydrocannabinol (THCA) in Urine Using Negative ESI Ionization Mode and Alternate Column Regeneration (ACR)

A. Szczesniewski. *Agilent Technologies, Wood Dale, IL*

**Background:** Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is suited for rapid, simultaneous analysis of multiple analytes. A fast, highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of Barbiturates and 11-nor-9-Carboxy-$\Delta^9$-Tetrahydrocannabinol (THCA) in urine using the negative ESI ionization mode. Barbiturates included; Amobarbital, Butalbital, Butabarbital, Hexobarbital, Pentobarbital, Phenobarbital and Secobarbital. Simple sample preparation techniques such as dilute and shoot and a short chromatographic configuration achieved good analytical sensitivity and was capable of quantitating all analytes over a wide dynamic range. **Methods:** An Agilent 6470 tandem mass spectrometer with Jet Stream technology in negative electrospray (ESI) mode and an Agilent Infinity II 1290 UHPLC system were utilized for this analysis. Use of the ACR was achieved by addition of second pump and 2 position 10 port switching valve for the reduction of method runtime that excluded column equilibration time. A 100 ul aliquot of urine was used for the analysis of Barbiturates and THCA. The Agilent Polaris C18-Ether, 100 x 2.0 mm, 3.0 µm column with a water:acetonitrile mixture containing a 5 mM Ammonium Acetate gradient achieved chromatographic separation in less than 3 minutes. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and an internal standard in the negative mode.

**Results:** The isobaric pair, Amobarbital and Pentobarbital, were not separated under these chromatographic conditions. Good linearity and reproducibility were obtained for the concentration range from 5 to 1000 ng/ml with a coefficient of determination >0.995 for all analytes. Excellent reproducibility was observed for all analytes (CV < 15%).

**Conclusion:** A fast, specific and accurate quantitative liquid chromatography mass spectrometry (LC/MS/MS) method was developed and verified for the simultaneous measurement of Barbiturates and THCA in urine.For Research Use Only. Not for use in diagnostic procedures.




## A-415

### A 5.0 Minute LC/MS/MS Method with Alternate Column Regeneration (ACR) for the Analysis of >100 Various Drugs and Their Metabolites in Urine

A. Szczesniewski. *Agilent Technologies, Wood Dale, IL*

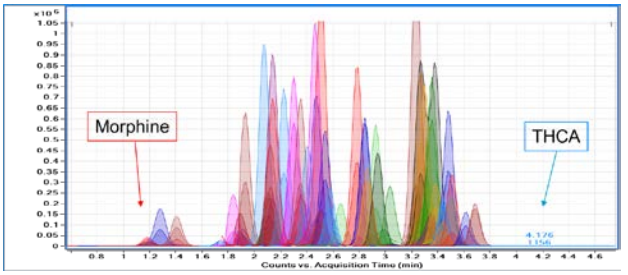
**Background:** Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is well suited for the rapid analysis of large numbers of analytes using a single method. A highly sensitive, specific and fast LC/MS/MS analytical method has been developed for the quantitation of over 100 drugs of the following drug classes: antidepressants, benzodiazepines, opioids, muscle relaxants, hallucinogens, stimulants. The described method achieves high analytical sensitivity and is capable of quantitating analytes over a wide dynamic range, in addition the Alternate Column Regeneration (ACR) hardware configuration was employed to significantly increase the sample throughput.

**Methods:** An Agilent 6470 tandem mass spectrometer with Jet Stream technology in positive and negative switching Electrospray mode and an Agilent InfinityII 1290 UHPLC system were utilized. ACR utilization was achieved by addition of second pump and 2 position 10-port switching valve for the reduction of method runtime that excluded column equilibration time. A 100ul urine aliquot was used for the analysis. Various columns were evaluated and an Agilent Poroshell 120 EC-C18 100x2.1mm, 1.9um with a water:methanol mixture containing 0.01% formic acid and 5mM ammonium formate achieved chromatographic separation using a less than

4.6 minute gradient. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standards in positive and negative modes.

**Results:** Good linearity and reproducibility were obtained with a concentration range from 1ng/ml to 1000ng/ml for most of the analytes with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. For some analytes, a quadratic curve fit was used. Excellent reproducibility was observed for all analytes (CV <15%) for all techniques and configurations.

**Conclusion:** A fast, sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of over 100 various drugs and their metabolites in urine. For Research Use Only. Not for use in diagnostic procedures.




## A-416

### Impact of Matrix-assisted Laser Desoprtion/Ionization Time of Flight Mass Spectrometry for Rapid Microbial Identification in a Community Hospital

S. E. Dawson[1], N. Collar[2]. [1]*Swedish Covenant Hospital, Chicago, IL,* [2]*North Park University, Chicago, IL*

In an effort to accelerate organism identification in positive blood cultures, and facilitate targeted antibiotic treatment sooner, our community hospital implemented the use of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF MS®).

**Goal:** The goal of this study was to determine if the implementation of MALDI reduced organism identification times, and subsequently resulted in any reductions in patient length of stay (LOS) or charges.

**Method:** All positive blood cultures from January-June 2014 (pre-MALDI group) were compared to those from January-June 2016 (MALDI group). Time from "positive bottle to organism ID" (PBID), LOS, and charges were determined for all specimens.

**Results:** The table below shows the average PBID time and range, the average LOS and the average charges.

| | n | Avg PBID(hr) | Time range PBID (hr) | Avg LOS days | Avg Charges |
|---|---|---|---|---|---|
| Gram Pos (GP) 2014 - ALL | 70 | 22.90 | 9.1-70.8 | 8.44 | $123,188 |
| Gram Pos (GP) 2016 - ALL | 71 | 12.46 | 3.3-49.40 | 8.3 | $95,958 |
| Gram Neg (GN) 2014 - ALL | 92 | 43.54 | 21.8-92.6 | 7.63 | $88,292 |
| Gram Neg (GN) 2016 - ALL | 71 | 9.76 | 3.73-22.1 | 6.95 | $79,758 |
| Gram Pos (GP)2014 – uncomplicated sepsis | 30 | 22.44 | 9.6-47.5 | 9.07 | $113,837 |
| Gram Pos (GP)2016 – uncomplicated sepsis | 26 | 13.36 | 6-19.7 | 7.92 | $90,504 |
| Gram Neg (GN) 2014 – uncomplicated sepsis | 53 | 43.04 | 21.8-92.6 | 7.62 | $84,172 |
| Gram Neg (GN) 2016 – Uncomplicated sepsis | 39 | 9.07 | 3.7-16.9 | 5.18 | $52,675 |
| Yeast 2014 | 8 | 40.12 | 19.4-69.9 | n/a | n/a |
| Yeast 2016 | 7 | 13.18 | 6-23.6 | n/a | n/a |
| Contaminants 2014 | 97 | 29.04 | 9.13-8.62 | n/a | n/a |
| Contaminants 2016 | 50 | 15.33 | 6-40 | n/a | n/a |

The average PBID time decreased by 45.6% for GP, 77.6% for GN, 40.5% GP for uncomplicated sepsis, 78.9% for GN uncomplicated sepsis, 47.2% for contaminants, and 67.1% for yeast. The "ALL" population patients included all diagnoses/procedures, many not associated with sepsis. The LOS and charges were dependent on the severity of the diagnosis and procedures. We investigated if the shorter PBID times provided better LOS outcomes for the subset of "uncomplicated" sepsis patients (no septic shock, severe sepsis or bacteremia). For this subset, the LOS for gram positive sepsis patients dropped from 9.07 days to 7.92 days and associated charges dropped 15.89% (unadjusted for inflation). The LOS for gram negative sepsis patients dropped from 7.62 days to 5.18 and charges fell 36.25% (unadjusted).

**Conclusion:** The implementation of MALDI TOF for identification of positive blood cultures markedly decreased the time to identification for all positive blood cultures. It appears from this study that the shorter time to ID, led to shorter LOS and lower charges for all patients and more significant decreases were noted with uncomplicated sepsis.

## A-417

### Improved Liquid Chromatography Mass Spectrometric Determination of Vitamin D Metabolites in Human Plasma/Serum by Phospholipid Removal with Zirconia Sorbents

X. Lu, D. S. Bell. *MilliporeSigma, Bellefonte, PA*

**Background (Objective)**

Liquid chromatography with mass spectrometry (LC/MS) has been gaining more and more popularity in the clinical analysis due to the unparalleled speed, selectivity and sensitivity. However, the application of LC/MS to the determination of vitamin D metabolites in plasma/serum has been compromised by the considerable matrix effects. The present study is attempt to the development of a simple and quick method to remove the highly abundant phospholipids as well as proteins in plasma/serum and thus reduce the matric effects.

**Method**

Zirconia sorbents packed into a 96-well plate were exploited for the selective removal of phospholipids from the matrices. Proteins in the matrices were firstly precipitated by the addition of organic solvent such as acetonitrile and methanol, into the plasma/serum samples. This can be done either inside or outside the 96-well plate. The protein-precipitated samples were then passed through the 96-well plate where the phospholipids were retained by the zirconia sorbents but vitamin D metabolites went through and were collected. The resulting samples were directly injected for LC/MS/MS analysis with a pentafluorophenyl (F5) phase HPLC column and triple quadrupole mass spectrometer.

**Results**

Four vitamin D metabolites including 25-OH vitamin D3 (D3), 3-epi-25-OH-vitamin D3 (epi-D3), 25-OH vitamin D2 (D2), 3-epi-25-OH vitamin D2 (epi-D2) spiked in human plasma, are separated and determined by the LC/MS/MS. Without phospholipid removal, the signals of the vitamin D metabolites in the plasma are about half of those of vitamin D neat standards in solutions. This indicates considerable matrix effects. Further study reveals multiple phospholipid species of high intensity, 1,000,000 to 7,000,000 cps, co-elute with the vitamin D metabolites. The zirconia sorbent-packed 96-well plate were exploited and found consistently removing >99.5% of the phospholipids from the plasma and the intensity of the phospholipids drops to <5,000 cps. Additionally, the signals of vitamin D metabolites in plasma are fully restored to that of the neat standards. The reproducibility of the recovery of the vitamin D metabolites ranges from 4% to 12%.

**Conclusion**

A method has been developed for the LC/MS/MS determination of vitamin D metabolites in human plasma with minimum matrix effects. The method utilizes zirconia sorbent-packed 96-well plate for quick and efficient phospholipid removal. The signals and recovery of vitamin D metabolites are significantly improved when phospholipids are removed from the plasma.

## A-418

### Comparison of blood sirolimus level measured by LC-MS/MS and immunoassay method

J. Cho, S. Lee, J. Rim, J. Kim. *Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: Sirolimus is a one of the widely used immunosuppressant for preventing organ rejection after kidney transplantation. Therapeutic drug monitoring of this agent is important because of its narrow window of therapeutic efficacy. In this study, we performed method comparison of analytical accuracy between liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and antibody-conjugated magnetic immunoassay (ACMIA).

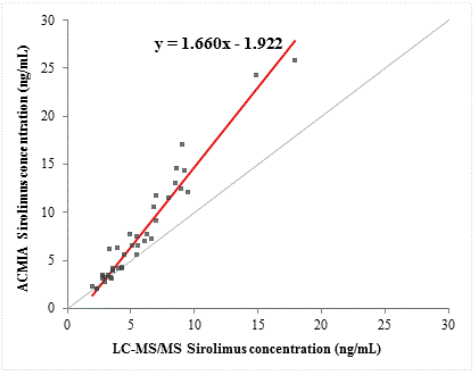
Methods: We used LC-MS/MS and Dimension RXL System (Siemens Healthcare Diagnostics) for measuring sirolimus concentrations. A total of 42 samples from patients treated with sirolimus were analyzed using these two methods. We also measured blood samples spiked with 6 different levels of sirolimus using these methods. We performed correlation and Passing-Bablok regression analyses between levels measured by two different methods with 95% confidence interval.

Results: For the real patient samples, the results measured by ACMIA showed significant (about 66%) deviation in sirolimus concentrations from those of LC-MS/MS. We obtained the following relationship: $y = 1.660\ x - 1.922$ (correlation-$r$ = 0.975), where $y$ is the value obtained with LC-MS/MS and $x$ is that obtained with ACMIA. However, for artificially spiked samples, these two methods did not show significant deviation: $y = 1.099\ x - 1.969$ (correlation-$r$ = 0.996) (Fig. 1).

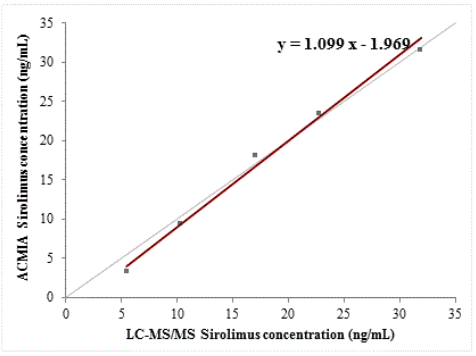
Conclusion: Sirolimus concentrations measured by ACMIA showed good agreement with those measured by LC-MS/MS for artificially spiked samples, but not for real patient samples. Compared to LC-MS/MS, 66% positive deviation in ACMIA was observed. The difference in agreement between sample types might be attributed to cross-reactivity of ACMIA with sirolimus metabolites (such as 12-hydroxy sirolimus, 39-O-demethyl sirolimus, and 27-39-O-didesmethyl sirolimus). So, we recommend the use of LC-MS/MS for measuring sirolimus concentrations to prevent cross-reactivity by its metabolites.

Fig. 1. Method comparison of analytical accuracy between LC-MS/MS and ACMIA. These are the results using the real patient samples (A) and artificially spiked samples (B) measured by these two methods.

(A)




(B)




## A-419

### Development of an LC-MS/MS Method for Measurement of a Steroid Panel in Serum for Clinical Research

X. Xie. *Thermo Fisher Scientific, San Jose, CA*

**Background**

Over the past few years there has been a growing interest to use liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure steroids in serum for clinical research. LC-MS/MS offers the potential for more reliable measurement and capability of measuring multiple steroids simultaneously compared to other detection systems, such as immunoassays. However, the challenges are time-consuming sample pre-treatment, matrix interference and isobars separation. We developed a simple and fast solid phase extraction (SPE) sample preparation method for an 11-steroid panel (11-deoxycortisol, 17-OH progesterone, aldosterone, androstenedione, corticosterone, cortisol, cortisone, estradiol, estrone, progesterone and testosterone) and evaluated the analytical performance on an LC-triple quadrupole MS/MS system. This analytical method is to demonstrate analytical capabilities of instruments and workflow, and it has not been validated and it is not intended to be used for reporting anything else other than analytical performance.

**Methods**

Extraction was performed on a micro 96-well SPE plate. Calibrators/QCs were spiked into neat solution or charcoal stripped serum and mixed with water and methanol. The mixture was loaded directly onto the SPE plate; no preconditioning was required. After washing the plate with 30% methanol, the elution was performed with two volumes of 25 µL of methanol each. Eluates were diluted with 50 µL water. 50 µL of the diluted eluate was injected for LC-MS/MS analysis. Extracted compounds were separated on a reverse phase column chromatographically followed by analysis on a triple quadrupole mass spectrometer with heated electrospray ionization. The total LC run time is 7 min. Data were acquired in selected-reaction monitoring (SRM) mode. Two SRM transitions for each analyte/IS were measured with polarity switching. Ion ratios were calculated for confirmation.

**Results**

The whole SPE process takes less than 20 minutes and no pre-conditioning, evaporation or reconstitution is required. Comparing to conventional SPE method which involves those steps, our method is simpler and faster. We optimized the washing step for SPE and determined that 30% methanol yielded the best overall recovery rate. The recovery rate ranged from 42% (aldosterone) to 95% (testosterone). Lower limit of quantitation (LOQ) of androstenedione is 1 pg/mL. LOQ of testosterone is 2 pg/mL. LOQ of 11-deoxycortisol, 17-OH progesterone, cortisone, estradiol, estrone, and progesterone is 5 pg/mL. LOQ of aldosterone, corticosterone, and cortisol is 10 pg/mL.

**Conclusion**

We demonstrated a simple and fast SPE method for sample pre-treatment of an 11-steriod panel with acceptable recovery rate. The LOQs of 1-10 pg/mL indicate LC-MS/MS is a sensitive and selective analytical method for simultaneous measurement of multiple steroids in serum for clinical research.

## A-420

### A Collaborative Approach for Mass Spectrometry Education for Medical Laboratory Scientists

J. A. Meixner[1], E. Palavecino[2], G. Pomper[3], S. Wong[3]. *[1]Winston-Salem State University,, Winston-Salem, NC, [2]Department of Pathology Wake Forest University School of Medicine, Winston-Salem, NC, [3]Clinical and Translational Mass Spectrometry Center Department of Pathology Wake Forest University School of Medicine, Winston-Salem, NC*

**Background:** Laboratory scientists, until recently, have had limited exposure to the principles, practice and methodologies of mass spectrometry. Since the 80's, gas chromatography/Mass spectrometry (MS) was the method of choice for drug confirmation for Workplace Drug Testing, Clinical and Forensic Toxicology. Recent technological advances have rapidly expanded clinical and translational applications of mass spectrometry and LC-MS/MS. These included the use of MALDI-TOF for microbiology, selected areas of clinical chemistry such as endocrine applications, and Therapeutic Drug Monitoring such as immunosuppressants, signaling the emerging Next Generation MS. Thus, the clinical laboratory is challenged to implement new tests using this methodology. Selected laboratory staff have gained mass spec proficiency by on the job training, but it is estimated that more personnel will be needed. In order to increase training and education of MS, professional societies have offered workshops and lectures to meeting attendees. Hands-on workshops have also been offered by some institutions. Because of our own needs of expanding LC-MS/MS capabilities, we developed the current collaborative and focused approach for training Medical Laboratory Scientists (MLS) in MS, which evolved as an extension of the overall educational missions of both Wake Forest Baptist Health (WFBH) and Winston Salem State University (WSSU).

**Methods:** WFBH and WSSU have recently modified the general clinical pathology rotation for their MLS students to offer both greater exposure and more in-depth hands-on training opportunities for students. The educational training contents were developed in a complementary collaborative planning process. The basic principles and introduction to MS were incorporated as part of the third and fourth year curriculum. Students were introduced to chromatography, MALDI-TOF and triple quadrupole MS instruments by didactic lectures at WSSU. This was complemented by the general clinical laboratory rotations at WFBH which provided limited

"shadowing" opportunities. From this initial exposure and with feedbacks from the faculty and staff, a select number of students was identified to continue focused training. On-site clinical presentations of selected area of clinical applications of MS for microbiology and clinical chemistry/toxicology were offered.

**Results:** Based on approximately 1. 5 years of collaboration, we have two students doing the general clinical laboratory rotation. From this group, one student was selected for additional training in Mass Spec. The student was able to participate in the validation of a newly developed assay for clinical use and also participate in research projects related to MS. At the end of the rotation, the student was competent in the basic areas of Mass Spec. The student presented her experiences in MS training at WFBH to her fellow students and increased their interest for this additional training. We now have new students interested in a summer internship in MS.

**Conclusion:** In developing a collaborative and complementary program, medical laboratory scientists are able to gain introductory knowledge and skill sets. For selected individuals, the exposure might evolve into more advanced training, enhancing clinical MS applications and the availability of trained laboratory personnel.

## A-421

### Steroid detection in small volume blood by LC-MS/MS

N. Bulayeva[1], R. English[2], R. Lieberman[3], B. Figard[2]. *[1]Concord Life Sciences, Houston, TX, [2]Shimadzu, Houston, TX, [3]Shimadzu, Baltimor, MD*

Background: Liquid chromatography- tandem mass spectrometry (LC-MS/MS) has become a successful immunoassay substitute for steroid detection in clinical laboratories. The LC-MS/MS platform provides superior specificity and the possibility to detect a vast number of hormones in a small amount of blood in just one acquisition. However, the sensitivity of the LC-MS/MS assay is still equal or even lower than that of immunoassays. The aim of the present study is to demonstrate the ability of LC-MS/MS to reach a higher level of sensitivity in patient samples using a small volume collection device for blood.

Method: An LC-MS/MS quantitative method was set up on a Shimadzu 8060 triple quadrupole mass spectrometer for 16 steroids (Cerilliant, Round Rock). Steroids were spiked in charcoal strip FBS serum (Fisher Scientific), which was tested negative for all hormones, present in study. Sample preparation was done by solid-phase extraction using SPE trace T-20 column (Tecan SP) and Biotage positive pressure manifold. After elution, samples were dried under nitrogen steam and reconstituted in a water/methanol solution. 10 ul of reconstitute was injected directly into the LC-MS system, which was operating in both positive and negative electrospay ionization mode. Compounds (including isobaric) were separated on a Restek Biphenyl column, with a total run time of 6 minutes (including column reequilibration). Concentration of steroids from vein and capillary blood across 40 individuals was compared using set-up LC-MS/MS assay.

Results: Method was clinically validated, and demonstrated acceptable accuracy (>90%) and precision (CV>80%). Linearity range for all compounds in this study covered published normal reference ranges, and LOQ's that were compatible with immunoassay, available for the Abbott i1000 chemistry analyzer. Method performance was confirmed by cross-validation with CAP proficiency testing samples and with 20 patient results from Abbott i1000 analyzer. Hormone concentration from vein and capillary blood demonstrates significant correlation ($R^2$> 0.98).

Conclusion: The current sensitivity level for steroid detection, which was demonstrated by owr LC-MS/MS assay allows for a successful substitute to the classical immunochemistry assay in clinic. The hormones concentration in capillary blood (finger stick) near identical to conventional serum venipuncture level. Therefore finger-stick could be an excellent source of blood for hormone testing due to convenience, cost, and timing of collection.

## A-422

### Determination of serum progesterone by Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry: a modification of JCTLM approved reference measurement procedure

Y. Wang[1], T. Zhang[1], W. Zhou[2], J. Zeng[2], J. Zhang[2], H. Zhao[2], W. Chen[1], C. Zhang[1]. *[1]National Center for Clinical Laboratories, Beijing hospital, National Center of Gerontology , Beijing Engineering Research Center of Laboratory Medicine. Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]National Center for Clinical Laboratories, Beijing hospital, National Center of Gerontology , Beijing Engineering Research Center of Laboratory Medicine., Beijing, China*

**Background:** The routine methods for progesterone measurement in clinical practice are mainly based on immunoassays. It is needed to establish higher order reference measurement procedures to provide an accuracy base to which routine methods can be compared . The LC/MS is a powerful tool to detect low concentration substances with high accuracy and precision, usually without derivation. There has been a JCTLM-list LC/MS/MS reference method developed by NIST. The present study was to modify the NIST method to reduce analysis time and serum volume. **Methods:** Progesterone calibrator solution was prepared from NMIJ 6003-a (NMIJ, Japan). [2,3,4-$^{13}C_3$] Progesterone, purchased from Cambridge Isotope Laboratories was added to the samples to create an approximately 1:1 mass ratio. In order to reduce the volatilization of calibration, the calibrators were prepared with the mixed solution of ethanol and deionized water. After simple liquid/liquid extraction to isolate progesterone from serum, the samples were analyzed on an API 5000 triple quadrupole mass spectrometer coupled with an Agilent 1200 LC system. The progesterone was separated through Symmetry C18 column with mobile phase of 0.01% acetic acid and acetonitrile. MS detection was performed in positive electrospray ionization mode. Multiple reaction monitoring was used to detect progesterone and its corresponding internal standard transitions. The pretreatment processes were evaluated by extraction rates. Ion suppression was assessed by comparing signal intensity of IS post-spiked into extracted plasma with that of aqueous IS solution of the same concentration. The accuracy of the method was assessed by comparing with national serum reference material GBW 09197 and RELA 2015. Structure analogues of progesterone were tested to determine whether they had interference with progesterone. Imprecision, limit of detection (LOD) were also evaluated to validate the LC/MS/MS procedure. **Results:** The whole analysis time was within 7 min. The results of this method agreed with the certified value within the uncertainty of the measurements for the GBW 09197 (5.00±0.13 nmol/L). The relative bias for sample 2015 RELA HM A was -1.01% compared to the target value. The serum volume used was within 1.2ml (for concentration of 0.15ng/ml ~39.7ng/ml). The extraction rates of hexane and cyclodextrin were 83% and 75%, respectively. Excellent precision was obtained with within-set coefficients of variation (CVs) ranging from 0.02 to 1.4%. No apparent ion suppression was observed. The detection limit at a signal-to-noise ratio of ~3 was 0.09pg of progesterone.

**Conclusion:** Compared with the existing reference measurement procedure , the present procedure for progesterone showed a less analysis time and sample volume. This method is precise, accurate, and could be served as an essential component in development of serum progesterone reference system in China.

## A-423

### Determination of serum cortisol by isotope dilution liquid chromatography tandem mass spectrometry

T. Zhang, W. Chen, C. Zhang, J. Zeng, W. Zhou, Y. Yan, J. Zhang. *National Center for Clinical Laboratories, Beijing hospital, National Center of Gerontology , Beijing Engineering Research Center of Laboratory Medicine, Beijing, China*

**Background:** To develop a candidate reference method for the measurement of cortisol in human serum based on isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS).

**Methods:** The internal standard [9,11,12,12-d4]cortisol was added to the serum sample and equilibrated with endogenous unlabeled cortisol. The cortisol was selectively isolated from the serum matrix by precipitation with dextran sulfate and manganese chloride. After centrifugation for 30min on 1500g the serum cortisol and labeled cortisol were extracted with ethyl acetate-hexane. The upper layer was transfer to another vial and evaporated to dryness under the nitrogen. The residue were reconstituted with mobile phase and analyzed by liquid chromatography tandem mass spectrometry system with multiple reaction monitoring (MRM). The concentration of serum cortisol is calculated by the theory of bracketing method.

**Results:** The within-run, between-run and total coefficients of variation ranged from 0.29% to 0.63 %, 0.56 % to 0.97 % and 0.72% to 1.15 %, and the averages were 0.47%, 0.76% and 0.94%, respectively. The analytical recoveries ranged from 99.0% to 100.9%. The results of analyzing the certified reference material ERM DA-192 and DA-193 showed biases of 0.6% (ranged from 0.3%~0.8%).

**Conclusion:** An ID-LC/MS/MS method for measuring serum cortisol has been developed. The method is highly precise and accurate and may be used as a candidate reference measurement procedure.

## A-424

### Identification of FLT3 Internal Tandem Duplications by Liquid Chromatography/Tandem Mass Spectrometry

R. McCall, B. Pang, J. Lee, C. Furdui, S. Wong. *Wake Forest Baptist Health, Winston-Salem, NC*

**Background:** Using sophisticated molecular methods, several mutations of prognostic significance have been identified in cytogenetically normal cases of acute myeloid leukemia (AML). The genetic loci most commonly affected, in order of relative frequency, include the coding regions for FMS-related tyrosine kinase 3 (*FLT3*), nucleophosmin family member 1 (*NPM1*), and CCAAT/enhancer-binding protein alpha (*CEBPA*). *FLT3* mutations are heterogenous and can involve either the juxtamembrane domain (JM) or the tyrosine kinase domain (TKD) of the protein product. Current methods of identifying *FLT3* mutations are based on polymerase chain reaction (PCR) amplification and subsequent analysis using a variety of techniques (capillary electrophoresis, etc.) which all impose significant cost on the clinical laboratory. With an ever increasing amount of information being stored in nucleic acid and protein databases, more rapid identification and accurate quantification of molecular species is now possible. Mass spectrometry (MS) is one technique that allows for the timely characterization of such entities while avoiding the tedious benchwork historically required for molecular identification.

**Methods:** Purified FLT3 protein (OriGene Technologies, Rockville, MD) was resuspended according to the manufacturer's instructions for subsequent LC-MS/MS analysis. Paraffin-embedded bone marrow samples from 56 AML patients were sectioned, stained with hematoxylin, and placed on standard glass slides. Separate areas of the bone marrow clot sections with the highest blast fractions were identified and laser capture microdissection (LCM) performed using a XT-TI system (Arcturus Engineering Inc., Sunnyvale, CA). Following LCM, protein extraction was carried out using the Liquid Tissue® MS Protein Prep Kit (Expression Pathology, Rockville, MD) for each specimen. Samples were analyzed on a Q-Exactive HF mass spectrometer (Thermo Scientific, Rockford, IL) coupled to a Dionex Ultimate-3000 nano-UPLC system (Thermo Scientific, Rockford, IL).

**Results:** A small oligopeptide EYEYDLK (480.2 > 375.2) was identified using data dependent acquisition and the top 20 most abundant peptide fragments. This small peptide is centered around arginine 595, the most frequently involved amino acid in FLT3 internal tandem duplications (ITDs), and demonstrates linear concentration-dependent changes in signal intensity.

**Conclusions:** Mass spectrometry is a promising modality for proteomic studies spanning a wide range of disease states. Acute myeloid leukemia is one area that may benefit from this technology as FLT3 mutational status becomes rapidly available and therapeutic regimens personalized according to the results of such molecular analysis.

## A-425

### Evaluation of Clinical, Genetical, and Steroid Profile Features of Cases with 3Beta-Hydroxysteroid Dehydrogenase Type 2 Deficiency

T. Guran[1], C. Kara[2], Z. Atay[1], A. Akbarzade[1], G. Can Yılmaz[2], E. Çelebi Bitkin[2], T. Akcay[3], M. Yildiz[3], A. Guven[4], G. Çatlı[5], G. Yeşil[6], S. Baş[1], P. Vatansever[7], G. Haklar[7], Ö. Sirikçi[7], S. Turan[1], A. Bereket[1]. *[1]Division of Endocrinology and Diabetes, Department of Pediatrics, Marmara University, İstanbul, Turkey, [2]Division of Endocrinology and Diabetes, Department of Pediatrics, 19 Mayis University, Samsun, Turkey, [3]Pediatric Endocrinology and Diabetes Clinic, Kanuni Sultan Suleymen E&R Hospital, İstanbul, Turkey, [4]Pediatric Endocrinology and Diabetes Clinic, Amasya University/Goztepe E&R Hospital, Amasya/Istanbul, Turkey, [5]Division of Endocrinology and Diabetes, Department of Pediatrics, 9 Eylul University, İzmir, Turkey, [6]Division of Genetics, Department of Pediatrics, Bezm-i Alem University, İstanbul, Turkey, [7]Department of Biochemistry, Marmara University, İstanbul, Turkey*

**Background:** 3-beta hydroxysteroid dehydrogenase 2 (3β-HSD2) is the enzyme which catalayzes the conversion of D4 steroids (pregnenolone; Preg,17-hydroxypregnenolone;17OHPreg, dehydroepiandrosterone;DHEA) to D5 steroids (progesterone; Prog, 17-hydroxyprogesterone; 17OHProg, androstenedione; AS) and is coded by the *HSD3B2* gene. 3β-HSD2 deficiency is the rarest cause of congenital adrenal hyperplasia and results in deficiency of glucocorticoid, mineralocorticoid, and sex steroids. Deficiency of adrenal sex steroids and increase of their precursors may cause sexual maturation disorders in both genders and premature adrenarche and hyperandrogenism in females. The impact of this disease on steroid profile is not known. We aimed to investigate the clinical, genetic and steroid profile properties of 3β-HSD2 deficiency.**Methods:** Patients with 3β-HSD2 deficiency (as confirmed by genetic testing or clinical and laboratory findings) were included in this multicentric study. The treatments of patients using glucocorticoids/mineralocorticoids, were discontinued for 48 h. Blood samples obtained between 08-09 A.M., were analyzed with liquid chromatography - tandem mass spectrometry (LC-MS/MS). (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratio was used for the diagnosis of 3β-HSD2 deficiency, and the ratio was compared to the healthy control ratios. *HSD3B2* gene sequence was analyzed in patients with elevated (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratios. (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratios were calculated and compared in patients with and without *HSD3B2* mutation, in 3β-HSD2 heterozygote individuals (parents of patients with *HSD3B2* mutation), and in patients with genetically confirmed 21-hydroxylase deficiency patients for the diagnosis and differential diagnosis of 3β-HSD2 deficiency.

**Results:** Of the 29 patients suspected to have 3β-HSD2 deficiency, 12 had *HSD3B2* mutation. Four of the six mutations found, were new mutations. (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratios could be calculated in 7 patients with *HSD3B2* mutation and was significantly elevated compared to other groups (median; 4.3, $p<0.0001$). Genetic testing was not done in ten patients with normal (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratios. *HSD3B2* mutation was not found in 7 patients with elevated (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratios (n=7, median; 0.16, IQR; 0.09-0.23) compared to healthy controls (n=43, median; 0.03, IQR; 0.02-0.08) (p=0.0002) . **Conclusion:** LC-MS/MS is the gold standard in the measurement of steroid hormone analysis. (Preg+17OHPreg+DHEA)/(Prog+17OHProg+AS+cortisol) ratio is very useful in the diagnosis of 3β-HSD2 deficiency. In patients with a similar steroid profile to that of 3β-HSD2 deficiency, further genetic analyses are required to elucidate the etiology of diseases such as hyperandrogenism and polycystic ovary syndrome and the effects of 3β-HSD2 action.

## A-426

### Development of a novel, high-sensitivity LC-MS/MS serotonin assay for assessing platelet function using a minimal amount of whole blood

S. Chan[1], X. Yi[2], E. Wysocki[1], R. Bridgman[1], J. Gutierrez[1], K. Mikrut[1], E. K. Y. Leung[1], J. Miller[1], K. T. J. Yeo[1]. *[1]The University of Chicago, Chicago, IL, [2]Houston Methodist Hospital and Research Institute, Houston, TX*

**Background:** Light transmission aggregometry (LTA) is presently the gold standard employed in patients with suspected bleeding disorders for *in vitro* investigation of an underlying platelet function abnormality. LTA instruments equipped with a second luminescence channel ("lumi-aggregometer") additionally can simultaneously measure released ATP that occurs upon secretion of platelet dense granules. The dense granules contain ATP, ADP, additional phosphates, serotonin (5-HT), and calcium.

The released ADP is thought to be most critical, since it activates additional platelets that then contribute to formation of the hemostatically essential platelet aggregate. Performance of lumi-aggregation employing a battery of platelet stimuli typically requires up to 50 mL of patient blood, with a platelet count of at least 150,000/µL. The development of a novel LC-MS/MS approach was undertaken in an effort to extend the opportunity for the study of platelet dense granule secretion to newborns, as well as to adult patients with decreased platelet counts.

**Methods:** 200 µL of citrated whole blood was incubated with exogenous deuterated serotonin ($D_4$5-HT). Upon $D_4$5-HT uptake, blood was aliquoted, and separately incubated with a variety of classic platelet stimuli, closely paralleling the approach used in LTA. Following stimulation, blood was centrifuged and the releasate obtained. A platelet lysate was used to assess total $D_4$5-HT uptake. For serotonin sample quantitation, calibrators and quality controls (QC) were prepared by spiking $D_4$5-HT into modified Tyrode's buffer (MTB). Internal standard (IS) methylated-5HT (10 ng/mL) was added into samples followed by precipitation using ascorbic acid (50 mg/mL) and perchloric acid (70%), followed by centrifugation. The supernatant fraction was subjected to solid phase extraction. The eluent was concentrated using evaporation technique followed by reconstitution with 5% methanol/95% $H_2O$ with 0.1% formic acid (FA) prior to injection. Samples were separated under a gradient elution on a Triart C18 Column (50 x 0.5 mm, 3µm, YMC America, Inc. PA) with a flow rate of 25 µL/min and a total run time of 4 min using the Eksigent microLC 200 (Sciex, MA). Analytes and IS were detected by the Sciex QTrap 6500 mass spectrometer (Sciex, MA) with an ESI source.

**Results:** This assay showed excellent analytical measuring range from 50-2000 pg/mL ($r^2$>0.99). QC samples prepared at the lower limit of quantitation, as well as low, mid and high QC levels yielded an inter-assay precision showing a CV of 9.6%, 4.8%, and 5.5%, respectively. Intra-assay precision showed 6.7%, 7.6%, and 3.6% respectively. Accuracy study demonstrated 75% extraction recovery, while showing 28% ion suppression.

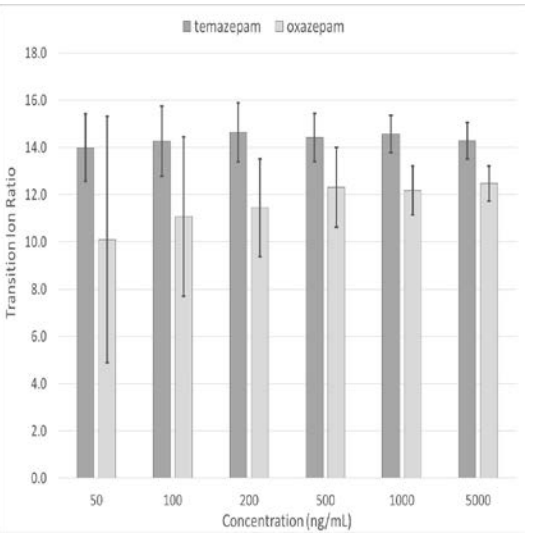
**Conclusion:** This novel methodology permits extensive study of platelet dense granule secretion using extremely small blood volumes. The use of exogenous, non-radioactive serotonin has allowed us to construct a highly sensitive, and robust serotonin release assay to assess platelet function in patients being evaluated for a bleeding disorder. This approach offers a means of overcoming serious limitations of current platelet function testing by expanding the availability to pediatric patients and to adult or pediatric with decreased platelet counts.

## A-427

### Variation of transition ion ratios for urine benzodiazepine analysis by liquid chromatography-tandem mass spectrometry

D. R. Bunch, A. J. McShane, S. Wang. *Cleveland Clinic, Cleveland, OH*

**Background:** Transition ion ratio (TIR) is the ratio of fragment-1 over fragment-2 from the same precursor and is frequently monitored for liquid chromatography tandem mass spectrometry (LC-MS/MS) quantification. If used correctly, TIR monitoring should improve the specificity of an LC- MS/MS assay. The Clinical and Laboratory Standards Institute (CLSI) C50-A guidelines give a static percent allowable TIR deviation based on the TIR level. For example, a TIR of 10-20% has a static allowable deviation (SAD) of 30%. Anecdotally, we have observed failures of these rules for some of our LC-MS/MS assays. **Objective:** To investigate whether TIRs always follow the SADs defined by the CLSI guidelines and whether TIRs may be concentration dependent for certain analytes and instruments. **Method:** Multiple spiked human urine lots with known levels (calibrators and quality controls) of 7-aminoclonazepam (Precursor ion: 286m/z->Product ion 1: 121m/z; Product ion 2: 222m/z), lorazepam (321->229;194), nordiazepam (271->140;208), α-hydroxyalprazolam (325->279;243), α-hydroxytriazolam (359->176;242), oxazepam (287->241;104), and temazepam (301->177;255) were analyzed by an LC-MS/MS method along with patient samples. TIRs for the calibrators and QCs on a Thermo TSQ™ Quantum Ultra from July 2016 to February 2017 were monitored. The TIRs were extracted and compiled using a Perl script with statistics and graphs produced using Microsoft Office Excel. **Results:** The mean TIR was 24.4%, 33.2%, 16.3%, 95.5%, 11.7%, and 14.4% for 7-aminoclonazepam, lorazepam, nordiazepam, α-hydroxyalprazolam, α-hydroxytriazolam, oxazepam, and temazepam, respectively. One of 7 displayed concentration dependent TIR, while four of seven showed concentration dependent CV for TIR. Figure 1 demonstrates an analyte (temazepam) of which TIR met the SAD requirement and another analyte (oxazepam) of which TIR was concentration dependent and failed the specified criteria by CLSI guidelines at the lowest concentration. **Conclusion:** TIR may be concentration dependent for certain analytes and instruments in LC-MS/MS analysis. TIR acceptance criteria should be assessed during method development for each individual analyte.




## A-428

### Can HPLC-MS be replaced by direct coupling of SPME to MS for Clinical Applications?

J. Pawliszyn. *University of Waterloo, Waterloo, ON, Canada*

**Background:** This new era of analytical chemistry, where sample preparation devices are directly and efficiently coupled to mass spectrometry (MS), has given rise to a growing branch of innovative research where micro/nano-sampling/extraction approaches enabling determination of target components in complex matrices facilitating on-site analysis. Herein, I discuss diverse Bio-SPME-MS strategies recently developed in my group to address challenging clinical, forensic and environmental applications including the determination of in-vivo tissue metabolites, controlled-substances in small volumes of biofluids, contaminants at trace levels in environmental samples, task traditionally involving LC/MS. Furthermore, this work thoroughly investigates exemplary cases in which the mere coupling of SPME to MS is not sufficient to answer relevant analytical questions and the use of a separation step is necessary. **Methods:** Coated Blade Spray and SPME-nano-spray analyses were performed using a TSQ Quantiva (Thermo Scientific). SPME-Transmission Mode (SPME-TM) analyses were performed using a DART-SVP ion source in positive/negative mode (IonSense Inc.) coupled to a TSQ-Vantage (Thermo Scientific) and a portable QDa (Waters Corporation). SPME-Open Port Probe experiments were performed using an API-4000 (SCIEX). Liquid chromatography methods coupled to high-resolution mass spectrometers (either Waters Xevo Q-TOF or Exactive-Orbitrap) were developed for metabolomics/lipidomics studies. Chromatographic methods coupled to tandem MS instruments were developed for method validation of the different SPME-MS technologies herein described. **Results:** In this work, we present diverse SPME-MS strategies recently developed in our laboratory for the analysis of complex sample matrices such as tissue and biofluids. These technologies include Coated Blade Spray (CBS), SPME-transmission mode-DART (SPME-TM-DART), SPME-nano-electrospray-ionization (SPME-nano-ESI), and SPME- open-port probe (SPME-OPP). Unlike direct-sample-to-MS approaches, SPME-MS provides a cleaner extract which allows for long-term operation of the instrument with minimal maintenance and reliable quantification. The total analysis time of biofluids, food and environmental samples does not exceed 10 minutes and sample volumes ranging between 1 µL and 10 mL were used. Sampling/sample-preparation is performed either by spotting the sample onto the SPME-device, or by immersing the SPME-device on a vial containing the sample. Despite short extraction times, limits of quantitation ranging between low-pg/mL to sub-ng/mL were obtained, while good accuracy, and linearity were attained for all the studied probes in a wide-range of samples (e.g. pharmaceuticals in waste-waters, therapeutic-drugs in blood droplets, and pesticides in orange juice). In the case of tissue analysis, although sampling times are relatively longer, Bio-SPME device act as a chemical biopsy tool by enriching small molecules carrying chemical information about the investigated system without removing tissue, thus providing a much cleaner extraction as the enrichment occurs via free form of analytes. In addition, I thoroughly discuss why SPME-MS facilitate successful fast quantitation of drugs/metabolites extracted in-vivo.

**Conclusion:** This presentation describes exemplary cases in which the mere coupling of SPME to MS is not sufficient to answer relevant analytical questions and they use of a chromatographic step is justified. Supplementary instrumental strategies that allow for removal of co-extracted interferences or source artifacts, such ion mobility and multiple reaction monitoring with multistage fragmentation, are also discussed in this presentation.

## A-429

### Coated Blade Spray: a technology breaking paradigms in direct coupling to mass spectrometry

G. Gomez-Rios[1], M. Tascon[1], N. Reyes-Garces[1], D. Rickert[1], E. Boyaci[1], J. Poole[1], K. Jaroch[2], B. Bojko[2], J. Pawliszyn[1]. *[1]University of Waterloo, Waterloo, ON, Canada, [2]Nicolas Copernicus University, Bydgoszcz, Poland*

**Background:** Coated Blade Spray (CBS) is a SPME-based technology designed for the enrichment of analytes of interest from complex sample matrices, which can be directly coupled with mass spectrometry instruments for rapid quantitative/qualitative analysis. Unlike other SPME-MS couplings, no-additional instrumentation is required as the blade acts as the extraction device/ionization-source. Moreover, when dealing with small volumes, no container is needed as the sample can be simply spotted onto the coated area. Due to the ultra-thin and biocompatible nature of the coatings, fast extraction/enrichment of the target analytes can be achieved with negligible adherence of matrix components onto the coated surface.

**Methods:** The analytical protocol consists of three steps: 1. analyte-enrichment: either by spotting the sample onto the CBS, or by extracting from a vessel containing the sample; 2. coating-cleaning: fast removal of matrix potentially adhered to the coated surface; and c. instrumental-analysis: applying 10µL of the elution/electrospray solution (e.g. 95/5/0.01 % methanol-water-formic acid) onto the CBS, which is placed 5 mm from the MS inlet. After 20s, a +4kV potential difference was applied resulting in ESI from the tip of the blade. Signal was integrated for 1-30s according to the application. Analysis were performed using either a Q-Exactive or a TSQ-Quantiva (Thermo Scientific, San Jose, USA).

**Results:** CBS is a SPME-MS technology designed for the ultrafast analysis of target compounds in small/large sample volumes of complex matrices such biofluids. Unlike direct-sample-to-MS approaches, CBS provides a cleaner extract which allows for long-term operation of the instrument with minimal maintenance and reliable quantification. The main goal of this work is to describe most recent advances on CBS-technology that break paradigms related to the direct coupling of SPME to MS. First, we present a CBS-autosampler that allows for processing of up to 96-samples simultaneously and the subsequent unsupervised MS-event of each CBS. This technology was assessed for the quantitative determination of drugs in urine, blood and plasma samples. Model analytes with a wide variety of physical-chemical and protein binding properties, including doping agents (e.g. clenbuterol), pain-management drugs (e.g. fentanyl), and therapeutic-drugs (e.g. tacrolimus) were selected for this study. Our results demonstrated that CBS can provide satisfactory linearity over 3 orders of magnitude (pg/mL to ng/mL) and great accuracy (85-120%) for the majority of the probes selected. Second, we introduce a ground-breaking strategy that allows lowering LOQ when determining therapeutic-drugs/controlled-substances in biofluids spots by neglecting the "solventless" philosophy of SPME. This approach is further exploited towards the concomitant analysis of immunosuppressive, antifungal and pain-panel drugs from a blood/plasma-droplet in the so-called lab-on-a-blade. Third, we describe diverse on-coating derivatization methodologies developed to enhance limits-of-quantitation for targeted analytes with poor ionization efficiency/high-instrumental background. Fourth, we present the application of CBS as a rapid tool for rapid discrimination of cell-cultures. Finally, we introduce recent fundamental studies on coating strategies for spot analysis, and blade geometry to improve inter-CBS reproducibility without the use of internal standard.

**Conclusion:** Herein, we describe the most recent advances on CBS-technology that allow for ultrafast quantitative analysis of small molecules in biofluids.

## A-430

### Simultaneous Measurement of ThioTEPA and its Metabolite TEPA in Serum and CSF by Turbulent Flow LC-MS/MS

B. Carroll, R. Schofield, M. Scordo, S. Giralt, L. Ramanathan, M. Pessin, D. Carlow. *Memorial Sloan-Kettering Cancer Center, New York, NY*

***Background:*** *N,N′,N″*-Triethylenethiophosphoramide (thioTEPA) is an alkylating agent that has been used in the treatment of multiple solid malignancies for over 20 years. Recently, interest in thioTEPA has been renewed as part of conditioning regimens prior to hematopoietic cell transplantation for hematologic malignancies owing to its potent myelosuppressive effects. Once administered thioTEPA is rapidly metabolized to *N,N′,N″*-triethylenephosphoramide (TEPA). ThioTEPA and TEPA have similar alkylating activity therefore it is necessary to monitor both compounds. Interestingly, thioTEPA and TEPA exhibit excellent central nervous system (CNS) penetration resulting in cerebrospinal fluid (CSF) concentrations comparable to simultaneous plasma concentrations making it an effective conditioning agent for use in CNS lymphoma.

***Objective:*** The objective was to develop a highly sensitive and specific turbulent flow LC-MS/MS (TFLC- MS/MS) method that is also suitable for application in a high volume clinical laboratory to simultaneously monitor thioTEPA and TEPA in plasma and CSF.

***Methods:*** Plasma and CSF samples were prepared by protein precipitation using methanol containing deuterated thioTEPA and TEPA as internal standards. TFLC-MS/MS analyses were performed on a Thermo Scientific TLX-2 HPLC system (TurboFlow® technology) interfaced to a TSQ Quantum Ultra mass spectrometer operated in the positive ion ESI mode. Chromatographic separation was achieved using a Cyclone-P TurboFlow®column (50 X 0.5 mm) and an Accucore®C18 (50 X 3 mm i.d.) analytical column. The HPLC gradient elution was 20-80% of 10 mM ammonium formate + 0.1% formic acid in methanol over 0.25 minutes which was then held for 1.5 minutes. Calibrators (6) were prepared in blank human plasma and CSF.

***Results:*** The analytical measurement range for thioTEPA and TEPA was established based on relevance to current practice at 19.5-2500 ng/mL with calibration curves linear over the AMR ($R^2 \geq 0.995$). The limit of quantitation of thioTEPA and TEPA was 5 ng/mL (CV <20%). Imprecision studies were conducted over a twenty-day period using concentrations of both drugs that spanned the AMR. Between-day CVs for both compounds in plasma and CSF did not exceed 15% over the entirety of the study. Similarly within-day (n=10) CVs did not exceed 10%. Given the lack of availability of a comparison method, the accuracy of the assay was assessed via recovery studies in patient plasma and CSF. Both thioTEPA and TEPA recoveries at three different concentrations were between 99 and 112% in plasma and CSF. Interferences from hemolysis, icterus and lipemia were evaluated in plasma and cross reactivity from other drugs was evaluated in both plasma and CSF.

***Conclusion:*** We have developed a fast, accurate and sensitive assay to measure thioTEPA and TEPA levels in plasma and CSF by TFLC-MS/MS. To our knowledge this is the most rapid method described to date for monitoring thioTEPA and TEPA in plasma with an analytical run time of less than five minutes and the only LC-MS/MS method developed to monitor thioTEPA and TEPA in CSF. The ability to rapidly and accurately monitor these drugs allows for timely dose adaptations in hopes of maintaining treatment effectiveness while mitigating excessive regimen-related toxicity.

## A-431

### A Comparison of Blood Collection Tube Types for Analysis of Testosterone by LC-MS/MS

T. D. Hawley, D. M. Goertz, K. Yaraei. *Pathology Associates Medical Laboratories, Spokane, WA*

**Background:** Testosterone is the major androgenic hormone and is present in both males and females. In males, the hormone is secreted by the testicular Leydig cells and is responsible for physical characteristics including external genitalia and secondary sexual characteristics. In females, it produced in the ovaries and is a precursor of estrogen. Testing for testosterone levels can be used to detect several medical conditions; however, because females, males on testosterone suppressing therapy, and children have very low levels compared to normal males, a more sensitive method of testing such as LC-MS/MS is required. Blood collection tube type is critical as it has been reported in literature that certain tube types can cause the value of testosterone to be falsely elevated due to the anticoagulants and/or serum separator gels. Pathology Associates Medical Laboratories (PAML) recently switched to a new LC-MS/MS method and evaluated different types of collection tubes.

**Methods:** A group of 10 volunteers consisting of both adult males and females were utilized. Five blood collection tubes were drawn from each participant; A serum tube (red top), serum separator tube (SST), lithium heparin, lithium heparin plasma separator tube (PST), and a K2 EDTA tube. Manufacturer instructions were followed for collection. Once the blood samples were centrifuged, the plasma and serum were aliquoted off except for the SST and PST, which only had an aliquot of 300 µL used. The serum and plasma were allowed to stay in contact with the gel in the SST and PST and aliquots of 300 µL were taken from them at 0 hours, 2 hours, 8 hours, and 24 hours after collection. The samples were then analyzed on an LC-MS/MS

instrument. **Results:** We evaluated testosterone results from all blood collection tube types compared to the red top tube as our predicate device, since red top tubes do not contain any anticoagulant or gel, and we also evaluated the effects of serum/plasma left sitting on the gel for up to 24 hours. The data was analyzed using EP Evaluator 11. The SST correlation had an R value of 0.9990, lithium heparin had an R value of 0.9998, PST Lithium Heparin had an R value of 0.9998, and K2 EDTA had an R value of 0.9997. To evaluate if the exposure to the SST or PST gel caused the value of testosterone to change over time, the aliquots at 0 hour, 2 hours, 8 hours, and 24 hours, were compared to the value at the time of collection, and the CV was less than 4.5% over a 24 hour period.

**Conclusion:** We have shown that different blood collection tubes can be used and that SST and PST gels up to 24 hours do not interfere with the assay if a robust LC-MS/MS method is used. Every lab should evaluate the blood collection tube types that they will be using with their testosterone method to ensure that no interferences are noted before testing patient samples.

## A-432

### Quantification of Infliximab in Human Serum by LC-MS/MS Using A Full-Length Stable Isotope Labeled Internal Standard

Z. Cao, K. Ray, P. Jalili. *MilliporeSigma, St. Louis, MO*

Background:

Infliximab, a chimeric monoclonal antibody used to treat rheumatoid arthritis, psoriatic arthritis and many autoimmune diseases by binding to tumor necrosis factor-alpha (TNFα) to reduce the inflammatory response. Clinical responses are different among patients due to inadequate amount of drug in blood and the formation of autoantibodies, which can also interfere with ELISA assays. Therefore, there is a growing demand for reliable LC-MS/MS assays to support quantification of serum Infliximab. The accurate quantitation of Infliximab is enabled by early introduction of an internal standard that behaves identically to the native target protein throughout the analytical workflow. We have developed a full-length stable isotope labeled Infliximab monoclonal antibody internal standard, which allows significant improvements in accuracy and reproducibility in routine quantification of serum Infliximab using LC-MRM assay. We demonstrate a lower limit of quantitation, without immunoenrichment, of 500 ng/mL with less than 15% CV and ±15% accuracy.

Methods:SIL-Infliximab was expressed in CHO cells which were grown in serum-free media enriched with 13C615N4 Arginine and 13C615N2 Lysine. The SIL-Infliximab was analyzed at the intact protein level and after trypsin digestion. Intact mass analysis (SEC-MS) was used to confirm the amino acid composition of the protein and level of glycosylation. The sequence and isotope incorporation were determined at the peptide level after trypsin digestion. For quantification, samples were prepared by spiking 20 µg/mL of SIL-Infliximab as an internal standard into human serum containing 0.5 - 250 µg/mL of an Infliximab target antibody. Samples were precipitated by adding saturated ammonium sulfate, reconstituted with 50 mM ammonium bicarbonate, and digested using trypsin. Tryptic peptides were separated on a Supelco BIOshell A160 Peptide C18, 2.7 µm fused core particle column; 10 cm x 500 µm. Detection was performed in MRM mode on Sciex QTRAP 5500 system. Transitions of four unique Infliximab peptides, GLEWVAEIR, SINSATHYAESVK, YASESMSGIPSR, and DILLTQSPAILSVSPGER, were monitored.

Results:Stable isotope labeled (SIL) full-length Infliximab monoclonal antibody has been produced with high purity and isotopic incorporation > 99%. The sequence was confirmed at peptide level using peptide mapping and at protein level by intact mass analysis. SIL-Infliximab was used as a full length internal standard for quantification of Infliximab in human serum using pellet digestion followed by microflow LC-MRM analysis. SIL-Infliximab was spiked into human serum containing 0.5 - 250 µg/mL of an Infliximab target antibody. Each standard was precipitated using saturated ammonium sulfate and digested using trypsin. The calibration curve was created using the ratio of area under the peaks of target antibody and internal standard. The linear regression coefficient was greater than 0.99 for four unique

Infliximab peptides. The lower limit of quantitation was determined to be 500 ng/mL with less than 15% CV and ±15% accuracy for triplicate calibration standards.

Conclusions:We demonstrate that the use of a full length SIL-Infliximab internal standard allows sensitive, accurate, and reproducible quantification Infliximab in human serum.

## A-433

### A clinical research LC-MS/MS method for busulfan pharmacokinetics and pharmacodynamics studies in plasma

S. Balloch, G. Hammond, L. J. Calton. *Waters Corporation, Manchester, United Kingdom*

**Background:** Busulfan is a bifunctional alkylating agent whose bioavailability varies greatly between individuals due to factors such as age, underlying diseases and drug-drug interactions. An accurate, sensitive and specific analytical method may play a role in assessing the pharmacokinetic and pharmacodynamic effects of busulfan administration in clinical research.

**Methods:** Samples (50µL) were deproteinised with busulfan-$^2H_8$ internal standard in methanol. Elution was achieved within three minutes using a Waters HSS-T3 C18 UPLC column (2.1x50mm, 1.8µm) on the Waters ACQUITY UPLC® I-Class with a water/methanol/ammonium acetate/formic acid gradient. Ammonium adducts of busulfan were analysed using electrospray ionization in positive mode with multiple reaction monitoring using the Waters XEVO® TQD mass spectrometer.

Matrix-matched calibrators (0.025-5 µg/mL) and quality control samples (0.05, 0.75, 1.5 and 3.5 µg/mL) were prepared by gravimetric weighings of independent stocks of busulfan. Due to known instability of busulfan in plasma, aliquots of in-house calibrators and quality control samples were stored frozen and thawed prior to use each time the method was used.

**Results:** Analytical sensitivity was calculated to be 0.020 µg/mL (n=10 extractions, over five occasions, ≤16.0% CV). Linearity was demonstrated over the concentration range 0.0175-6.51 µg/mL and system carryover was negligible in samples ≤10 µg/mL.

Precision studies (n=5, over five occasions) demonstrated repeatability and total precision ≤7.3%. A comparison was made by analysing anonymized plasma samples (n=40, range 0.20–2.28 µg/mL) against an independent UPLC-MS/MS method. Good agreement was evident from an ordinary linear fit comparison of r=0.998, a mean bias of 5.3% from Altman-Bland analysis and a Deming equation of y=1.01x+0.04.

The mean recovery for busulfan pooled plasma samples (n=3 at 0.05 and 3.5 µg/mL) was between 85.1-106.1% in the presence of high concentrations of endogenous compounds albumin, bilirubin, cholesterol, triglycerides and uric acid and exogenous Intralipid®. The mean recovery was between 93.2-103.0% in the presence of acetaminophen, fluconazole, ketoconazole, itraconazole, phenytoin, posaconazole and voriconazole.

Negligible matrix effects were observed at low (0.05 µg/mL) and high (3.5 µg/mL) concentrations as indicated by respective mean internal standard adjusted matrix factors of 0.99 (range 0.97–1.02) and 0.98 (range 0.95–1.00).

**Conclusions:** A method for the quantification of busulfan in plasma for clinical research has been developed. The method demonstrates good linearity, analytical sensitivity and precision with negligible matrix effects.

For Research Use Only, Not for use in diagnostic procedures.

## A-434

### Analysis of Monosialogangliosides in the Plasma of Patients with GM3 Synthase Deficiency by Using a Novel UPLC/MS/MS Assay

D. Liu[1], Q. Huang[1], X. Zhou[1], B. Xin[2], K. Cechner[2], H. Wang[2], A. Zhou[1]. *[1]Cleveland State University, Department of Chemistry, CLEVELAND, OH, [2]DDC Clinic, Center for Special Needs Children, Middlefield, OH*

**Background:** Gangliosides are a family of glycosphingolipids characterized by mono- or polysialic acid-containing oligosaccharides that are abundantly present in the central nervous systems of many living organisms. Their metabolic disruption and deficiency are associated with various neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. GM3 synthase deficiency is newly identified as an infantile-onset neurological disorder in the Old Order Amish population in the United States. It is caused by the premature biosynthetic termination of GM3 ganglioside and the downstream metabolites accumulation as a result of the genetic mutation of GM3 synthase. Oral administration of GM3 & GD3 enriched formula has been implemented as a potential therapy. In order to assess and monitor the efficacy of the treatment, we used a reverse-phase ultra-performance liquid chromatography (UPLC)/tandem mass spectrometry (MS) method in combination with chemical derivatization to determine monosialogangliosides present in the plasma of patients under treatment.

**Methods:** Ganglioside 500 (G500), a highly-concentrated and appropriately formulated dairy ganglioside product, was used as an oral supplement on those

children with GM3 synthase deficiency. The amount of administration was 1 to 2 g of G500 /kg of body weight. The children were assessed and monitored clinically after the treatment, particularly focusing on bioavailability and metabolism of gangliosides, and the gangliosides' therapeutic effects on the intellectual disability in this condition. An established UPLC/MS/MS method was used in combination with the DMTMM & PAEA chemical derivatization for signal enhancement as the primary means for implementing the intended study on collected plasma samples from the effected children. The samples were analyzed by using the Shimadzu Nexera UHPLC system interfaced to an AB Scix Qtrap 5500 mass spectrometer that operated in an ESI positive and Multiple Reaction Monitoring (MRM) mode to achieve detection with superior sensitivity and specificity.

**Results:** Plasma samples collected from different patients at various time points have been analyzed continuously to monitor the possible changes on ganglioside levels within the blood circulation of GSD patients before/after ingestion of the dosage of G500.The patients currently undergoing treatment generally show irregularly fluctuated pattern on the level of circulating GM3 from 80~200 ng/ml in their plasma samples in response to the progression of G500 therapy, while the level of circulating GM2 remained consistently undetectable throughout the entire study. The results partially support the improvement of clinical manifestation in treated patients.

**Conclusion:** In summary, we implemented a therapeutic intervention strategy based on oral administration of exogenous gangliosides to treat GSD, an inherited neurological disorder, and this study sheds a new light on fundamentally understanding the physiological and pathological functions of gangliosides in human neurological disorders.

## A-435

### Liquid chromatography-tandem mass spectrometry (LC-MSMS) quantitation of buprenorphine, norbuprenorphine and naloxone using the AC Extraction Plate-TM(ACP) - simple but sensitive

J. Stone[1], A. Sitarska[2], R. L. Fitzgerald[3]. *[1]Univ. of Calif. San Diego Health Center for Advanced Laboratory Medicine, San Diego, CA, [2]Tecan Schweiz AG, Männedorf, Switzerland, [3]Univ. of Calif. San Diego, Dept. of Pathology, San Diego, CA*

**Background:** Buprenorphine (BP) for treatment of opioid dependency has some advantages versus methadone. Urine testing to monitor compliance, parenteral administration or adulteration is a standard recommendation. Desirable features for LC-MSMS BP methods include ease of use, quantification limits <1 ug/L and detection of BP, norbuprenorphine (NBP) and naloxone (NX). Dilution methods are simple but less sensitive and solid phase extraction is sensitive but complex. We developed and validated a simple automated LC-MSMS method for BP, NBP and NX using the ACP with 0.5, 0.5 and 5 µg/L lower limits of quantification(LLoQ) respectively.

**Methods:** We investigated the acetonitrile:$H_2O$ ratio(5:95-95:5) and pH(2-11) of extraction, wash and elution/injection reagents to optimize extraction recovery and chromatography. The LC-MSMS was a Waters Acquity LC-XEVO TQS in positive ESI mode with a Waters BEH C18,2.1x50mm,1.7 µm column. Mobile phases A and B (MP-A/B) were 2 mmol/L ammonium acetate/0.1% formic acid and acetonitrile/0.1% formic acid. The LC gradient was 95:5 to 40:60 MP-A:MP-B, flow rate 0.5mL/min, run time 3.02min. MRMs were BP-468/396, 468/414, BP_D4-472/101, NB-414/101, 414/187, NB_D3-417/83, NX-328/212, 328/268, NX_D5-333/258. Injection volume was 7 µL. Six calibrators (BP, NB, NX) and two QC (BP-, NB- and NX-glucuronides) were prepared in drug free urine(DFU). The master-mix of beta-glucuronidase, Rapid Hydrolysis buffer (IMCSzyme®, IMCS-LLC,Irmo,SC) and internal standard(IS) was 20,000 U/mL glucuronidase and 80/80/2,400 µg/L of BP_D4/NBP_D3/NX_D5 respectively in 95:5 buffer:acetonitrile. Reagents were: Extraction(Na bicarbonate buffer, 1 mol/L, pH 9.2), Wash(1% $NH_4OH$) and Elution/Injection((80:20 $H_2O$:acetonitrile/1% formic acid). A Freedom EVO automated liquid handler with orbital shaker performed all extraction steps. The extraction protocol was addition of 200 µL urine and 50 µL of master-mix to an AC plate, then 2 min of mixing. Glucuronide hydrolysis was performed at 65 °C for 30 min. Addition of extraction reagent (post-hydrolysis) was followed by 10 min of mixing to partition the analytes into the non-polar coating of the ACP. The extraction mixture was discarded, followed by a wash step. The wash residue was discarded and elution reagent was added. After 5 min of orbital shaking the 100 µL eluate was transferred to an injection plate.

**Results:** Hydrolysis recovery was >95%. No interference was observed from 54 other drugs/metabolites at 3,000-50,000 µg/L. Within- and between run precision studies of the 6 calibrators (n=16), 2 QC and LLoQ patient pool (n=20) over 5 days yielded coefficients of variation <10%, bias <10% at means ranging between 0.5/0.5/5 to 98/102/1,029 µg/L for BP/NBP/NX respectively. Mean %recovery was 42/33/6 and mean %matrix effect was 52/65/92 for BP-BP_D4/NBP-NBP_D3/NX-NX_D5 respectively. Post-column infusion studies showed matching patterns of ion suppression between analytes and the corresponding IS. No carryover was found at 100/100/1,000 µg/L for BP/NBP/NX. The assay was linear from 0.5-100/0.5-100/5-1,000 µg/L(BP/NPB/NX). Patient samples were tested on 1:9 dilutions with DFU or undiluted. Deming regression statistics for patient sample testing (n=42) versus two other LC-MSMS methods were (BP/NBP) slope 1.01/1.05, intercept -1.6/10.9, Sy/x 6.1/32.9, % bias -0.1/1.1, ranges 7/8-605/982 µg/L).

**Conclusion:** This automated method has good precision and accuracy, low quantitation limits and expands the options available for BP urine monitoring.

## A-436

### Comparison of LC-MS based alpha 1 antitrypsin evaluation algorithm with isoelectric focusing based algorithm.

A. Bobr, D. L. Murray, M. R. Snyder. *Mayo Clinic, Rochester, MN*

**Background:** Alpha 1 antitrypsin (A1AT) deficiency affects 1 in 1500 to 3500 individuals of European ancestry. The disease develops due to levels of A1AT that are the result of mutations in the SERPINA1 gene. Multiple mutations of the gene have been described with the vast majority (>95%) of the disease caused by mutations resulting in the Z and S phenotypes. The current gold standard algorithm to diagnose A1AT deficiency is accomplished by documenting both low serum A1AT concentration and a disease associated phenotype by isoelectric focusing (IEF). Recently, as an effort to increase efficiency and cost effectiveness, our lab has implemented a LC mass spectrometry (MS) method to screen for S and Z phenotypes to decrease the number of samples to needing IEF. The material costs of a single MS based test is about 10 times less than cost of IEF test. Here we compare our experience with the LC mass spectrometry based testing algorithm (A1AT proteotyping) to the traditional IEF method during the same 1.5 year period.

**Methods:** The A1AT proteotyping algorithm utilizes both A1AT level and LC-MS S and Z detection as a screen prior to IEF. If a patient has a no detectable S or Z mutation and the protein level is greater than 100 mg/dL, no further testing performed as the patient is by definition not A1AT deficient. If the patient screen positive for an SS or ZZ phenotype, no further testing is done as the diagnosis has been established. However, patients with A1AT levels less than 100 mg/dL who are screen negative for S and Z mutations or those with levels below 70 mg/dL who are screen positive for heterozygous for S and Z phenotypes are reflexed to IEF to detect less common deficiency phenotypes.

**Results:** During a period spanning 7/2015 to 11/2016, our lab performed 5500 A1AT proteotypes. Of the 5500 tests, 96.7% of the samples were resulted from the LC-MS screen. Of the 113 samples reflexed to IEF for being less than 100 mg/dL and screen negative for the S or Z mutation (most likely MM phenotype), 88% had no other phenotype found by IEF while 3 IM, 7 PM and 2 F phenotypes were reported. For the screen positive S and Z heterozygous phenotypes reflexed for being below 70 mg/dl, 90% were confirmed MS and MZ while 2 FZ, 1IZ and 1 PZ phenotypes were reported.

During the same time period 16,324 tests were performed using the traditional IFE approach. In an effort to determine the number of deficient phenotypes that may have been missed by the proteotype algorithm, we retrospectively applied the algorithm to these specimens. In total, 4 potential risk phenotypes would have been missed (1 I, 2 IS and 1 IZ), which represents 0.02% of total tests.

**Conclusion:** In conclusion, the proteotype algorithm appears to be an efficient and cost effective method to detect A1AT deficiency.

## A-437

### Measurement of 17-hydroxyprogesterone and cortisol in dried blood spots by liquid chromatography-tandem mass spectrometry as a candidate confirmatory test for congenital adrenal hyperplasia

L. Han[1], N. P. Tavakoli[2], M. Morrissey[2], Z. Cao[2]. *[1]The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China, [2]Wadsworth Center, New York State Department of Health, Albany, NY*

**Background**: We developed and validated a liquid chromatography-tandem mass spectrometry (LC-MC/MC) method for measuring 17-hydroxyprogesterone (17-OHP) and cortisol in dried blood spots (DBS) as a confirmatory test for congenital adrenal hyperplasia (CAH). The most common cause of CAH is 21-hydroxylase deficiency leading to accumulation of its substrate, 17-OHP, and reduction of cortisol. Newborn screening (NBS) plays an important role in reducing morbidity and mortality, as early diagnosis and timely initiation of therapy can prevent adrenal crisis

caused by CAH. Most NBS programs use immunoassays to measure the concentration of 17-OHP in DBS. Although immunoassays are generally sensitive, the specificity is low leading to a low positive predictive value. A reliable second tier confirmatory test that will reduce the false positive rate while minimizing the chance of missing clinically significant cases would be beneficial.

**Methods**: Cortisol and 17-OHP of DBS (4 x 3 mm) were suspended in 0.5 mL water, extracted with 1.4 mL tert-butyl-methyl-ether, and separated on a C18 column (50x2.1 mm, 2.6 µm). The analysis was carried out using electrospray ionization tandem mass spectrometry in negative-ion mode for 17-OHP by monitoring m/z 329→285 transition for quantification, m/z 329→123 for qualification, and [2,3,4-$^{13}C_3$]17-OHP internal standard by monitoring the m/z 332→126 transition. Cortisol was detected in positive-ion mode by monitoring m/z 363→121 for quantification, m/z 363→91 for qualification, and [2,3,4-$^{13}C_3$]cortisol internal standard by monitoring m/z 366→124 transition. Concentrations of 17-OHP and cortisol in QC and patient DBS samples were determined using the LC-MS/MS method and compared with the 17-OHP concentrations measured using the AUTODELFIA Neonatal 17α-OH-progesterone immunoassay kit (Perkin Elmer).

**Results:** The LC-MS/MS method had a limit of detection, for 17-OHP and cortisol, of 5 and 2.5 ng/mL, and limit of quantification of 10 and 5 ng/mL, respectively. Both inter- and intra-assay imprecisions were < 12% at 20, 80, and 120 ng/ml for 17-OHP, and 30, 80, 120 ng/ml for cortisol. Recoveries were 91-109% for 17-OHP at 40, 80, 160 ng/ml and cortisol at 50, 100, 200 ng/ml, respectively. Linear response was obtained for 17-OHP ($R^2$ = 0.9938, range 10 - 160 ng/mL), and for cortisol ($R^2$ = 0.9963, range 5 - 200 ng/mL). No interference was observed in DBS samples spiked with 10 steroids at concentrations >10 times the upper limit of their corresponding reference ranges. Correlation analysis of results by LC-MS/MS versus AUTODELFIA showed $R^2$ = 0.9787 (AUTODELFIA = 0.9215*LC-MS/MS, n=43) for positive and negative QC materials, and $R^2$ =0.3649 (AUTODELFIA = 1.5305*LC-MS/MS, n=44) for patient samples. When using 56 ng/mL as the positive cut-off value for 17-OHP, a sensitivity of 82.4% and specificity of 96.3% was achieved. Combining this cut-off value with a cut-off value of 1.0 for the ratio of 17-OHP to cortisol, the sensitivity increased to 94.4% with a specificity of 100%.

**Conclusions:** The LC-MS/MS method for measuring 17-OHP and cortisol produced more reliable 17-OHP results than the immunoassay, possibly by specifically detecting the analytes of interest and being not subject to interfering materials existed in patient samples. The method can be used for confirming CAH.

## A-438

### A highly reproducible automated proteomics sample preparation workflow

Q. Fu[1], M. P. Kowalski[2], M. Mastali[1], S. J. Parker[1], K. Sobhani[3], I. v. Broek[1], C. L. Hunter[4], J. E. Van Eyk[1]. *[1]Advanced Clinical Biosystems Research Institute, Cedars Sinai Medical Center, Los Angeles, CA, [2]Beckman Coulter Life Sciences, Indianapolis, IN, [3]Pathology and Laboratory Medicine, Los Angeles, CA, [4]SCIEX, Foster City, CA*

**BACKGROUND:** Sample preparation for protein quantification by mass spectrometry requires multiple processing steps including denaturation, reduction, alkylation, protease cleavage, and peptide cleanup. Scaling these procedures for the analysis of numerous complex biological samples can be tedious and time-consuming, and there are many liquid transfer steps and timed reactions where technical variations can be introduced and propagated.

**METHODS:** An automated sample preparation workflow was established on a Biomek NX$^P$ Span-8 Laboratory Automation Workstation. The total processing time for 96 samples is 5 hours, including denaturation, reduction, alkylation and a 2-hour incubation with trypsin. Peptide cleanup is accomplished by online diversion during the LC-MS analaysis.

**RESULTS:** For albumin, complement C3, alpha-1-antitrypsin, alpha-1-acid glycoprotein 1, hemopexin, and apolipoprotein C-III and spiked β-galactosidase mean intra-day CVs for 5 samples ranged from 5.5%-8.9% for serum and 3.9%-6.2% for plasma, and mean inter-day CVs over 5 days ranged from 5.8%-10.6% for serum and 3.9%-6.0% for plasma. In an SRM assay targeting >70 proteins, 90% of the transitions from 6 plasma samples repeated on 3 separate days had total CVs below 20%. Similar results, with at least 93% of peptides having CVs below 20%, were obtained when the workflow was transferred to a second site. In an analysis of β-galactosidase-spiked plasma samples from 48 individuals, the average CVs for 3 β-galactosidase peptides were less than 10%.

**CONCLUSIONS:** An automated trypsin digestion workflow yields uniformly-processed samples in less than 5 hours. Reproducible quantitation of hundreds of peptides from >70 proteins was seen across replicates, days, instruments, and laboratory sites, demonstrating the broad applicability of this approach.

## A-439

### Characterization of Standard Reference Material 2924 C-reactive Protein Solution

E. L. Kilpatrick, L. E. Kilpatrick, M. S. Lowenthal. *National Institute of Standards and Technology, Gaithersburg, MD*

**Background:** The National Institute of Standards and Technology (NIST) has developed a standard reference material (SRM) - SRM 2924, C-Reactive Protein Solution. This material is a recombinant C-reactive protein (CRP) intended to serve as a "pure substance" calibrant providing traceability to SI units in the analysis of future serum-based reference materials containing CRP. The material was analyzed using a variety of techniques to determine concentration, molar mass, structure, stability and density using the highest order methods possible. This material will complement the existing reference material CRM 6201b produced by the National Metrology Institute of Japan.

**Methods:** The concentration of SRM 2924 was determined using amino acid analysis (AAA) using double isotope-dilution tandem mass spectrometry. Samples were selected for analysis using stratified random sampling from the sample boxes and were subjected to hydrochloric acid hydrolysis following spiking with isotopically labeled free amino acids (isoleucine, leucine, phenylalanine, proline and valine) and calibrated by similarly processed unlabeled amino acids. NMIJ CRM 6201-b, C-reactive Protein Solution, was included as a quality control material. The pentameric structure of CRP was analyzed using size exclusion chromatography (SEC) with detection by monitoring fluorescent detection (295 nm excitation, 350 nm emission). Retention times were calibrated using protein size standards. Molar mass of the material was assessed using electrospray ionization mass spectrometry with deconvolution. Density determinations were made using the Lang Levy pipet method. Optimal storage conditions were determined using AAA and SEC comparing frozen and refrigerated groups. Short term stability of the material was analyzed by incubating the material at a variety of times and temperatures and assessed by retention of pentameric structure.

**Results:** The concentration of SRM2924 was 20.6 µmol/L with an expanded uncertainty of 1.2 µmol/L (k=2.15) with good linearity of the calibrating curves ($r^2$ >0.999). The material concentration was determined to be homogeneous although slight differences were observed dependent upon from which box the material was selected. These differences were added into the overall uncertainty. Values for the QC material were within 2.8% of the certified value. Relative molar mass values determined to be 23028.0 (dimensionless) with an expanded uncertainty of 0.3 (k=2.20). This agrees with the theoretical value of 23027.8 based on the sequence and known post-translational modifications. Pentameric structure was assessed comparing the retention time of SRM2924 to calibrating proteins of known size. The principle peak was 99.6% of the total signal observed between the calibrating masses of 158,000 Dalton and 44,000 Dalton verifying the pentameric structure. Additional peaks were observed eluting at later times but were determined to be either preservative (sodium azide) or small levels of nucleic acids based on ultraviolet absorbance characteristics. Long term storage of the material will be at a temperature of -80 °C. Stability studies showed no effects on structure for short term temperature excursions.

**Conclusion:** The evaluation of SRM2924, C-reactive Protein solution was performed using the highest order methods available for a variety of characteristics and has been found to be acceptable as a secondary reference material with anticipated release in the second quarter of 2017.

## A-440

### Solid phase microextraction: a new tool for in vivo monitoring of metabolic changes occurring during deep brain stimulation

N. Reyes Garces[1], E. Boyacı[1], G. A. Gomez-Rios[1], B. Bojko[1], C. Hamani[2], J. Pawliszyn[1]. *[1]University of Waterloo, Waterloo, ON, Canada, [2]University of Toronto, Toronto, ON, Canada*

**Background:** Deep brain stimulation (DBS) is a medical therapy successfully used to treat several disorders including Parkinson's disease, essential tremor, depression, among others. Despite the fact that DBS has been used for decades, there is not a clear understanding about the neurophysiological mechanism behind it. Solid phase microextraction (SPME) is a sample preparation tool that has demonstrated its suitability for untargeted and in vivo studies. Taking advantage of such features, in this work we present the application of SPME and liquid chromatography-mass spectrometry as a strategy to elucidate the biochemical changes occurring in the brain hippocampus of rodents after electrical stimulation of their prefrontal cortex.

**Methods:** Four months male Fischer rats were used as subjects. 4 mm mixed mode and C18 SPME fibres were employed. The fibres were preconditioned in 1:1

methanol:water, rinsed in water, introduced into the brain for 30 min, and afterwards they were quickly rinsed in nanopure water. A special holder to ensure secure positioning of the fibres in the rats brain was used. The MM probes were desorbed in 1:1 acetonitrile:water and the C18 probes were desorbed in methanol that was later diluted with water. The extracts were run using liquid chromatography coupled to high resolution mass spectrometry (Orbitrap). Data analysis was done using XCMS online for features detection, alignment and annotation. Univariate statistical analysis was carried out using the Mann-Witney test. **Results:** Several metabolic features were found to be up-regulated or down-regulated after comparing baseline and post-DBS extracts . For brain extracts obtained with mixed mode coatings 105 and 93 features showed p-values < 0.01 and fold changes >1.5 in positive and negative modes, respectively. In the case of C18, 255 features in positive mode and 295 features in negative mode were found to meet the same threshold. One of the most interesting findings relates to the upregulation of glutamate and citrulline. Citrulline is coproduct of the enzymatic generation of nitric oxide (NO), a reaction that is catalyzed by nitric oxide synthase (NOS) and where arginine is the only substrate of all NOS isoforms. Based on these results it is suggested that an increase in the production of nitric oxide takes place after DBS of the prefrontal cortex. Taurine, uric acid and several lipids were also found among the dysregulated metabolites (p-values<0.01, q-value<0.05 and fold changes > 1.5). To the best of our knowledge, this work is the first metabolomics-based attempt to understand DBS mechanisms. **Conclusion:** By using an SPME and LC-MS based metabolomics platform it was possible to monitor in vivo metabolic changes occurring after DBS.

## A-441

### Enhanced Lipids Removal from Biological Matrices to Prepare Samples for LC/MS/MS Analysis

L. Zhao, D. Lucas. *Agilent Technologies, Wilmington, DE*

**Background:** Matrix components like proteins and lipids can significantly impact bioanalysis quality by liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS). Proteins can be removed effectively by protein precipitation, while phospholipids and other matrix components can take more effort and cost for efficient removal. The unremoved phospholipids and matrix interferences can cause ion suppression resulting in lower detection limits and poor method reliability. Using the Agilent EMR-Lipid method, protein precipitation can be performed in-well followed by pass-through cleanup for highly selective and efficient matrix removal, significantly improving the reliability of bioanalysis by LC/MS/MS.

**Methods:** In order to demonstrate the EMR-Lipid method on biological matrix cleanup, eight generic drug compounds were tested for the quantitation in human serum, including 5-Fluorouracil, Gemcitabine, Amphetamine, Metoprolol, Hydrocortisone, Warfarin, Androstenedione, Atorvastatin, and Diclofnac. These compounds were selected to cover different compound properties, from hydrophilic to hydrophobic, and acidic to neutral to basic. In addition, different biological matrices were tested for phospholipid removal, including human plasma, human serum, and animal plasma. The quantitative method was investigated for calibration curve linearity, inter- and intra-day accuracy and precision, method selectivity and recovery.

**Results:** Calibration range were 0.5 - 200 ng/mL including duplicated calibration curves, and the $R^2$>0.99 for all of compounds with >90% of calibration points within 20% of expected concentration at LOQ level and 15% at all higher levels. The accuracy and precision data were collected based on five levels of QCs, with average accuracy within 15% of expected concentration. Precision is demonstrated with <15% RSD for both intra-day (n=6 at each level) and inter-day (n=18 at each level) reproducibility. The absolute compounds recoveries were between 80-120% for all of compounds at level of 1 ng/mL. Among all of the tested biological matrices, >99% removal of phospholipids was obtained using the EMR-Lipid cleanup with protein precipitation. The post-column infusion test results demonstrated significantly reduced ion suppression in samples using the EMR-Lipid cleanup compared to samples without cleanup or other cleanup methods.

**Conclusion:** The results demonstrate that the Agilent EMR-Lipid cleanup method provides easy sample treatment workflow, highly selective and efficient lipid/matrix removal and reduces matrix ion suppression, and thus support the accurate and precise quantitation of multiple drug compounds in biological matrices. For Research Use Only. Not for use in diagnostic procedures.

## A-442

### A Validated Turboflow LC-MS/MS Method for Plazomicin - Its Role as a Reference Method in Diagnostics Development

M. Rudrabhatla[1], D. Luo[1], A. Thao[1], R. Diokno[2], N. Sandon[2], J. Seroogy[2], J. S. D. Zimmer[3], L. Ye[1], T. Rigl[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Achaogen, South San Francisco, CA, [3]Alturas Analytics, Inc., Moscow, ID*

**Background**: LC-MS/MS is a practical reference method to demonstrate immunoassay performance in complementary or companion diagnostics programs, where the drug is new and the immunoassay to be commercialized might, during the course of drug development, be found to be beneficial or even essential for therapeutic drug management. Here, we present the development and validation of a novel TurboFlow™ LC-MS/MS method for the detection and quantitation of plazomicin in human serum/plasma. This LC-MS/MS method will serve as a reference method for development of a QMS® Plazomicin Immunoassay on the Beckman Coulter® AU 680. Plazomicin is a next generation aminoglycoside antibiotic being developed to treat serious bacterial infections due to multi-drug resistant (MDR) Enterobacteriaceae, including carbapenem-resistant Enterobacteriaceae (CRE).

**Methods**: The method involves a simple single step extraction/protein precipitation from 50 µL serum/plasma using trichloroacetic acid (TCA), followed by centrifugation. The extract is then injected into the TLX-HESI-MS/MS in the positive ion mode using optimized TurboFlow and LC-MS/MS conditions. Cyclone P 0.5x50 mm TurboFlow and Hypersil Gold 50x2.1mm, 3 µm analytical columns are used. The TurboFlow clean-up and analytical separation were achieved within 7-minute run time. The method was validated as per the FDA guidelines on bioanalytical method validation. The validation studies used 9 calibrator and 4 control levels.

**Results**: A TurboFlow LC-MS/MS method for the sensitive detection and accurate quantitation of plazomicin in human serum/plasma was developed. A nine-point linear calibration curve ranging from 0-100 µg/mL with a correlation coefficient R of 0.999 was established. The lower limit of quantification (LLOQ) was established at 0.08 µg/ml. Minimal carryover was observed. The method demonstrated intra-day precision from 0.56% to 5.87% CV and inter-day precision from 1.96% to 4.91% CV, with recovery bias less than ±10%. The method validated that plazomicin analyte in human serum/plasma can be subjected to three freeze/thaw cycles. The auto sampler reinjection stability achieved 24 hours at $4^0$C. For dilution integrity a recovery bias less than 7.24% and coefficient of determination ($R^2$) at 0.9986 were obtained. This indicated that the samples could be accurately quantitated after diluting 10-fold. No interferences were observed when tested with other aminoglycosides or potential interfering substances. Matrix effects were minimal. Method comparison studies performed using plazomicin clinical samples between the in-house TurboFlow LC-MS/MS method and a separate LC-MS/MS method, developed and validated at Alturas Analytic, Inc., typically used in pharmacokinetic studies, yielded a Passing-Bablok's regression equation of y = 1.043x - 0.4103 with an R of 0.9657 (n=62).

**Conclusions**: The developed TurboFlow LC-MS/MS method for the quantitation of plazomicin in human serum/plasma is highly sensitive, specific, robust and accurate. TurboFlow clean-up saves a significant amount of time in sample preparation. Due to its excellent performance characteristics, the method can be successfully used as a reference method for the development of the QMS® Plazomicin Immunoassay on the Beckman Coulter® AU 680.

This project has been partially funded under BARDA Contract No. HHSO100201000046C.

## A-443

### Liquid Chromatograpy Tandem Mass SpectrometryAnalysis of Edibles Containing Δ9-tetrahydrocannabinol

H. Horng, K. Vo, K. Li, C. Smollin, K. Lynch, A. Wu. *UCSF, San Francisco, CA*

Cannabis and its principal active constituent, Δ9-tetrahydrocannabinol (THC), are becoming increasingly available as edibles resembling commercially available products. In this case, we describe a population of predominantly pediatric patients who were inadvertently exposed to a THC-containing gummy product and the liquid chromatography tandem mass spectrometry (LC-MS/MS) method used to quantitate the levels of Δ9-tetrahydrocannabinol (THC) and the 11-nor-9-carboxy-Δ9-THC (THC-COOH). Twelve children and nine adults were identified, with 16 patients having detectable serum THC and THC-COOH. In general, pediatric patients had more severe symptoms and longer hospital length of stays, and uniquely, a majority presented with leukocytosis and elevated lactic acid levels. For the sample preparation of serum samples, aliquots (50 µl) were taken and added directly into microcentrifuge

tubes and quenched with a solution of acetonitrile containing 11-nor-9-carboxy-Δ9-THC-$D_3$ internal standard (150 µl). Samples were then centrifuged and the supernatant fraction (150 µl) was transferred to HPLC vials for LC-MS/MS analysis. Sample preparation for the gummy candies involved weighing, melting, and water incubation, followed by the preparation steps described above. Serum and gummy candy samples were analyzed by LC-MS/MS (API 5000) analyses using a reverse phase column (Thermo BDS Hypersil C8, 5.0 µm, 4.6 x 50 mm) via electrospray positive ionization. The detection of THC and THC-COOH, and 11-nor-9-carboxy-Δ9-THC-$D_3$ (internal standard), were carried out by multiple reaction monitoring (MRM) transitions $MH^+$ m/z 315 to m/z 41, $MH^+$ m/z 345 to m/z 299, and $MH^+$ m/z 348 to m/z 302, respectively via a gradient system of water/0.1% formic acid and acetonitrile/0.1 formic acid, 15% ACN to 100%, over 6 min at a flow rate of 0.5 ml/min. The elution times of 4.5 min and 3.8 min were obtained for THC and THC-COOH, respectively. Concentrations were determined by plotting peak area ratios of THC and THC-COOH to the internal standard versus the concentration of THC and THC-COOH. The acquisition of mass spectral data was acquired with Analyst software (version 1.5.2). The measurable concentration range is 1 to 1000 ng/ml and the lower limit of quantitation was equivalent to the lowest point of the standard curve. Interday precision and accuracy were determined through the analysis of quality control (QC) samples at 3 concentrations (800, 200, and 30 ng/ml hair). Precision, defined by the coefficient of variation (CV) and accuracy, defined by relative error (RE) were less than <15%. Each gummy candy contained approximately 1.92 mg of THC (206 µg/g THC). Both THC and its metabolite, THC-COOH, were detected in all serum samples available for analysis. All but three patients had serum THC-COOH concentrations that were greater than THC concentrations, which is consistent with the published literature on THC pharmacokinetics after oral ingestion. Of the samples obtained, THC concentrations ranged from 17.9-106 ng/mL in pediatric patients, and 27.5-91.9 ng/mL in adult patients. Serum THC-COOH concentrations ranged from 10.9-195 ng/mL in pediatric patients, and 27.5-159 ng/mL in adult patients. Six patients had quantifiable serum THC and metabolite levels, which were not reflected in preliminary urine immunoassays.

## A-444

### Making the Right Call: Distinguishing Residual M-proteins from Monoclonal Antibody Therapies Used in the Treatment of Multiple Myeloma

M. C. Kohlhagen, D. R. Barnidge, J. R. Mills, D. L. Murray. *Mayo Clinic, Rochester, MN*

**Background:** Diagnosis and management of Multiple Myeloma (MM) is largely supported by the identification and quantification of the disease-associated M-protein. The most commonly encountered M-protein is an intact monoclonal immunoglobulin (Ig) of the IgGκ isotype accounting for up to 40% of M-proteins. Recently, the breakthrough therapeutic monoclonal antibodies (t-mAbs), daratumumab and elotuzumab, were FDA-approved for treatment of refractory MM. Furthermore, additional t-mAbs (isatuximab) have entered phase III clinical trials for treatment of MM. These t-mAbs are of the IgGκ isotype and are given at doses where a majority of patients will achieve steady-state concentrations that exceed the detection limit of immunofixation electrophoresis (IFE) and in a subset of patients will also be detectable by protein electrophoresis (PEL). Each of these therapies migrates within the γ-region making it challenging to distinguish between residual IgGκ M-proteins and t-mAbs using conventional methods with certainty. One solution is to identify t-mAbs through their accurate molecular mass, which is distinct for each t-mAb, using mass spectrometry (MS). The objective of this study was to develop and assess the analytical performance of different MS methods to differentiate residual M-proteins from t-mAbs used in the treatment of MM.

**Methods:** Serum was immunoenriched for IgG antibodies using a camelid-derived single domain antibody fragment coupled to agarose beads that recognize all IgG subclasses independent of the light chain subclass. Bound antibodies were washed with PBS/water and eluted with 50 mM TCEP in 5% acetic acid to dissociate bound Igs into heavy and light chain components. Dissociated Igs were spotted onto a MALDI plate and analyzed using a Bruker Microflex LT MALDI-TOF instrument and were also analyzed by a high resolution, accurate mass (HRAM) LC-MS method using microflow liquid chromatography coupled with an ESI-QTOF mass spectrometer. Patient samples with IgGκ M-proteins that co-migrated with daratumumab, elotuzumab or isatuximab were used for spike-in studies. Each M-protein was diluted to a range of M-protein concentrations (0.1g/dL- 1g/dL) with pooled healthy donor serum and then spiked with a range of concentrations (range: 0 g/dL - 0.1 g/dL) of co-migrating t-mAbs (N=173). In addition, residual serum samples were collected from patients with a history of IgGκ MM who had received daratumumab (N=17), elotuzumab (N=2) or isatuximab (N=2) therapy. All samples in this study were evaluated by IFE, MALDI-TOF and HRAM-MS.

**Results:** T-mAbs could be resolved from endogenous M-proteins using HRAM LC-MS in 100% of spike-in samples (173/173). MALDI-TOF MS could resolve 83% of samples (143/173). In samples collected from patients receiving a t-mAb, the presence of an interference was ruled out correctly in 100% of cases (21/21) by both MS methods. In contrast, blinded reviewers were uncertain in nearly all samples evaluated by IFE.

**Conclusion:** HRAM LC-MS could differentiate t-mAbs from residual M-proteins in all samples evaluated in this study. In contrast, both MALDI-TOF MS and traditional IFE were unable to resolve all cases with a high degree of certainty. HRAM LC-MS provides a reliable method to differentiate residual M-proteins and exogenous t-mAbs.

## A-445

### In situ metabolomics analysis analysis of grafts by SPME-LC-HRMS: the minimum invasive approach to reliable assessment of organ quality and function

I. Stryjak, B. Bojko. *Nicolaus Copernicus University, Bydgoszcz, Poland*

**Background:** Since introduction of immunosuppressant drugs transplantation became routinely used life-saving procedure. However, still there are problems, which transplant surgeons need to face. Among them is limited access to reliable tools for assessment of quality of donor organ and not fully understood mechanisms of reperfusion injury. Currently, the assessment is mainly done by visual inspection supported by single biopsy collection and histological testing in some cases. Metabolomics, which is recently used for characterization of biological specimens including tissue samples would be an excellent tool aiming better evaluation of organs quality, but standard sample preparation approaches for tissue analysis require collection of additional biopsy, which would increase the risk of organ damage. To address the problem, minimum invasive tool for tissue sampling was proposed. Device of the size of acupuncture needle coated with extraction phase, named solid phase microextraction fiber, and combining in situ extraction of metabolites and metabolism quenching was used to obtain wide range of small molecules of different chemical and physical properties.

**Methods:** The pilot studies involved sampling of six kidneys harvested from rabbits. Sampling times were 0, 2, 4, 6 and 21 hours after harvesting. Organs were stored at static hypothermic conditions. For the studies probes with 7 mm C18/SCX coating was used. Time of sampling was 30 min followed by desorption in 250 uL of acetonitrile:water 1:1 (v/v). The extracts were analyzed on Q Exactive Focus (Thermo Fisher Scientific) in positive and negative ionization mode. Reversed phase chromatographic separation was performed using Discovery HS F5, 2.1 mm x 100 mm, 3 µm column (Supelco). Compound Discoverer 2.0.0.303 was used for data processing and analysis. For the significance test the p-value of <0.05 and two-fold change were set.

**Results:** The results showed change in metabolic profile already after 2 h of preservation. Comparable metabolic profile, which was found after fourth hour was followed by dramatic change after sixth hour and it was similar after 21 h. The most significant change in metabolic profile during the first four hours was associated with level of nicotinamide and nicotinamide mononucleotide, which protective role against ischemia and oxidative stress was already proven in heart transplant. Also, increased concentration of cystein and cysteinylglycine participating in extracellular reduction of glutathione suggested strong antioxidative response form the tissue. The findings reflect an ischemic preconditioning phenomenon reported mainly for heart and liver tissue. The decline of these metabolites as well as some of the amino acids also playing protective role against ischemic injury like arginine, lysine or guanine after 6 h of static hypothermic preservation showed that protective capacity of the tissue has been finished. Moreover, a significant decrease of guanosine, inosine and adenosine level was observed indicating utilization of the compounds as alternative energy source during glucose deficiency.

**Conclusions:** The pilot study showed that the proposed methodology has a potential in discovery of biomarkers for graft quality. However extensive study and appropriate validation have to be used to achieve the final goal in the future.

**A-446**

## Parallel Reaction Monitoring using Q-Exactive Exhibit Improved Sensitivity and Selectivity in Sub-amole Testosterone Quantification

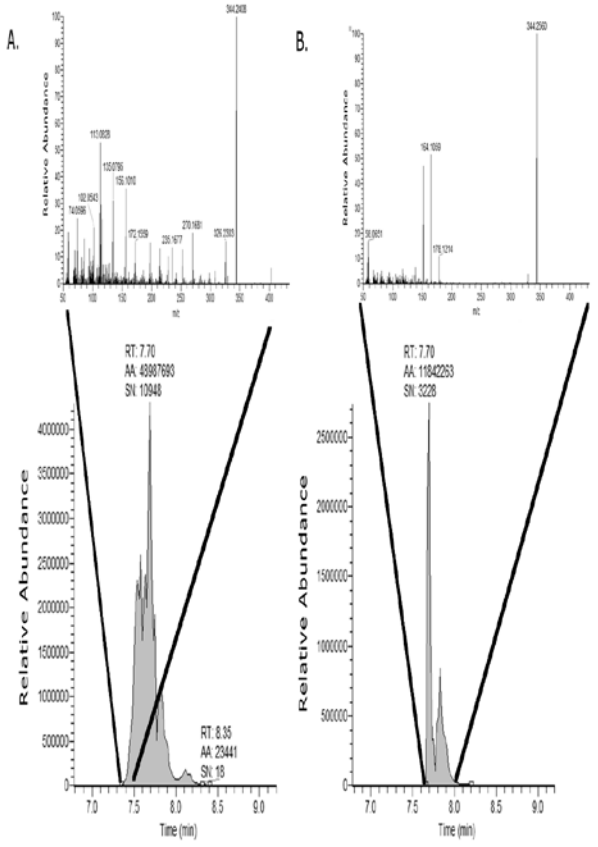
Y. Zhou, D. Bunch, S. Wang. *Department of Laboratory medicine, Cleveland Clinic, Cleveland, OH*

**Background:** Precise and accurate measurement of low level testosterone in highly complex clinical samples remains challenging. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the technology of choice for this analyte due to its high sensitivity and specificity. However, wide range of interferences present in a complex biological specimen impair reliable quantification by LC-MS/MS, especially at the sub pg/mL level for free testosterone in circulation. Parallel Reaction Monitoring (PRM) using high-resolution mass spectrometry (HRMS) not only has target quantitative analysis capabilities, but also performs MS/MS full scan at the same time, offering additional confidence of analyte identification.

**Methods:** Testosterone in serum was extracted, dried, and derivatized with Amplifex™ Keto reagent (AB SCIEX). The resulting solution was injected onto an EASY-Spray™ PepMap™ RSLC (C18 15 cm x 75 µm ID column) and eluted with a 40-90% acetonitrile gradient in 0.1% formic acid over 12 min at a flow rate of 300 nL/min. The analytical system was a Thermo Scientific™ EASY-nLC 1000™ HPLC system with EASYSpray™ source and coupled to a Q-Exactive mass spectrometer (Thermo Scientific). The Q-Exactive was set for time scheduled parallel reaction monitoring.

**Results:**A blank Seracon serum was spiked with 1.0 pg/mL testosterone. After derivatization, 10 µL resulting solution was analyzed using the nLC-MS method. Ion chromatograms were constructed using a mass tolerance window of 500 mmu (A) and 2 mmu (B), respectively. As shown in Figure, the chromatogram for 500 mmu mass tolerance window had unresolved peaks with significant interference, while the 2 mmu mass tolerance provided two nice isoform peaks with much less interference.

**Conclusion:** HRMS was demonstrated to reduce interference, resulting in reliable quantification of low abundance testosterone in a complex biological matrix.



# Tuesday, August 1, 2017

Poster Session: 9:30 AM - 5:00 PM

Nutrition/Trace Metals/Vitamins

## A-447

### Assessment Of Oxidative Stress And Antioxidant Status; And Their Correlation With Glycated Hemoglobin In Diabetics As Well As In Healthy Controls

M. Sigdel[1], D. R. Pokharel[1], N. Chaudhary[2], N. Chapagain[2], R. Baral[2], S. Timilsina[2], S. Tiwari[2], R. Chalese[3], N. K. Yadav[1], S. K. Jha[1], L. B. Sapkota[4], P. Shukla[1]. *[1]Manipal College of Medical Sciences, Pokhara, Nepal, [2]School of Health and Allied Sciences, Pokhara, Nepal, [3]Institute of Medicine, Kathmandu, Nepal, [4]Chitwan Medical College, Chitwan, Nepal*

**Background:** Several studies have indicated lopsided redox balance due to pro oxidant environment as one of the important etiological factors for diabetes. Some recent researches also indicate a causal relationship with oxidative stress. So far, no detailed study has been undertaken on this aspect in Nepali populations. We, therefore, aimed this maiden study to compare the magnitude of oxidative stress as well as antioxidant capacity and correlate them with the glycated hemoglobin - a marker of hyperglycemia in type two diabetes mellitus (T2DM) and in healthy controls.

**Methods:** Cross sectional study involving 125 subjects with T2DM and 125 healthy controls. Plasma total peroxide (TP) and Total antioxidant capacity (TAC) were measured to evaluate the oxidative stress and antioxidant status respectively. Oxidative stress index (OSI) was calculated as the ratio of TP to TAC. Fasting blood sugar (FBS), Glycated haemoglobin (GHb), and post prandrial blood sugar (PPBS) were also measured. Statistical analysis was performed using SPSS 17.0.

**Results:** Medians of FBS, PPBS, GHb, HbA1c, mean blood glucose (MBG), plasma TP and OSI levels were significantly higher (P = <0.001) in T2DM patients compared to healthy group whereas plasma TAC levels were significantly lower (P = <0.001) in T2DM group. In case of diabetic group, GHb showed significant positive correlation with TP ($\rho$ = 0.51; P = <0.001) and OSI ($\rho$ =0.54; P = <0.001) whereas with TAC it showed significant negative correlation ($\rho$ = - 0.53; P = <0.001). However, in case of control group, GHb showed weak positive correlation with TP ($\rho$ = 0.02; P = 0.904), OSI ($\rho$ = 0.05; P = 0.727) as well as with TAC ($\rho$ = 0.01; P = 0.951) which were statistically insignificant.

**Conclusion:** The study showed an increase in oxidative stress and decrease in antioxidant capacity in diabetes and also indicated a positive correlation between the degree of hyperglycemia and oxidative stress. So, evaluation of oxidative status and choosing the appropriate treatment may help to support antioxidant defense in diabetic patients.

## A-448

### Oral Administration Of Cyanocobalamin Causes Higher Increase In Circulating Holotranscobalamin Than Hydroxocobalamin: An Indo Danish Study With Different Doses Of Cobalamin

E. Greibe[1], N. Mahalle[2], V. Bhide[2], C. Heegaard[3], S. Naik[2], E. Nexo[1]. *[1]Department of Clinical Biochemistry and Institute of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark, [2]Deenanath Mangeshkar Hospital and Research Center, Pune, Maharashtra, India, [3]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark*

**Background:** Most studies on cobalamin absorption are carried out with cyanocobalamin, but little is known as to whether such studies reflect the absorption of the natural forms of the vitamin present in food items, such as hydroxocobalamin. **Objective:** Here we investigate the uptake of oral intake of cyanocobalamin and hydroxocobalamin in an Indian (low cobalamin status) and Danish (high cobalamin status) population.

**Methods:** The CobaSorb test was used to estimate cobalamin absorption by measurement of circulating holotranscobalamin before and after three daily doses of cobalamin for two days. In the deplete population (n = 59, divided into six groups), the test was performed twice on each participant using a cross-over design with three different doses (1.5 µg, 3 µg, and 6 µg) of cyanocobalamin and hydroxocobalamin. In the replete population (n = 42), the test was performed three times with doses of (3 µg, 6 µg, and 9 µg) cyanocobalamin in 28 individuals, and twice with doses of 9 µg cyanocobalamin and 9 µg hydroxocobalamin in 14 individuals. Holotranscobalamin was measured by an in-house ELISA.

**Results**: In the cobalamin-deplete population, doses of 6 µg cyanocobalamin and hydroxocobalamin showed higher increase in holotranscobalamin than doses of 1.5 µg and 3 µg with no difference between 1.5 µg and 3 µg. Cyanocobalamin showed a 2-3 times higher increase in holotranscobalamin than hydroxocobalamin for all three doses (1.5µg:p<0.0001; 3 µg: p = 0.0002; 6µg: p<0.0001). In the cobalamin-replete population, doses of 3 µg cyanocobalamin showed a lower increase in holotranscobalamin than doses of 6 µg ($p$ = 0.03) and 9 µg ($p$ = 0.005) with no difference between 6 µg and 9 µg ($p$ = 0.89). Cyanocobalamin (9 µg) showed a twofold increase in holotranscobalamin than hydroxocobalamin (9 µg) ($p$ < 0.0001).

**Conclusions:** We show that administration of cyanocobalamin result in a twofold increase in circulating holotranscobalamin than administration of hydroxocobalamin independent of cobalamin dose or cobalamin status. In addition, our data suggests that the maximal uptake capacity is reached only by doses of above 3 µg cobalamin administered three times per day in both cobalamin replete and deplete individuals. Our results underscore the importance of using equimolar doses of the same form of cobalamin while comparing the uptake of free as compared to e.g. food-bound cobalamin.

## A-449

### Comparative Study of Iodine Status between Community School Children and Adult Population in South-Western, Nepal

N. Gautam[1], U. Shrestha[1], K. K. Agrawal[2], A. C. Jha[1], A. Jayan[1]. *[1]Universal College of Medical Sciences, Department of Biochemistry, Bhairahawa, Nepal, [2]Universal College of Medical Sciences, Department of Internal Medicine, Bhairahawa, Nepal*

**Background:** The present study was designed to assess and compare the iodine status between the community school children and community dwelling adult population residing in south-western Lumbini zone, Nepal. The thyroid status was also assessed based on hormonal level and presence of goiter in that community and correlated with median urinary iodine (MUI) concentration.

**Methods:** A cross sectional comparative study was conducted on 170 primary school age children of 8-12 years and 151 adult populations of 20-50 years. Spot urine samples iodine was estimated by Ammonium Persulphate Digestion, WHO method and thyroid hormones in sera were analyzed by spectrophotometric ELISA method. Household salt iodine content was estimated by Iodometric titration method. At low, medium and high urinary iodine, intra assay CVs were 5.99%, 4.9% and 4.93% and inter assay CVs were 13.8%, 8.41% and 6.35%.

**Results:**Our results showed overall population had frequency of 26(15.29%) school children and 13(8.6%) adult had iodine deficiency. Out of which 8(4.7%) children/0(0%) adult had severe, 6(3.5 %) children/ 3(1.98%) adult had moderate and 12(7.1%) children/10(6.62%) adult had mild iodine deficiency. Though the maximum population had optimal iodine level, but 50(29.41%) children/73(48.34%) adult had more than adequate requirement iodine and 39(22.94%) children/25(16.55%) adult had excessive iodine intake. The median urinary iodine concentration (MUI) in school children was 204.65 µg/L as compared to adult 252.95 µg/L (p=0.0001). The overall goiter prevalence was 5(2.94%) in children/2(1.32%) in adult among them 3(60%) children had iodine deficiency and no iodine deficiency observed in adult. 20(11.76%)children/57(38%) adult had subclinical hypothyroidism with MUI 206.4 µg/L/224.9 µg/L and 8(4.7%)children/41(27%) adult had overt hypothyroidism with MUI 203.3 µg/L /281.0 µg/L showing more than adequate requirement of iodine status in hypothyroid patients. There was positive correlation MUI with TSH (r=0.269, p=0.0001), negative correlation with $fT_3$ (r=-0.328, p=0.0001) and negative correlation with grading of goiter (r=-0.198, p=0.01).

**Conclusion:** Our study showed spectrum of iodine status with iodine deficient, more than adequate requirement to excess iodine nutrition in both community children and adult. The high iodine intake as assessed by MUI may trigger the hypothyroidism in children as well as adult. Universal salt iodization and awareness programs regarding adverse effects of iodine excess should be continued to minimize the risk to the vulnerable age groups.

## A-450

### Burden of Vitamin D Deficiency and its Association with Insulin Resistance in Ghanaian Type 2 Diabetics

L. A. Fondjo[1], W. K. B. A. Owiredu[1], S. A. Sakyi[1], E. F. Laing[1], M. A. Adotey-Kwofie[2], E. 0. Antoh[1], E. Detoh[3]. *[1]Knust/Kath, Kumasi, Ghana, [2]Knust, Kumasi, Ghana, [3]Nkawie Government Hospital, Kumasi, Ghana*

**Background:** There is increasing interest in the non-skeletal role of vitamin D. Contemporary data suggests that vitamin D deficiency is related to several infectious and chronic conditions; with a possible influence on glucose homeostasis. There is growing incidence of diabetes mellitus among Ghanaians with a large percentage of the diabetics being overweight. In recent times, there has been attention on modifiable determinants for prevention of diabetes mellitus. Current data suggest vitamin D supplementation as a risk modifier for type 2 diabetes mellitus (T2DM), as it improves insulin secretion and reduce insulin resistance in T2DM. The vitamin D status and the association between vitamin D deficiency and diabetes has not been explored in Ghanaian population. This study provides preliminary information on vitamin D status among Ghanaian type 2 diabetics and assessed its association with glucose homeostasis. **Methods:** Briefly, in this case control study, 118 clinically diagnosed Ghanaian type 2 diabetics patients 25 years and above, of more than six months duration attending Diabetic Clinic at the Nkawie Government Hospital, Kumasi, Ghana, were selected as subjects between October and December 2015. Hundred healthy non-diabetics with fasting blood glucose (FBS) less than 6.4mmol/L living in Nkawie district were were selected as controls. Pregnant women and those with chronic illness or were on vitamin D supplementation were excluded. Structured questionnaires were administered to obtain socio-demographic data. Anthropometric data and venous blood samples were obtained from both subjects and controls to estimate their FBS, Lipid profile spectrophotometrically and intact parathyroid hormone, Insulin, vitamin D by using commercial ELISA kits. Statistical analyses were performed using SPSS v20.0 Statistics. **Results:** The average age of the cases was 58.81years and 57.79 years for the controls, more females were diabetic compared to males (n = 93/25). The indices of obesity (BMI, WC, HC, BAI) were significantly higher in the cases compared to the controls. There was vitamin D deficiency of 92.4% among the cases and 60.2% among the non-diabetic controls. Vitamin D deficiency did not significantly associate with **HOMA-β** [T2DM: $r^2=0.0209$, $p=0.1338$ and Control: $r^2=0.0213$, $p=0.2703$] and **HOMA-**IR [T2DM: $r^2=0.0233$, $p=0.1132$ and Control: $r^2=0.0214$, $p=0.2690$] in both the controls and the subjects**. Conclusion**: Vitamin D deficiency is prevalent in both T2DM and non-diabetics. There is no association between vitamin D deficiency and insulin resistance or beta cell function in our study population. Vitamin D supplementation among type 2 diabetics is recommended.

**KEYWORDS**: vitamin D, diabetes, HOMA-IR, HOMA- β, lipids, obesity.

## A-451

### Analysis of serum uric acid level and the prevalence of hypouricemia based on a multicenter study in Chinese population

Y. Yin. *Peking Union Medical College Hospital,Peking Union Medical College&Chinese Academy of Medical Science, Beijing, China*

**Context:**

Hypouricemia may lead to nephrolithiasis or acute renal failure, however, it did not get enough attention as hyperuricemia and often ignored by clinicians because of the low incidence.The total prevalence rate of hypouricemia(≤119μmol/L) varied from 0.61% to 0.97% in western countries and 0.15% to 0.34% in Japanese outpatients and healthy individuals. No data about the prevalence of hypouricemia in China based on large population was reported until now.

**Objective:**

The objective of this study was to examine the prevalence of hypouricemia based on a representative multicenter population in China.

**Methods:**

Data came from the Chinese Physiological Constant and Health Condition (CPCHC). Participants included 34724 participants (16440 males and 18284 females) aged from 8 to 98 years old, which were recruited from six representative provinces (20 cities) of China during October to November 2011. A physical examination was performed and fasting blood was collected for biochemical tests.Uric acid was measured with a Beckman AU Series Automatic Biochemical Analyzer, using Beckman AU reagents. Hypouricemia was defined as a serum uric acid concentration less or equal than 119μmol/l(2.0mg/dl).

**Results:**

The prevalence of hypouricemia in this population was 0.58% with a range of 2~119μmol/l. Of the total subjects, females had significant higher rate of hypouricemia than males (0.83% vs 0.31%, P<0.01), participants from Ningxia Hui Autonomous Region had significant higher rate of hypouricemia than other volunteers(P<0.01).The mean serum uric acid concentration of the total population was 304.24±85.37μmol/l. Females had significant lower uric acid concentration than males (268.29±68.85 vs 343±68.85μmol/l, P<0.01). Volunteers aged from 8 to 18 had higher uric acid level than that of individuals aged from 19 to 59 years old. Participants came from Sichuan and Yunnan province had significant higher uric acid concentration than those from other provinces. Higher uric acid levels were observed in participants with the increase of BMI.

**Conclusion:**

Hypouricemia in chinese population is not rare compared with other countries. However, less attention paid to hypouricemia in our country.

Sex, age, region and BMI had significant effect on the level of uric acid.

## A-452

### Impact of paper mill effluent discharge on the physico-chemical and microbiological qualities of Imo River at Owerrinta, Abia State, Nigeria.

J. N. Dike-Ndudim[1], E. E. Ejiogu[2], K. Obasi[2], A. C. Udebuani[2], F. O. U. Osuala[2]. *[1]Imo State University, Owerri, Owerri, Nigeria, [2]Federal University of Technology, Owerri, Owerri, Nigeria*

**Background:** Pollution of aquatic environment from industrial processes is one of industrial problems in Nigeria. Most industries discharge their effluents into water bodies. Such effluents may contain dissolved substances and microorganisms. This improper waste disposal leads to pollution of surface and ground water, hence unsafe to users and increases water-borne diseases. Water pollution affects the aesthetic status of water, the health of aquatic organisms and humans. Toxic pollutants can also alter the genetic makeup of an organism leading to their death or deformities. Consequently this research set out to assess the impact of paper mill effluent discharges on the physico-chemical and microbiological status of Imo River at Owerrinta.

**Methods:**River water samples from several locations and effluent sample from discharge point of the paper mill industry were aseptically collected and investigated physicochemically by Hach's and Atomic Absorption Spectrophotometer (AAS) techniques and microbiologicall by pour plate method on nutrient, MacConkey and Sabouraud agar.

**Results:** Study revealed that the river water at 30meters before the entry of effluents had a higher value of dissolved oxygen (5.90mg/l), pH range value, 6.5, Low values of suspended solids, alkalinity, total hardness, sulphate, nitrate, calcium, chemical oxygen demand, and biochemical oxygen demand. At the point of entry of the effluent, there was a decline in dissolved oxygen content (2.62mg/l) and increase in other factors. At about 150meters from the point of discharge of effluent, quantitatively, most of the parameters fall within the two earlier stations. The total heterotrophic aerobic bacteria counts ranged from $175.0x10^6$ (cfu)/ml and $125x10^4$(cfu)/ml to $8.75X10^6$ and $1.8X10^3$ (cfu)/ml. The fungal counts for all the samples ranged from $0.65X10^6$ (cfu)/ml to $3.5x10^6$ (cfu)/ml. Identified and predominant microorganisms are *Klebsiella species, Pseudomonas spp., Streptococcus faecalis, Bacillus species and Chromobacterium violaceum for* bacteria**,** and *Candida spp. and Aspergillus fumigatus* for the fungal isolates.

**Conclusion:** Physicochemical analysis showed that the effluent samples had the highest values of most of the parameters assessed, followed by the samples of the region of effluent discharge and lastly by the samples at the downstream. Analyses of the result revealed that a relationship exist between biochemical oxygen demand (BOD) and nitrate, whereas there is no relationship between total alkalinity and total hardness. The presence of microorganisms of public health and economic importance is indication of risks to users.

## A-453

**Impact of a single oral dose of 100,000 IU vitamin D3 on profiles of serum 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 in adults with vitamin D insufficiency**

L. Saleh[1], J. Tang[2], J. Gawinecka[1], W. Fraser[3], A. von Eckardstein[1], A. Nowak[4]. *[1]Institute for Clinical Chemistry, University Hospital of Zurich, Zurich, Switzerland, [2]I2Bioanalytical Facility, Bob Champion Research and Education Building, James Watson Road, University of East Anglia, Norwich Research Park, Norwich, United Kingdom, [3]2Bioanalytical Facility, Bob Champion Research and Education Building, James Watson Road, University of East Anglia, Norwich Research Park, Norwich, United Kingdom, [4]Division of Internal Medicine, University Hospital of Zurich, Zurich, Switzerland*

**Background:** We investigate the effect of a high dose of vitamin D3 on circulating concentrations of 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 in healthy individuals with self-perceived fatigue and vitamin D insufficiency (25(OH)D3 <50 nmol/L). **Methods:** 107 study participants (age 20-50 years) were randomized to receive a single 100,000 IU dose of vitamin D3 (n= 52) or placebo (n= 55). Vitamin D metabolite concentrations in serum were measured before, and 4 weeks after, supplementation. **Results:** Overall, 52% of participants receiving vitamin D3 attained a serum 25(OH)D3 level >75 nmol/L. Among individuals who received vitamin D3, there were significant increases in serum concentrations of 25(OH)D3 and its metabolites 24,25$(OH)_2$D3, 3-epi-25(OH)D3, and 1,25$(OH)_2$D3 at 4 weeks; however, inter-individual variability in these changes was substantial. Positive correlations between serum 25(OH)D3 and 24,25$(OH)_2$D3 and 3-epi-25(OH) D3, and a significant negative correlation between serum 1,25$(OH)_2$D3 and 3-epi-25(OH)D3, were found 4 weeks after supplementation. The 24,25$(OH)_2$D3/25(OH) D3 and 24,25$(OH)_2$D3/1,25$(OH)_2$D3 ratios were significantly increased, compared with baseline, in participants receiving vitamin D3. Baseline 25(OH)D3 concentration was the only factor predictive of the change in 25(OH)D3 after supplementation. **Conclusions:** Administration of a single high dose of vitamin D3 leads to a significant increase in concentrations of 25(OH)D3, 24,25$(OH)_2$D3, 3-epi-25(OH)D3 and 1,25$(OH)_2$D3; induction of the catabolic pathway predominates over the production of 1,25$(OH)_2$D3. Due to the high inter-individual variation in the 25(OH)D3 response to supplementation, any given dose of vitamin D is unlikely to achieve optimal vitamin D status in all treated individuals.

## A-454

**Vitamin D nutritional status and bone turnover biomarkers in acute lymphoblastic leukemia survivors**

E. Delvin[1], S. Morel[1], V. Marcil[1], C. Laverdière[1], N. Alos[1], M. Lecours[1], F. Rauch[2], D. Sinnett[1], C. Nyalendo[3], É. Levy[1]. *[1]Ste-Justine Hospital Research Centre, University of Montreal, Montreal, QC, Canada, [2]Shriners Hospital for Children, Mc Gill University, Montreal, QC, Canada, [3]Ste-Justine Hospital, University of Montreal, Montreal, QC, Canada*

**Objective:** The remarkable progress in the treatment of childhood acute lymphoblastic leukemia (ALL) has lead to an ever-increasing survival rate. This success story is unfortunately linked to increased risks of impaired accumulation of skeletal mass during childhood and adolescence, predisposing patients to impaired bone mass in early adulthood. This study aims at characterizing the vitamin D status and bone metabolism biomarkers in a large cohort of childhood ALL survivors.

**Methods:** The study population consists of 251 patients [median age at entry: 21.9 years] of French-Canadian origin with an established genetic founder effect, and diagnosed when younger than 19 years with ALL. They were all treated with the Dana Farber Cancer Institute protocols without hematopoietic stem cell transplantation. The median (2.5-97.5%$^{ile}$) post-treatment time was 13.0 (4.4-23.8) years. The Institutional Review Board of Sainte-Justine Hospital approved the study and the investigations were carried out in accordance with the principles of the Declaration of Helsinki.

Patients' dietary intakes were evaluated after filling a food frequency questionnaire using the Canadian Nutrient File Database. Serum 25-hydroxyvitamin $D_3$ ($25OHD_3$), for assessing the vitamin D nutritional status, was measured by a QTOF-MS method. Total calcium ($Ca_t$), inorganic phosphate ($P_i$) and alkaline phosphatase (Alk Phos) were measured on an Abbott's Architect *c*System and intact Parathyroid hormone (iPTH) on an Immulite 2000.

Serum bone resorption [Carboxy-terminal collagen type-1 telopeptide (CTx,)] and formation [Pro-collagen type-1 amino-terminal pro-peptide (P1NP)] biomarkers were measured by an automated chemiluminescent immunoassay (IDS Immunodiagnostics). Bone mineral density was measured by dual X-ray absorptiometry (DXA) using the Lunar Prodigy absorptiometer.

**Results:** The food frequency questionnaires revealed that the total vitamin D intake varied greatly (44-2132 IU/d), that only 16.8% of the participants consumed vitamin D supplements, and that 74% were below the RDI (400 IU/d). For those who took supplement (n = 42), the median (2.5 – 97.5$^{\%iles}$) intake was 600 IU/d (21.2-1972 IU/d). Only 14 patients had daily calcium intakes below the RDI (800 mg/d). Out of the 248 patients for whom $25OHD_3$ was measured, 16 were vitamin D deficient (<30 nM) and 66 insufficient (≥30 - <50 nM). Although the mean total body or lumbar (L1-L4) BMD Z-scores were with the normal range, 3 male participants and 2 female participants had Z-scores ≤ 2.5 SD, and 36 male participants and 21 female participants had Z-scores between ≤1.0 and <2.5 SD, classifying them respectively as osteoporotic and osteopenic. No correlation was observed between serum 25OHD concentration and serum bone turnover concentrations.

**Conclusions:** These data demonstrate that this group of ALL young adult survivors were not at higher risk of vitamin D deficiency or insufficiency than the general Canadian population. However a fair number exhibited osteopenia and a small percentage osteoporosis despite their young age. **(449 words)**

## A-455

**The relation between vitamin D deficiency in the first rimester of pregnancy and Bacterial vaginosis in Egypt**

N. S. El beltagy. *Alexandria University, Alexandria, Egypt*

**Background:** Adequate vitamin D intake is essential for maternal and fetal health during pregnancy, Epidemiological data indicates that many pregnant women have sub-optimal vitamin D levels. Notably, vitamin D deficiency correlates with preeclampsia, gestational diabetes mellitus, and bacterial vaginosis, and an increased risk for C-section delivery. Recent work emphasizes the importance of non classical roles of vitamin D in pregnancy and the placenta. The aim of the present study was to evaluate the association between vitamin D deficiency and bacterial vaginosis in the first trimester of pregnancy. **Methods**: a cross sectional study was conducted on 100 pregnant women attending Elshatby Maternity University Hospital during their first antenatal visit between April- December 2014 (6 months). Women were subjected to a pelvic speculum examination at the initial obstetrical visit to ascertain for the presence of BV and vaginal swab was taken to be evaluated by gram's stain for presence of bacterial vaginosis. Plasma 25-OH-D concentration, the major circulating form of vitamin D, was assayed using a commercially available ELISA kit. **Results**: the age of the included pregnant woman ranged from 20-32 with a mean of 24.9±2.91 years, vitamin D deficiency was considered if the serum level <10 IU. insufficient level between 10-30 IU and sufficient level with 30-100 IU in serum. Most of the studied women were less than or equal 25 years (60.0%). The gestational age ranged from 8.0-12.0 with a mean of 9.93±1.59 weeks. Most of the studied women had a BMI more than 25 kg/cm2 (95.0%). 82 cases were positive for bacterial vaginosis, while the other 18 cases(18%) were negative for bacterial vaginosis. The majority of the patients had vitamin D deficiency (53.0% of the patients), while 35.0% of them had insufficient vitamin D. Only 12 cases (12.0%) had sufficient amount of vitamin D. The level of vit D ranged from 1.54-40.0 IU with a mean of 13.97±9.57 IU. There was a significant relation between the incidences of vit. D deficiency and the presence of bacterial vaginosis. **Conclusions:** There was association between vitamin D deficiency in the first trimester of pregnancy and bacterial vaginosis, all subjects with sufficient serum vit .D were free from bacterial vaginosis.( 66.7%). All patients with deficient serum vitamin D level have bacterial vaginosis (64.6% of the patients).

## A-456

**Association of 25 OH-Vitamin D and hsCRP in adults with Essential Hypertension**

M. Prasad[1], D. Rajeswari,[1], K. Ramalingam[1], J. Peela[2], J. Naidu[1], P. Srilakshmi[3]. *[1]Narayana Medical College and Hospital, Nellore, AndhraPradesh, India, [2]St.Matthews University, Grand cayman Ky1 -1204, Cayman island, B.W.I., PO Box 30992, Regatta Office park, Leeward 3,B.W.I, GA, [3]Mamatha Medical College and Hospital, Khammam, Telangana, India*

**Background:** Essential hypertension is a typical example of complex, multifactorial trait and a well studied risk factor for cardiovascular disease. hs-CRP is well known marker of inflammation resulting in atherosclerosis and cardiovascular disease. Recent studies showed that 25-OH-vitamin-D has an anti-inflammatory role and decreased levels may increase the cardiovascular risk in subjects with essential hypertension.

**Methods:** Thirty adults Both male and female with essential hypertension (Systolic blood pressure ≥140mmHg and Diastolic blood pressure ≥ 90mmHg) 30 age and sex matched healthy controls both male and female between 24 -60 years of age were recruited from General Medicine department. 25-OH-Vitamin D levels were assessed by High Performance Liquid Chromatography and hs-CRP was estimated by Turbidometric method.

**Results:** In subjects with essential hypertension 25-OH-vitD levels (mean ± SD 28.35± 2.05 $p<0.05$) ng/mL were significantly low when compared to control subjects and hs-CRP levels (mean ± SD 9.32 ± 1.04 $p<0.05$) mg/L were significantly increased when compared to control subjects . Our study also showed a significant negative correlation between 25-OH-vitD and hs CRP levels ( r = -0.4) ($p<0.01$).

**Conclusion:** Our study concludes 25 OH-Vitamin D levels are significantly decreased and hs-CRP levels are increased in subjects with essential hypertensives. Negative correlation between 25-OH Vitamin D and hsCRP levels which leads to cardiovascular risk in subjects with essential hypertension. The effects of Vitamin D supplementation on morbidity and mortality of large group of population with essential hypertension.

## A-457

### Seminal plasma total antioxidant capacity (TAC), magnesium and calcium levels of infertile men

E. R. Ekong, I. B. Eze, O. W. Egbe. *University of Calabar, Calabar, Nigeria*

**Background**: Poor quality semen of infertile men may be attributable to testicular production of abnormal spermatozoa, post testicular damage in the epididymis or ejaculate from abnormal accessory reproductive gland secretions. The secretions are central to the reproductive potential of semen, especially in acrosome reaction and capacitation processes during fertilization. This study aimed at the assessment of the levels of seminal plasma total antioxidant capacity (TAC), magnesium (Mg) and Calcium (Ca) and their influences on sperm quality of infertile men. **Methods**: Seventy one infertile men attending clinics in General Hospital Calabar, further classified into oligospermic (n=33), asthenozoospermic (n=30) and azoospermic (n=8) based on the WHO classification and forty nine apparently healthy fertile men who served as control were recruited for this study. Informed consents were obtained from all the participants of this study. Semen were obtained from participants following 3-5 days of abstinence from sexual intercourse by self-help into wide mouth bottles held close with the body; delivered within 30 minutes to the laboratory for analyses. Semen quality was evaluated and seminal plasma harvested after centrifugation. Seminal plasma TAC, Mg and Ca levels were determined by colorimetry. Data were analyzed using SPSS statistical package, variations among groups were determined by ANOVA, differences between groups by Student's t-test and association between parameters by Pearson's correlation. Results expressed as mean± SD, significant at $p<0.05$. **Results**: The percentage motility, sperm count, TAC, Mg and Ca levels of the fertile group (69.80±7.29%, ±84.4065.08 $x10^6$/ml, 0.56±0.20mmolTrolox equiv./L, 0.55±0.12mmol/L and 3.17±2.07mmol/L respectively) were significantly higher ($p<0.05$) than (28.38±15.44%, 7.03±5.66$x10^6$/ml, 0.29±0.18mmolTrolox equiv./L, 0.21±0.15mmol/L and 1.86±1.19mmol/L respectively) of the infertile group. Comparison of the normospermic, asthenozoospermic, oligospermic and azoospermic groups, shows that in all the parameters measured the values for the normospermic were significantly higher ($p<0.05$) than those of the various infertile groups. Percentage motility, sperm count and volume vary significantly among the infertile groups, while TAC, Ca and Mg levels did not vary significantly. Azoospermic group had the highest level of Mg (0.25±0.21mmol/L) and lowest level of Ca (1.26±0.55mmol/L). Asthenozoospermic and oligospermic did not differ significantly in all the parameter measured. In the fertile group, Mg and Ca correlated negatively, (r=-0.322, p=0.024). Motility and sperm count correlated positively in both oligospermic (r=0.478, p=0.005) and asthenozoospermics (r=0.605, p=0.001). TAC and motility correlated positively in oligospermic group (r=0.377, p=0.031). **Conclusion**: Spermatozoa travelling the male reproductive tract, are kept viable in fluids secreted by the various accessory glands. Magnesium and calcium from the prostate gland may play important roles in acrosome reaction and capacitation necessary for fertilization of the oocyte, through mechanisms which appear to involve modification of intracellular calcium and other ions, lipid transfer/remodeling in sperm plasma membrane. While low seminal plasma Ca and Mg may be responsible for loss of motility, failed acrosome and capacitation reactions, supraphysiological levels may propagate chemical sterilization. Seminal plasma TAC represents the net oxidative system balance in semen, which prevents spermatozoa death that may occur at oxidative stress level. The study shows that abnormal seminal TAC, Calcium and Magnesium may affect sperm qualities and characteristics associated with fertility

## A-458

### Performance Evaluation of the Atellica™ Vitamin B12 Assay*

H. Zhang, G. Caicedo Montoya, A. Zhou, J. Conklin, H. Leipold, J. Freeman. *Siemens Healthcare Diagnostics, Tarrytown, NY*

**Introduction:** The ADVIA Centaur® Vitamin B12 (VB12) assay (Siemens Healthcare Diagnostics Inc.) is an in vitro diagnostic immunoassay for the quantitative detection of vitamin B12 in serum or plasma. Clinical and laboratory findings for B12 deficiency include neurological abnormalities, decreased serum B12 levels, and increased excretion of methylmalonic acid. The primary objective of this study was to demonstrate the analytical performance of a similar VB12 assay on the Atellica™ Immunoassay (IM) Analyzer** a high-throughput analyzer in development by Siemens Heathineers.

**Methods:** The Atellica™ VB12 Assay uses the same reagents as the ADVIA Centaur VB12 assay. The ADVIA Centaur® VB12 Assay is a "competitive" immunoassay using direct chemiluminescent technology. Vitamin B12 from the patient sample competes with vitamin B12 labeled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in the Solid Phase. The Atellica VB12 Assay requires an ancillary reagent that contains a release agent (sodium hydroxide) and DTT to release vitamin B12 from the endogenous proteins in the sample. Precision of the Atellica VB12 Assay was evaluated according to CLSI protocol EP05-A3. Method comparison of the ADVIA Centaur VB12 Assay on the Centaur XP and the Atellica VB12 Assay followed CLSI protocol EP09-A3. Limit of blank (LoB) and limit of detection (LoD) were evaluated according to CLSI protocol EP17-A2.

**Results:** Observed repeatability for the Atellica VB12 Assay ranged from 4.1 to 1.3% CV and within-lab precision ranged from 5.9 to 2.4% CV over sample result ranges of 151 to 1708 pg/mL. Quantitative comparison of the ADVIA Centaur VB12 Assay on the Centaur XP and the Atellica VB12 Assay yielded the following regression equation: Atellica VB12 Assay = 0.997(ADVIA Centaur VB12 Assay) + 7.054 pg/mL, with 139 serum samples ranging from 47.58 to 1935.78 pg/mL; r = 0.994. The LoB and LoD were determined to be 37.60 and 53.51 pg/mL, respectively, with two reagent lots tested.

**Conclusion:** The Atellica VB12 Assay has demonstrated analytical performance capable of measuring vitamin B12 with accuracy and precision for use in the detection of B12 deficiency.

*Under development. Not available for sale. Future availability cannot be guaranteed.

**Not available for sale. Not CE Marked.

HOOD05162002576680

## A-459

### Biotin Interference in Automated Immunoassay Methods: A Perfect Storm

E. W. Holmes[1], J. Garbincius[2], K. M. McKenna[2]. *[1]Depts. of Pathology and Pharmacology, Loyola University Medical Center, Maywood, IL, [2]Clinical Laboratories Loyola University Medical Center, Maywood, IL*

**Background:** Streptavidin/biotin and biotin/anti-biotin reactions are present in many FDA-cleared immunoassays (IA's) that are now being used for patient care. A marked increase in the use of biotin supplements over the past three years and an increase in the size of the doses that are being taken have led to a steady increase in reports of falsely increased or decreased test results owing to biotin interference in subjects taking OTC biotin supplements in doses of >1000 mcg /day. Currently, the most popular doses used in the US are 5000 or 10000 mcg capsules taken 1 to 3 times/day. 100,000 mcg capsules are now available with suggested doses of 1 to 3/day! This study was undertaken to gain a better understanding of the risk of erroneous test results in patients taking biotin supplements. **Methods:** In June of 2016 we reviewed the current package inserts (IFU's) for 417 methods performed on eight immunoassay platforms(Roche Elecsys®, Ortho Vitros®, Siemens Dimension®, Siemens Centaur®, Beckman Coulter Access®/DXI®, Abbott Architect i2000®, Siemens Immulite 2000®, Diasorin Liaison XL®) to determine which methods were potentially vulnerable to biotin interference and to identify the manufacturer-reported interference thresholds(IFT's) above which exogenous biotin caused a significant (> +/- 10%) difference in the test result. A method was considered to be potentially vulnerable to biotin interference when it utilized a streptavidin/biotin reaction, an anti-biotin/biotin reaction, or a pre-bound avidin/streptavidin or biotin/anti-biotin reagent in the analysis.

**Results:** Our review showed that the methods performed by 2 of the platforms were not vulnerable to biotin interference, but that 173 of the 319 methods (54 %) performed by the other six platforms were. The distribution of the IFT's among the vulnerable methods was (number of methods, IFT(nmol/L): 31,< 50 nmol/L; 36, 15-150 nmol/L; 33, 150-250 nmol/L; 46, 250-500 nmol/L; and 7, > 2000 nmol/L. The IFU's for 20 of the vulnerable methods neither presented an IFT nor mentioned that biotin interference was an analytical limitation of the test! Previously published data from single-dose pharmacokinetic studies and reports of analytical interference in patients receiving 10000 mcg biotin/day for the treatment of genetic disorders of biotin metabolism, predict that methods with IFT's of <200 nmol/L are at risk for generating inaccurate results in subjects who take biotin supplements at doses of 1000, 5000, and 10,000 mcg/day. Subjects that take doses of >100,000 mcg/day are at high risk for markedly inaccurate results and should be tested using interference-free methods.**Conclusion:** The emergent problem of biotin interference has yet to be widely recognized and fully appreciated by the clinical laboratories that perform the testing, or the healthcare providers that order lab tests and interpret test results. The combination of increased biotin supplement use by and the design limitations of many of the currently available clinical immunoassays is a "perfect storm" that has the potential to have a significant, negative impact on patient care. This source of analytical interference needs be promptly and effectively addressed by laboratories and clinical diagnostics manufacturers.

## A-460

### Prevalence of hyperuricemia and its relevant risk factors in healthy adolescents in China

X. cheng, S. Yu, J. Wu, L. Qiu. *Peking Union Medical College Hospital, Peking Union Medical College, Beijing, China*

**Background:** Hyperuricemia (HUA) is a risk factor for metabolic disorders, such as gout. In this study, we investigated the serum uric acid (SUA) level and HUA rate and relevant risk factors in healthy adolescents in six provinces/regions of China.

**Methods:** A total of 14,022 healthy adolescents aged 8 to 18 years were randomly selected from six provinces in China, and biochemical markers such as SUA, blood glucose, blood lipids, liver function, and kidney function were determined. Moreover, high UA levels, hypertension, and obesity were defined and stratified according to applicable international guidelines.

**Results:** A significant difference was observed in the SUA levels of healthy adolescents from different provinces with different genders, places of residence, and ethnicities. The overall HUA rate was 15.5%. The HUA rate was higher in healthy adolescent boys than that in girls (17.5% vs 13.5%, $P < 0.001$). Moreover, the HUA rate increased with age in boys and peaked at the age of 15 to 16years, whereas it decreased with age in girls and peaked at the age of 11 to 12 years. The HUA rate was highest among healthy adolescents in Yunnan (21.1%) and was higher in urban centers than in other areas (21.1%). In addition, HUA rate was highest in Korean Chinese (21.2%) and lowest in Mongolian Chinese (7.6%) and Hui Chinese (6.4%) adolescents, with significant differences from the rates of Han Chinese adolescents. The levels of biochemical markers such as alanine aminotransferase (ALT), total protein (TP), albumin (ALB), γ-glutamyl transferase (GGT), creatinine (CR), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), calcium (CA), creatine kinase (CK), amylase (AMY), and Urea were significantly higher in adolescents with HUA than in those without HUA. We used a logistic stepwise regression model to select six risk factors for HUA, including gender, place of residence, ethnicity, obesity, exercise, and hypertension. After controlling for these factors, the odds ratio (OR) of HUA was 1.41-fold higher in boys than in girls; 1.52-, 1.33-, and 1.79-fold higher in urban centers than in the suburbs of large cities, small-to-medium cities, and agricultural and pastoral areas; and 1.55 (1.02-2.35)-, 0.44 (0.32-0.59)-, and 0.29 (0.20-0.43)-fold higher in Tujia, Mongolian, and Hui Chinese than in Han Chinese adolescents.

**Conclusion:** The HUA rate in healthy adolescents in China is related to economic development and lifestyle. Thus, improving quality of life and implementing HUA prevention measures will help to reduce the risk of HUA.

## A-461

### Survey of Vitamin D status and the relationship with routine biomarkers in apparently healthy younger adults in Beijing

L. Qiu, S. Yu. *Peking Union Medical College Hospital, Beijing, China*

**Background:** vitamin D deficiency was prevalent globally. However, data of vitamin D deficiency status among younger adults of Han nationality in China is still not thoroughly analyzed. In this study, we analyzed the vitamin D status among apparently healthy younger adults of Han nationality in Beijing and the relationship with routine clinical biomarkers to provide reliable data for preventing vitamin D associated diseases in the process of aging.

**Methods:** This is an observational study. Participants included 287 apparently healthy young adults(143 males and 144 females) with an average of 32.2±6.9 years old(19~44 years). We measured 25-hydroxyvitamin D (25OHD) using liquid chromatography tandem mass spectrometry method, vitamin D with deficiency, insufficiency and sufficiency was categorized as 25OHD <20 ng/ml, 20-30 ng/ml, ≥30 ng/ml, respectively. ALT, Ca, P, Cr, Glu, TG, TC, iPTH was analyzed using automatic analyzers.Statistical analysis was performed using SPSS17.0.

**Results:** The median 25OHD level in the total studied younger adults was 16.0(2.5%~97.5%: 6.1~29.0) ng/ml while in males that was 17.9(8.3~32.3) ng/ml and in females that was 14.4(5.4~26.4) ng/ml. Males had significant higher level of 25OHD than females ($P<0.01$). Of the total subjects, the rate of vitamin D with deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and sufficiency (≥30 ng/ml) was 72.8%,25.1%,2.1%, respectively, while that of males was 65.0%,30.8%,4.2%, respectively, and that of females was 80.6%,19.4%,0%, respectively. Females had significantly higher rate of 25OHD deficiency ($P<0.01$). With adjusting sex, age and BMI, serum iPTH and Glu was significantly negatively correlated with 25OHD while Cr showed significantly positively correlation with 25OHD.

**Conclusion:** Vitamin D deficiency is prevalent in younger adults of Han nationality in Beijing, especially in females.

## A-462

### Quantitation of Vitamin B1 in Whole Blood Using a Simple HPLC Method with Internal Standard

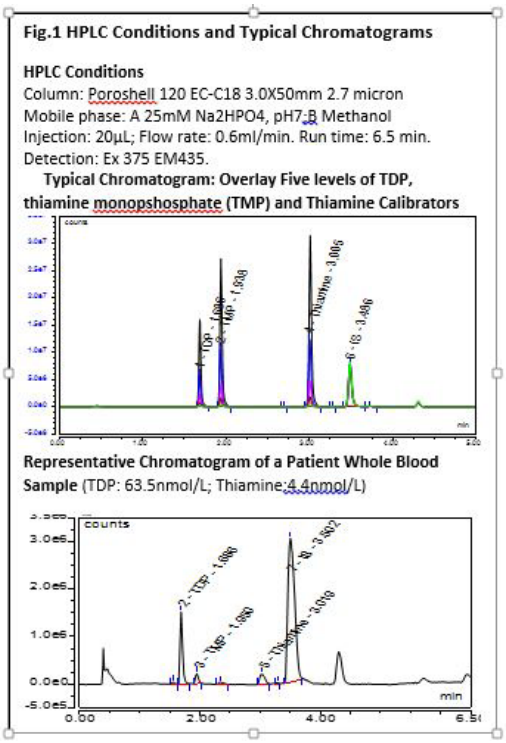
X. Zhang, X. Tang, T. M. Daly. *Cleveland Clinic, Cleveland, OH*

**Background:** Measurement of whole blood thiamine diphosphate, the bioactive form of vitamin B1, has been considered the most effective way of detecting vitamin B1 deficiency. Free thiamine is the predominant form present in human plasma, and reflects recent dietary intake of vitamin B1. Current HPLC-MS/MS methods usually detect total thiamine after a lengthy enzyme reaction to convert TDP to thiamine using acid phosphatase. The published HPLC methods lack a suitable internal standard (IS) and often include the use of ion pairing reagents in separation or a methyl-tert-butyl ether wash in sample preparation. Our objective was to develop a simple HPLC method that simultaneously measures TDP and thiamine in whole blood with an IS to improve reproducibility.

**Methods:** Three chemicals (pyrithiamine, amprolium, actylaneurine) that share some structural similarity to thiamine were evaluated for suitability as an internal standard. For sample preparation, 250ul whole blood after freeze and thaw was treated with 750ul of 6.7% trichloroacetic acid (TCA) to precipitate protein. After centrifugation and filtration, the supernatant was derivatized with 0.04% alkaline potassium ferricyanide solution. The derivatized samples were analyzed using Vanquish UHPLC (Thermo Fisher Scientific) with fluorescence detection.

**Results:** Our data showed that pre-derivatized amprolium was a suitable IS. It generated strong fluorescence at the same wavelength as TDP and thiamine, and does not exist in human blood. The intra- and inter assay precision was: TDP 2.3-2.5% and 4.0-4.8%; thiamine 3.4-5.0% and 2.1-6.5% respectively. The analytical measurement range was 1.7-442.3 nmol/L (TDP) and 1.7-375.4 nmol/L (thiamine). Method comparison of TDP with a reference laboratory HPLC method showed r=0.9625, slope=1.021, intersect=0.982 (n=53). In addition, our data showed that thiamine concentration in whole blood mirrored plasma thiamine levels and can be used to determine recent vitamin B1 uptake.

**Conclusion:** This is a simple and reliable method for evaluating vitamin B1 nutritional status.




## A-463

### Reducing utilization of 72-hour fecal fat testing through order restriction and simple alternative tests

L. de Koning[1], M. Korostensky[2], S. R. Martin[2], C. Naugler[1]. *[1]Calgary Laboratory Services, Calgary, AB, Canada, [2]University of Calgary, Calgary, AB, Canada*

**Background:** At Calgary Laboratory Services (CLS), 72-hour quantitative fecal fat testing has been available for patients located in Calgary and Southern Alberta for many years. As with other clinical laboratories, this test continued in use despite being made obsolete by the gradual introduction of other tests that could rule in or rule out fat malabsorption. In 2014, a review of clinical appropriateness, utilization and cost was performed at CLS to develop a plan to eliminate and replace the test. This included a research study to evaluate the predictive power of stool specimen characteristics, including weight, qualitative and quantitative (via automated image analysis) measures of stool lipid droplets, for abnormal fat excretion by the 72-hour test. **Methods:** A literature review was performed in consultation with adult and pediatric gastroenterologists to identify the major clinical uses as well as the most appropriate users of the 72-hour test. Laboratory tests and clinical information that provided similar rule-in or rule-out information as the 72-hour test were identified. Ordering restrictions and testing recommendations were proposed with the regional heads of adult and pediatric gastroenterology. Memos were circulated that detailed the agreed-upon changes in advance of go-live. To develop an alternative to the 72-hour test, all historic (2009-2016) 72-hour quantitative fecal fat results (mmol/day) and specimen data were extracted from the CLS laboratory information system, and 100 banked 72-hour stool specimens were examined microscopically for oil-red-o stained neutral fat droplets via (1) qualitative (≥5 droplets / 400X field) manual reading by technologists and (2) quantitative automated image analysis using ImageJ. Logistic regression and ROC-curve analysis were used to identify predictive power of specimen weight and stool lipid droplet measures for abnormal fat excretion (>=21 mmol/day for >=7 years of age; >=7 mmol/day for <7 years of age). **Results:** From 2009-2014, CLS performed ~100 72-hour fecal fat tests per year. On January 1 2015, an ordering restriction preventing adult specimens to be run without approval by clinical biochemist was enacted, reducing workload to 20 tests per year. Tests were cancelled for physicians that did not contact the lab, and contacting physicians were informed of the availability of alternatives, In specimens tested from 2009-2016, fecal weight was a moderately strong predictor of fat malabsorption in patients ≥7 years (Area under curve = 0.74; n = 423) and < 7 years (Area under curve = 0.77, n = 113). In a sample of 91 specimens >=7 years of age, fecal weight alone was a similarly strong predictor (Area under curve = 0.70). Additionally considering either a qualitative (area under curve = 0.76) or quantitative measure of stool fat globules (area under curve = 0.77) improved discrimination over fecal weight alone.

**Conclusion:** Engagement with clinician stakeholders reduced 72-hour quantitative fecal fat testing, resulting in more appropriate lab utilization and clinical evaluation. Based on the results of our research study, we propose to reflex all specimens submitted for fecal fat analysis for reporting of fecal weight and qualitative stool fat globules.

## A-464

### Analysis of Vitamin A ordering patterns in a Canadian City

J. Boyd, S. Chin, H. Sadrzadeh. *Calgary Laboratory Services, Calgary, AB, Canada*

**Background:** Vitamins A is a lipophilic vitamin that plays a role in vision. Measurement of Vitamin A is most commonly performed for assessment of deficiency, such as patients who have malabsorptive syndromes. In rare cases, it may be measured for toxicity. Recently, measurement of Vitamin A has become popular as part of the 'wellness' movement and tests are ordered regularly without the usual clinical indications. Over the past few years, the increasing number of requests for Vitamin A at our laboratory has required the addition of a second batched run per week. The objective of this study was to quantify and evaluate increases in Vitamin A ordering at our laboratory. **Methods:** A request for data was submitted to our institutional data team for Vitamin A data between 2010 and 2015 including patient age, sex, test ordered, test result, collection date, and ordering physician. All data was de-identified as per our institutional privacy policy. Analysis was performed using Microsoft Excel 2013.

**Results:** Vitamin A requests increased from 2431 in 2010 to 4369 in 2015. Of these orders, 89% were from family physicians, 8% from specialists and 3% were from hospital inpatients. When analyzed by ordering physician, it was determined that 10 physicians were responsible for over 73% of all Vitamin A requests. Of these ten physicians, 7 listed integrative or functional medicine as a specialty on their websites. Further analysis of the ordering patterns for these 7 physicians determined that many of their patients had repeat Vitamin A's ordered in less than a year regardless if the previous result had been normal or not. In 2015, the number of Vitamin A's ordered with a previous normal result performed within 12 months was 1253, which accounts for 28% of the 2015 workload. **Conclusion:** Vitamin A is frequently ordered by physicians practicing functional or integrative medicine and may be target for utilization measures at our institution.

## A-465

### Vitamin B12. Is the normal range a sufficient range?

F. L. Soares[1], J. K. Miqueloto[2], E. M. R. Lima[2], G. Campana[2], M. P. Campagnoli[2]. *[1]School of Medicine - Pontifical Catholic University of Paraná, Curitiba, Brazil, [2]DASA, Curitiba, Brazil., Curitiba, Brazil*

Background: Vitamin B12 is a water-soluble compound, not synthesized by the human body and available in foods of animal origin.

Cyanocobalamin deficiency can lead to hematological, neurological and cardiovascular disorders by interfering with homocysteine metabolism and the body's methylation reactions.

Serum dosage of this vitamin has some methodological restrictions of sensitivity and specificity, and clinical symptoms of deficiency may occur even with serum levels into the range of reference values, therefore, the contribution of the laboratory results still uncertain for the clinical follow-up of these patients.

Our work aims critically analyze the distribution of serum vitamin B12 from outpatient dosage in the historical database of our laboratories, since several scientific studies question the reference values or were performed with small population samples.

Methods: This is a retrospective observational study of the laboratory database tests performed between January 2016 and January 2017, in Curitiba, PR, Brazil.

The database is composed of serum dosage results and personal data (gender and date of birth) of individuals who had their exams requested by their attending physicians who are not linked to the laboratory.

In case of serial dosages, only the first result of each patient was considered.

The laboratory analysis of Vitamin B12 was carried out on serum samples by direct chemiluminescence method ADVIA Centaur VB12 Siemens, with sensitivity and

specificity determined by the manufacturer's package insert, suggesting reference range between 211 and 911 pg/ml, detection limit between 45 and 2000pg / ml.

Results: A total of 104,271 patients were evaluated, of which 73,756 (70.73%) were female.

We split the sample by age into 03 groups: Children < 18 years old (12.93%) Adults > 18 and < 60 years (68.16%) and Elderly > 60 elderly (26.91%).

The mean serum level in the evaluated population was 461.87 ± 240.62 pg/mL with a median of 410 pg/mL in a range of 73 to 2000 pg/mL.

Similar results were found when the analysis was performed by subgroups of sex and age.

The quartile assessment suggests a range of normality between 326 (p25) and 524 (p75) pg/mL and in the percentile assessment we found a range between p3 and p97 similar to that suggested by the manufacturer's package insert, from 220 to 977 pg/mL.

Conclusion: The values found in this population were similar to those described in several scientific studies and in the manufacturer's package insert.

Due to the size of the population studied, we believe that the values are adequate for a first analysis, however, tests for this purpose vary widely in sensitivity and specificity.

Thus, the laboratory diagnosis still leaves doubts about its potential contribution in the clinical and therapeutic decisions of patients with results between p3 and p25 of the values found and it is necessary to establish a gold standard interpretation criteria.

We believe that values closer to the median may be more adequate to corroborate with clinical diagnosis, however the correlation with other laboratory dosages may clarify this hypothesis more accurately.

## A-466

### The association of the vitamin A, vitamin E level and potential biomarkers of mRNA expression with the extent of coronary lesion

C. T. O. Fernandes[1], V. H. R. Duarte[1], J. N. G. De Araújo[1], A. M. G. Silva[1], M. S. M. Cruz[1], M. K. R. N. Duarte[1], I. C. C. Santos[1], J. C. Santos[1], A. C. Araújo[2], A. M. Q. S. Santos[1], C. H. B. Costa[1], J. M. Oliveira[3], M. H. Hirata[4], R. D. Hirata[4], K. D. S. Ribeiro[1], A. D. Luchessi[1], V. N. Silbiger[1]. *[1]Federal University of Rio Grande do Norte, Natal, Brazil, [2]Federal University of Rio Grande do Norte, Natal, Brazil, [3]Onofre Lopes University Hospital, Natal, Brazil, [4]School of Pharmaceutical Sciences, São Paulo, Brazil*

Atherosclerosis is a chronic inflammatory process associated with the majority of cardiovascular diseases (CVD). The oxidative stress is an important event in the pathogenesis of these diseases that are major cause of morbidity and mortality worldwide. Antioxidants, like vitamin A and vitamin E, are substances capable of preventing the deleterious effects of oxidation and recent studies showed that they might have a protective role in CVD. It has been suggested that this substances are associated with decreased risk of CVD and atherosclerosis. 'Omics' analysis can contribute to this field by providing fundamental information to better understand complex biological systems, such as atherosclerosis. Particularly, transcriptomics is a relevant tool for the identification of diagnostic/prognostic biomarkers for CVD. Thus, the objective this study is evaluated the association of the vitamin E, retinol and potential biomarkers of mRNA expression with the extent coronary lesion. The study included adults aged 30-74 years undergoing elective cinecoronariography for the first time. Fasting blood samples have been collected for biochemical analysis. The concentration of vitamin E was determined by high-performance liquid chromatography (HPLC). The atherosclerotic burden was measured through Friesinger score. This score is determined by separately scoring each of the three main coronary arteries within a range of 0 to 15. For this analysis, the FS was divided into three categories: 0-4, 5-9 and 10-15. The gene expression was performed using mRNA of blood peripheral cells follow relative quantification by real-time PCR. The sample consisted of 177 adult patients; 99 patients (55.9%) were male, and 78 patients (44.1%) were female. Regarding to the Friesinger index, the patients were stratified into three groups: 0-4 (n=90), 5-9 (n=50) and 10-15 (n=37). Patients at group 10-15 presented higher age compared to patients at the group 0-4 (p=0.005) and this patients presented less glucose compared to patients at the group 5-9 and group 10-15 (p<0.001 and p=0.015, respectively). Patients in the 5-9 group had higher levels of the vitamin E/cholesterol and vitamin E than patients in the 0-4 group (p=0.035 and p=0.035, respectively). No statistical difference was found in relation to vitamin A. Regarding mRNA relative expression five gene were different mRNA expressed according to the severity of extent of coronary lesion. The *AREG*, *BCL2A1* and *IL18R1* increase the mRNA expression by Friesinger index categorization, higher values were observed in patients 10-15 group than 0-4 (p=0.014; p=0.035 and p=0.017, respectively). Moreover, higher values of *BCL2A1*, *BCL2L1* and *MYL4* mRNA expression was in patients 5-9 group than 0-4 (p=0.012; p=0.004 and p=0.011, respectively). In conclusion, the transcriptional profiling from whole-blood cells presented here suggests a potential use of *AREG*, *BCL2A1*, *BCL2L1*, *IL18R1* and *MYL4* as gene expression biomarkers for stages of atherosclerosis and consequently for CVD. Moreover, the involvement of vitamin E with these genes and the extent of coronary lesion open new possibilities for future studies to evaluated their applicability as biomarkers.

## A-467

### Changing from solvent-based to serum-based calibration for the CDC serum 25-hydroxyvitamin D LC-MS/MS assay provides quality improvement

K. L. Maw, K. L. Powell, M. R. Sternberg, R. L. Schleicher. *Centers for Disease Control and Prevention, Atlanta, GA*

During validation of a 25-hydroxyvitamin D assay, which quantitates serum concentrations of 25-hydroxyvitamin $D_3$ [$25(OH)D_3$], 25-hydroxyvitamin $D_2$ [$25(OH)D_2$], and the C3-epimer of 25-hydroxyvitamin $D_3$ [epi-$25(OH)D_3$] using liquid chromatography-tandem mass spectrometry, solvent and serum were shown to provide equivalent calibration matrices. However, in practice we experienced greater variation in analytical measurements (namely, slope, imprecision, bias) using solvent-based calibration. Thus, we reinvestigated using serum as the calibration matrix.

Solvent-based calibrators for $25(OH)D_3$, $25(OH)D_2$, and epi-$25(OH)D_3$ were prepared in 70% methanol-water; serum-based calibrators were prepared by spiking these analytes in a mixture of sera. The sera were selected because they provided low concentrations of these analytes. Based on 15-19 analytical runs per matrix, the precision for serum-based calibration slopes improved overall but particularly for the epi-$25(OH)D_3$ (CV from 13% to 4%) and $25(OH)D_3$ (CV from 7% to 4%) ($p \leq 0.05$). When comparing the CVs of 3 QC pools used daily, we noted smaller CV differences between the matrices within-run and larger CV differences between the matrices among-runs with serum-based calibration providing better QC precision across runs. NIST Standard Reference Materials were used to assess bias. Average percent bias for $25(OH)D_2$ and $25(OH)D_3$ were smaller for serum-based compared to solvent-based calibration ($p \leq 0.05$).

Quality improvement for this assay was realized by changing the calibration matrix from solvent to serum even though method validation showed that the matrices were interchangeable.

## A-468

### Obesity and Co-morbidities among School Children in Kumasi, Ghana.

E. K. Togbe, M. F. Annani-Akollor, F. N. Prempeh. *Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

**Introduction and Objective:** Obesity in children and the risk of developing diabetes mellitus and comorbidities continue to be on the increase worldwide. Research on obesity in children therefore continues to be of great public health concern, but data on its prevalence and interventionary measures are scanty in Ghana. Our objective is to do a pilot study to assess the situation for further research work. **Methods:** Eighty-five school children aged 5 to 16 years were chosen randomly from two different schools in the Kumasi Metropolis. Parental consent was sought on behalf of all children for the exercise and an experienced medical laboratory scientist and a nurse undertook the measurements. Fasting plasma glucose (FPG) was assayed by glucose oxidase spectrophotometric means. Weight and height were measured using standard equipment and body mass index (BMI) was determined as Weight (kg)/Height $^2$ (m) and calculated as percentiles. Arterial blood pressure (BP) was measured using mercury sphygmomanometer. A structured questionnaire on lifestyle of participants was administered. Analysis was conducted using IBM-SPSS® version 23. **Results:** Eight children (9.4%) were overweight, 3, (3.5%) were obese and 6, (7.1%) were underweight. Fifteen children (17.6%) had Fasting Plasma Glucose between 5.6 and 9.0 mmol/L and therefore classified as having impaired fasting glucose by American Diabetic Association criteria of 5.6mmol/L to 6.9mmol/L, 2, (2.4%) had BPs greater than 140/90 mmHg. Television viewing and lack of outdoor games had significant association on obesity ($p = 0.013$ and 0.015 respectively). There was positive linear relationship between FPG and BMI ($r = 0.23$; $p = 0.003$). **Conclusion:** Majority of the participants were "normal" by the parameters we measured, but the presence of overweight, obesity, in the presence of underweight, impaired fasting glucose, lack of physical activity by the children and absence of health promoting recreation centres for school-going children are important health issues for authorities and other stake holders as well as future researchers need to address.

## A-469

### Biomarkers of fat-soluble vitamin status from NHANES 2003-2006: covariate analysis

R. L. Schleicher, M. R. Sternberg, C. M. Pfeiffer. *CDC, Atlanta, GA*

Monitoring the nutritional status of the U.S. population to inform public health policy is one of the key goals of the National Health and Nutrition Examination Survey (NHANES), an ongoing representative survey of the non-institutionalized, civilian population. Nutritional biomarkers (usually found in blood or urine) impart useful information about a person's recent or long term nutrient intake. Although non-dietary factors can show weak-to-moderate associations with these biomarkers, dietary intake and supplement use are usually the primary determinants of their concentrations.

The fat-soluble vitamins A and E are important to human health. The most commonly used biomarkers for these nutrients are retinol (vitamin A) and α-tocopherol (vitamin E). For each of these biomarkers, particular serum concentrations (medical decision points) are associated with risk for deficiency. Serum concentrations below these cutpoints are associated with visual loss (vitamin A) or neurological injury (vitamin E).

To develop up-to-date population reference intervals and estimates of the prevalence of persons at risk for vitamin deficiencies, biomarker concentrations of fat-soluble vitamins were measured in a representative sample as part of the NHANES conducted in 2003–2006. To further study these data, linear regression models were used to assess the association between biomarkers of fat-soluble nutrient status and sociodemographic, lifestyle, and vitamin intake in adults 20 years and older.

Through systematic modeling advancing from model 1) simple linear regression, to model 2) multiple linear regression using sociodemographic and lifestyle factors, to model 3) adding total intake of specific vitamins, we acquired novel information about the amount of variability in nutritional biomarker concentrations that is explained by these variables. All results to follow were significant at $p \leq 0.05$. For vitamin A, age (r=0.23) and alcohol intake (r=0.20) were modestly correlated with serum retinol concentration whereas for vitamin E, age (r=0.41) and smoking (r=-0.25) were moderately correlated with serum α-tocopherol. Overall, after controlling for socioecomonic and lifestyle variables (model 2), race-ethnicity was the strongest non-intake determinant for vitamin A whereas age was strongest for vitamin E.

Through additional modeling (model 3), we explored the relationships between nutritional biomarkers, diet, and supplements. Race-ethnic differences in serum biomarker concentrations were substantially attenuated after adjusting for total intake of these vitamins. Less so for serum retinol than for α-tocopherol, in the overall subpopulation of adults, total intake of preformed and pro-vitamin A sources and supplements accounted for 4% of the variability in the biomarker, whereas, for vitamin E, intake from supplements (16.8%) and total intake from foods and supplements (23.9%) accounted for substantial variability in serum α-tocopherol. Adjusting for all significant covariables accounted for 17.4% of the variability in serum retinol and 36.2% of the variability in serum α-tocopherol concentrations.

Similar systematic approaches are being applied to water-soluble vitamins and iron status indicators in NHANES 2003-2006.

## A-470

### Linoleic acid-enriched diet and chronic ethanol exposure activated hepatic NLRP3 inflammasome contributing to liver inflammation and injury in mice

Y. Wang, Y. Liu, W. Feng, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

**Background/Aim:** Chronic alcohol consumption leads to a spectrum of liver abnormalities, including fatty liver, steatohepatitis, fibrosis and cirrhosis. Fatty liver (steatosis) is the accumulation of triglycerides and other lipids in hepatocytes. Steatohepatitis is characterized by a combination of steatosis and inflammation. Inflammasome activation with subsequent release of IL-1β, a critical pro-inflammatory cytokine, is an important mechanism contributing to alcohol-induced liver inflammation. Both alcohol and dietary factors may affect hepatic inflammasome activity. The aim of the present study was to examine the effects of chronic ethanol administration and different types of dietary fatty acids on hepatic inflammasome activation in mice.

**Materials and Methods:** C57BL/6N male mice were fed either an unsaturated fat (USF, ω6-PUFA, linoleic acid, enriched) or a saturated fat (SF, medium chain fatty acid enriched) diets. Animals received control (SF or USF) or ethanol containing diets (SF+EtOH or USF+EtOH) for 8 weeks. Liver injury was evaluated by plasma ALT activity. Liver steatosis was assessed by liver tissue histological examination, and biochemical measurement of hepatic triglycerides. Plasma LPS levels were measured as a marker of endotoxemia. Chloroacetate esterase staining was used to evaluate necroinflammatory changes. Macrophage infiltration was determined by F4/80 staining. Hepatic pro-inflammatory cytokine and chemokine expression was assessed by qRT-PCR. Plasma IL-1β (ELISA), and hepatic NLRP3, ASC, caspase-1, and IL-1β mRNA (qRT-PCR) and protein (WB) levels were measured as markers of NLRP3 inflammasome activation.

**Results**: Compared to SF+EtOH, long term of USF+EtOH feeding resulted in an early stage of alcoholic liver disease characterized by hepatic steatosis with elevated hepatic triglyceride levels ($64.23 \pm 8.8$ *vs* $93.7 \pm 7.3$ mg/g liver, $p<0.05$), and liver injury with increased plasma ALT levels ($27.27 \pm 1.9$ *vs* $44.91 \pm 2.8$ U/L, $p<0.05$). USF+EtOH-induced liver steatosis and injury were accompanied by neutrophil and macrophage infiltration, and increased hepatic inflammation with elevated levels of pro-inflammatory cytokines, including TNF-α, MCP-1, MIP-2, PAI-1. USF+EtOH but not SF+EtOH feeding caused endotoxemia assessed by elevated LPS levels. Compared to USF feeding, USF+EtOH administration resulted in up-regulation of hepatic markers of NLRP3 inflammasome, including NLRP3, ASC, caspase-1, and IL-1β mRNA. Mice fed SF+EtOH did not reveal elevated markers of NLRP3 inflammasome activation.

**Conclusion:** The data demonstrate the differential effects of diverse types of dietary lipids on ethanol-mediated liver injury, and suggest that dietary USF, specifically rich in linoleic acid, may contribute to EtOH-mediated hepatic inflammation via inflammasome activation. The detailed mechanisms need to be further investigated.

## A-471

### Food Intolerance (IgG-Mediated Food Sensitivity Reaction) Status of Community-based Patients

W. Hui, A. Ptolemy, D. Bailey, G. Waite, H. Li. *Dynacare, Brampton, ON, Canada*

**Background:** IgG-mediated food sensitivity reactions (food intolerance) may have a significant impact on patient health. Unlike the immediate reactive symptoms of food allergy, manifestations of food intolerance often appear hours or days after food consumption. These symptoms can be vague and nonspecific. Severe headache, irritable bowel syndrome, migraine, skin or respiratory conditions have been linked to food intolerance. Food consumption, as a root cause of a patient's ill health, is not always correctly identified. The relative prevalence of population (age and gender) or food-specific intolerances is not widely known.

**Objective:** Assess the results of food intolerance tests performed by our regional reference laboratory to characterize the patient population receiving this test and their food intolerance status as a potential diagnostic aid.

**Methods:** Food intolerance testing was performed using a FoodPrint® Microarray 200+ Food IgG assay. This colorimetric microarray-based ELISA quantifies IgG antibodies to *N*=222 foods in patient serum. IgG food antibodies were classified as: positive, >29 U/mL; borderline, 24 to 29 U/mL; and negative, <24 U/mL.

**Results:** From January 2012 to December 2016, 26479 food intolerance tests were performed. Female and male patients received 72% (*N*=19052, median age 43y, age range 1 to 98y) and 28% (*N*=7427, median age 41y, age range 0 to 105y) of these tests, respectively. Patient age distribution was bimodal with 8y (*N*=182) and 42y (*N*=657) having the highest testing frequencies. From January-December 2016, the relative rates of positive, borderline and negative results were 11.5%, 3.3% and 85.1%, respectively, with no difference noted by gender. There was a negative correlation between patient age and IgG food antibody positivity rates or general food intolerance. Four rates of intolerance were noted for food items: level 1, frequently intolerant foods, were positive in >33% of individuals and consisted of 23 foods, including wheat; egg white; milk (cow); pea; milk (sheep); cola nut; yeast (Brewer's); pistachio; casein; bean (red kidney); barley; milk (goat); agar agar; corn (Maize); bean (white haricot); almond; hazelnut; sunflower seed; cashew nut; gliadin; brazil nut; aloe vera; and peanut; level 2, commonly intolerant foods, were positive in 10-33% of individuals and consisted of 34 foods; level 3, infrequently intolerant foods, were positive in 1-9% of individuals and consisted of 82 foods; and level 4, tolerant foods, were positive in <1% of individuals and consisted of 83 foods. The most intolerant foods were identical for females and males, with positivity rates ranging from 38-93%.

**Conclusion:**

Food intolerance is widely observed in our patient population. Foods with relatively higher rates of intolerance include: grains, especially those containing higher gluten content; dairy and egg; vegetables with high protein content (pea and beans); nuts and seeds; and plants with high polysaccharide content (agar agar or aloe vera).

Leafy vegetables, fruits, seafood and meats had relatively lower levels of intolerance. Physicians may use this population-based information with the frequency of a patient's food consumption to evaluate potential IgG-mediated food sensitivity reactions.

## A-472

### Vitamin D metabolite metrology at NIST: Past, present and future

C. Q. Burdette, S. S. Tai, J. E. Camara, F. Nalin, M. Bedner, M. A. Nelson, B. E. Lang, J. H. Yen, K. Lippa, K. W. Phinney, L. C. Sander. *NIST, Gaithersburg, MD*

Vitamin D is a nutrient essential for bone health; however, vitamin D deficiency/insufficiency is estimated to exist within 10% of the US population. Routine blood tests to monitor vitamin D can give inconsistent or even inaccurate results, complicating diagnosis and treatment of vitamin D deficiency. This variability calls into question the ability of vitamin D assays to accurately identify individuals affected by vitamin D deficiency. National Institute of Standards and Technology (NIST), the National Institute of Health Office of Dietary Supplements (NIH-ODS) and the Centers for Disease Control and Prevention (CDC) entered into a collaboration - and as part of a broader Vitamin D Standardization Program (VDSP) - with a goal of standardizing laboratory measurements of vitamin D metabolites to ensure comparability in national health surveys. Standardization ensures accurate and consistent detection and treatment of vitamin D deficiency, regardless of location or laboratory procedure. As a consequence of these efforts by NIST and its partners, confidence in serum-based measurements of vitamin D metabolites has been improved. These accomplishments lower health care costs by reducing the need for retesting, and provide greater certainty in the data collected in national health surveys to support clinical and policy decision making. Since 2010, NIST has provided metrological support for these critical vitamin D metabolite measurements through: 1) the development of Standard Reference Materials (SRMs) for use as primary calibration materials and quality control samples; 2) the development of Joint Committee for Traceability in Laboratory Medicine (JCTLM)-recognized higher-order Reference Measurement Procedures for the measurement of vitamin D metabolites in clinical samples; and 3) administration of a quality assurance program for vitamin D metabolites. Furthermore, team members have provided critical measurements for test samples used in VDSP efforts, and Vitamin D External Quality Assessment Scheme (DEQAS), a PT program administered in the UK, with samples distributed quarterly to 1200 international participants. This effort has explicitly allowed DEQAS to move to an accuracy-based program for vitamin D assessments. The Chemical Sciences Division of NIST has established "Best in the World" capabilities for the determination of vitamin D metabolites in human serum. Much of this service to the clinical community has been delivered in the form of SRMs, intended for use in providing SI traceability and calibration, as well as method validation of these critical measurements. To date, the re-issued SRM 972a Vitamin D Metabolites in Human Serum has sold over 1250 units to hundreds of clinical diagnostics and health monitoring laboratories, both nationally and internationally. Other SRMs include SRM 2972a 25-Hydroxyvitamin D2 and D3 Calibration Solutions, and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level). New isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC/MS/MS) methods for the determination of emergent metabolites of vitamin D status, such as 24R,25-dihydroxyvitamin D3, have been more recently developed. This measurement capability is being applied to the evaluation of patient samples for DEQAS samples, and to the development of new calibration solution and serum-based SRMs. Candidate ID-LC-MS/MS methods for other metabolites, such as 1,25-dihydroxyvitamin D3, are also currently under investigation.

## A-473

### Utilization of vitamin $B_{12}$ and folate tests in a major reference laboratory suggests a lack of evidence-based ordering practices by community physicians

J. L. Gifford, M. L. Abbott, L. De Koning, H. Sadrzadeh. *Calgary Laboratory Services, Calgary, AB, Canada*

**Objective:** To assess the evidence-based utilization of vitamin $B_{12}$ and folate testing in a large reference laboratory.

**Introduction:** Vitamin $B_{12}$ and folate are important enzyme cofactors that play key roles in the utilization of methyl-groups central to DNA synthesis and multiple metabolic reactions. Megaloblastic anemia is a cardinal feature of both vitamin $B_{12}$ and folate deficiency. Clinically, low levels of vitamin $B_{12}$ are associated with decreases in energy and neurological effects that include tingling and numbness of extremities, abnormalities in walking, and cognitive changes. At-risk populations include vegetarians; the elderly; patients with intestinal malabsorption, gastric or intestinal surgery; and those with pernicious anemia. In pregnancy, folate deficiency can lead to neural tube defects and at-risk populations include those with dietary insufficiency and malabsorption syndromes. In our laboratory, tests for vitamin $B_{12}$ and folate are ordered at very high numbers by physicians (>240,000 tests per year), despite the fact that deficiency is extremely rare due to fortification of food and the availability of these vitamins in the diet.

**Methods:** De-identified patient results from venous blood samples for the period of December 30, 2014 to June 30, 2016 were obtained from the Laboratory Information System. Specific test results queried included those for vitamin $B_{12}$, folate, and mean corpuscular volume (MCV). Additional information collected included age, collection location, and ordering physician speciality. The data was analyzed in Excel v.14.0.

**Results:** In the time period, our institution performed 332,644 vitamin $B_{12}$ measurements. Of these, 4.0% were below and 10.1% were above our current reference interval (RI; 155-700pmol/L). 90.5% of all vitamin $B_{12}$ measurements had a matched MCV, of which only 2.8% indicated macrocytosis (MCV >100fL); 0.1 % of vitamin $B_{12}$ measurements below the RI had an associated macrocytosis. During this period, 29,551 serum folate measurements were performed. Of these, all but two (*N*=29,549) had a matched vitamin $B_{12}$ measurement. Of the total folate tests performed, 2.0% were deficient (RI >12.0nmol/L). Analogous to that seen with vitamin $B_{12}$, 83.7% of folate test orders had a matched order for MCV. Although only 0.36 % of all folate results were associated with a macrocytosis, 18.3 % of patients with deficient levels of folate had a macrocytosis. In our study, 0.3% of patients with vitamin $B_{12}$ results below the RI had an associated deficiency in folate. Only 0.1 % (*N*=7) were deficient in both vitamin $B_{12}$ and folate and had a macrocytosis. Finally, greater than 80% of the above tests were ordered by community physicians.

**Conclusions:** In our institution, greater than 85% of vitamin $B_{12}$ and folate tests ordered by community physicians are normal and do not add any value to the overall diagnosis and should be eliminated.

## A-474

### Conundrums of Testing Algorithms for Vitamin B12 Deficiency

L. Liu[1], B. Schupay[2], C. Bullman[2], O. M. G. Peck-Palmer[1], D. W. Gutkin[3]. *[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, PA, [3]eterans Affairs Pittsburgh Healthcare System, Pittsburgh, PA*

**Objective:** Testing algorithms are essential tools for test utilization management to reduce unnecessary testing and provide high-quality, cost-effective patient care. With the intention to implement vitamin B12 testing utilization management, we found various algorithm recommendations. These algorithms differ in the threshold value of vitamin B12 to initiate reflex testing and more importantly, in whether to include homocysteine as a follow-up test. When homocysteine is included, these algorithms also differ in defining vitamin B12 deficiency as having elevations in both methylmalonic acid (MMA) and homocysteine, versus in either MMA or homocysteine.

**Methods:** We evaluated the testing algorithms performance for our inpatient population and to determine the best testing strategy for our population. From January 2016 – December 2016, we analyzed 914 vitamin B12 tests (reference range: 180-914 pg/mL) that were ordered concurrently with MMA (reference range: 0-378 nmol/L) and homocysteine (reference range: 6.6-17.5 µmol/L) at our medical center.

**Results:** The mean vitamin B12 concentration was 468.6 ± 280.6 pg/mL and the median age of the patients was 68 (male: 92%; female: 8%). Stratified serum vitamin B12 concentrations were assessed for percentage of elevated MMA (>400 nmol/L) and/or homocysteine concentrations (>18 µmol/L). We found that 4% of the patients with a serum vitamin B12 above 914 pg/mL have elevated MMA concentrations. This percentage remains the same in patient with a serum vitamin B12 above 400 pg/mL, but increases as vitamin B12 concentration decreases. This supports the concept that a serum concentration above 400 pg/mL represents vitamin B12 replete status in this population. Importantly, we also found that in the equivocal range of vitamin B12 concentrations from 150-400 pg/mL, 28% of patients have elevated homocysteine concentrations, 13% have elevated MMA concentrations, but only 2% have both elevated homocysteine and MMA concentrations. Because the percentage of patients with elevated homocysteine is twice as high as those with elevated MMA concentrations, we further investigated whether the patients with elevated homocysteine but normal MMA concentrations represent true vitamin B12 deficiency by reviewing hematologic indices (red blood cell count, hemoglobin and MCV), neurologic symptoms including cognitive and mood changes, peripheral neuropathies, and whether abnormalities can be reversed by vitamin B12 supplement. Interestingly, only 10% of the these patients may represent true vitamin B12 deficiency, while over

50% of these patients have conditions including folate deficiency, renal deficiency, alcohol abuse or use of medications such as fibrates and hydrochlorothiazide that can elevate homocysteine concentrations.

**Conclusion:** Our data suggest that for evaluating vitamin B12 deficiency, homocysteine should be used in patients with equivocal vitamin B12 results, but normal MMA concentrations, and when all other compounding factors that can increase homocysteine levels can be excluded. Furthermore, each institution needs to analyze data from their own patient population before implementing testing algorithms.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Animal Clinical Chemistry

## B-001

**Antidiabetic Activity of Aqueous *Kalanchoe pinnata* Preparation: Potential Mechanism of Action**

L. E. Rios, F. Omoruyi, J. Sparks. *Texas A&M University Corpus Christi, Corpus Christi, TX*

**Background:** The aqueous preparation of *Kalanchoe pinnata* is traditionally used in the management of Type 2 diabetes mellitus, but the effectiveness in curtailing the indices of Type 2 diabetes is not clear. In this study, we evaluated hematological and oxidative stress indices, and enzymes involved in carbohydrate metabolism in the liver of Type 2 diabetic rats treated with aqueous preparation of *K. pinnata*. **Methods:** Six rats were fed a normal diet, while 24 rats were fed a high fat diet (HFD) for twenty-one days. Diabetes was induced in eighteen of the rats fed HFD by a low dose of streptozotocin administration on day fourteen and diabetes was confirmed on day 21. Animals were then divided into five groups (n = 6) as follows: non-diabetic group; non-diabetic control group fed HFD; diabetic group; diabetic plus *K. pinnata* (0.14 g/kg body weight/day); diabetic plus metformin (300 mg/kg body weight/day). Animals were euthanized by decapitation after treatment for 28 days and blood and liver were collected for assays. **Results:** Type 2 diabetic rats treated with *K. pinnata* preparation lost significant ($P < 0.05$) weight. *Kalanchoe pinnat*a consumption resulted in decreased serum glucose. There were also significant ($P < 0.05$) increases in white blood cell count and hemoglobin levels. Serum reduced glutathione (GSH) levels, superoxide dismutase and hepatic pyruvate kinase activities were significantly ($P < 0.05$) elevated. Hepatic malic enzyme and glucose-6-phosphate dehydrogenase activities were not significantly ($P > 0.05$) altered in Type 2 diabetic rats treated with aqueous *K. pinnata* preparation. **Conclusion:** Overall, our data showed that the consumption of aqueous preparation of *K. pinnata* in Type 2 diabetic rats decreased body weight and serum glucose levels. Similarly, the observed increase in superoxide dismutase activity and GSH levels in the diabetic rats treated with *K. pinnata* preparation may be protective against oxidative stress associated with the disease. The observed increase in hepatic pyruvate kinase activity in diabetic rats treated with *K. pinnata* preparation may be indicative of improved glucose metabolism via the glycolytic pathway with subsequent decrease in blood glucose.

## B-002

**Evaluation of oxidized linoleic acid metabolites in rodent models of alcoholic liver disease**

D. Warner[1], A. Feldstein[2], C. McClain[1], I. Kirpich[1]. *[1]University of Louisville, Louisville, KY, [2]University of California San Diego, San Diego, CA*

**Introduction/Goal:** Liver dysfunction as a result of alcohol consumption is a significant health problem for which there are no current FDA-approved therapies. Alcoholic liver disease (ALD) encompasses a range of severities from steatosis to steatohepatitis, and further to irreversible damage, fibrosis and cirrhosis. Progression from early to late stages may be influenced by dietary factors such as the types of dietary fatty acids and therefore present an opportunity for intervention. It has been previously shown that rodents placed on diets high in unsaturated fat (USF, enriched predominantly in dietary polyunsaturated fatty acid, linoleic acid [LA]) when combined with ethanol showed an increase in the production of oxidized LA metabolites (OXLAMs). These OXLAMs may lead to the enhanced liver injury by mechanisms that remain to be determined. Therefore, a determination of plasma OXLAM burden may be a beneficial diagnostic tool in the assessment of ALD severity. The goal of the present study was to test the hypothesis that ethanol-induced oxidation of LA and subsequent increase in hepatic and circulating OXLAMs exacerbate liver inflammation and injury via shifting hepatic macrophages toward the pro-inflammatory (M1) phenotype. **Materials/Methods:** Two animal models of ALD (chronic and chronic-binge ethanol administration) were used in this study. Male mice (C57BL/6) were fed a liquid diet that was enriched in USF (primarily corn oil/LA-enriched) or saturated fat (SF, medium chain triglyceride and beef tallow-enriched) and supplemented with 5% (v/v) ethanol or isocaloric maltose dextrin for 10 days followed by a single "binge" of 5g/kg ethanol administered by oral gavage. Alternatively, mice were placed on SF- or USF-enriched liquid diets (control or ethanol-containing) for 8 weeks. Plasma and hepatic concentrations of OXLAMs were determined by LC-MS and hepatic gene expression was assessed by qRT-PCR. **Results:** Lipidomic analysis by mass spectrometry demonstrated that plasma and hepatic concentrations of LA and OXLAMs (9- and 13-hydroxyoctadecadienoic acids [9-HODE and 13-HODE]) were significantly higher in mice fed USF+ethanol compared to controls and those mice fed SF+ethanol in both models. This was correlated with enhanced liver damage as determined by plasma ALT activity and increased hepatic neutrophil/macrophage infiltration. qRT-PCR analysis for macrophage type M1 and M2 cytokine gene expression revealed that M1-associated proinflammatory cytokines (*Tnf-α* and *Il-1β*) were elevated in mice provided the USF+ethanol diet but showed no changes in M2-associated (*Tgfβ* and *Arg-1*) cytokine gene expression. These changes may be a direct effect of HODEs on macrophage gene expression because RAW246.7 cells (a mouse macrophage cell line) expressed more *Tnf-α* and *Il-1β* following incubation with 9-HODE. **Conclusions:** Increased plasma OXLAM levels were found in both experimental animal models of ALD and were correlated with greater liver injury in mice fed ethanol and a diet high in LA. Furthermore, increased macrophage polarization to a pro-inflammatory state may be one mechanism by which LA metabolites lead to greater ethanol-induced liver injury. Therefore, plasma OXLAM concentrations may be a predictor of liver inflammation resulting from ethanol-induced oxidation of dietary fatty acid, LA.

## B-003

**Developing a policy/protocol for testing of non-human and forensic samples by a clinical core laboratory**

G. Pomper[1], S. Appt[2], T. Register[3], E. Palavecino[1], M. Beaty[1], P. Lantz[4], J. McLemore[4], J. Hausman[5], E. Wilson[5], D. Dennard[5], E. Oliphant[5], S. H. Wong[1]. *[1]Wake Forest School of Medicine, Pathology, Wisnton-Salem, NC, [2]Wake Forest School of Medicine, Pathology, Comparative Medicine, Preclinical Translational Services/Center for Industry Research Collaboration, Wisnton-Salem, NC, [3]Wake Forest School of Medicine, Pathology, Comparative Medicine, Preclinical Translational Services/ Center for Industry Research Collaboration, Wisnton-Salem, NC, [4]Wake Forest School of Medicine, Patrhology and Forensic Pathology, Wisnton-Salem, NC, [5]Wake Forest Baptist Health, Wisnton-Salem, NC*

**Background:** Testing of non-human samples provides data for translational studies using various animal models. Analysis of postmortem forensic autopsy samples serves as an important adjunct for death certification. If these tests are performed in a clinical laboratory for patients' samples, safeguards must be considered so that patient testing is not adversely affected by inadvertent contamination. The Comparative Medicine and Forensic Pathology sections of our Department and a few investigators had occasional test requests. Consequently, we conducted multiple discussions and planning sessions. We consulted with colleagues from other medical centers and diagnostic companies. Consequently, we developed an initial policy/procedure for testing these samples with focus on avoiding cross contamination. **Methods:** Chemistry and Hematology Sections of our Core Laboratory accept veterinary and postmortem samples with the full understanding that validation studies for analysis of non-human or human cadaveric specimens had not been established. The Microbiology section does not accept veterinary samples for culture. However, serology testing for infectious diseases could be accepted for testing by the chemistry analyzers. Feline samples were unacceptable due to viscosity. After internal and external consultations, we classified two test groups : A) Established veterinary testing based on tests/analyzers used previously by veterinary lab customers; B) No previously established testing for veterinary and forensic postmortem samples. The following protocol was developed with sequential steps/considerations. Investigators contact the central processing department to obtain an approval form, indicating the projected volume, frequency of testing, desired turn-around-time, and funding information. Section managers use this information to schedule testing, and to increase reagent and disposable orders. Acceptable samples include blood, urine and cerebrospinal fluid. Group A veterinary samples are tested without further review. Group B tests are reviewed by the section director who makes the final decision. Upon approval, the investigators notify central processing about tests, sample size and delivery. Samples are batched and analyzed during the weekend on a non- STAT basis. These steps safeguard against mix-up with human clinical samples and avoid cross contamination. After testing, five aliquots of water are used to rinse the instrument, followed by testing of quality control samples and 5 previously tested patient samples. These steps ascertain acceptable QC values and non-significant differences of the 5 patient samples.

**Results:** Based on the experience of applying this protocol over the past 4 months, investigators initially had frequent inquiries about account information, sample transport scheduling and testing details. A lead time of 2 - 4 weeks was recommended

for new accounts. Initial testing was limited to basic metabolic panel, comprehensive metabolic panel, lipid profile, automated complete blood count with or without differential, troponin I, and BNP. Results have been acceptable by the two Sections. **Conclusion:** The above policy/protocol has been incorporated into the clinical pathology core laboratories. With weekend testing, occasional short staffing and instrumentation problems can present a challenging but manageable situation without delay to routine patient service. These initial steps would benefit from future reviews and improvements.

## B-004

### Effect of Neutral Sphingomyelinase Inhibition on ER Stress and Apoptosis in Liver Ischemia-Reperfusion Injury

M. Aslan, H. Tuzcu, B. Unal, E. Kırac, E. K. Konuk, F. Ozcan, G. O. Elpek, N. Demir. *Akdeniz University, Antalya, Turkey*

**Background:** Previous studies have revealed the activation of neutral sphingomyelinase (N-SMase)/ceramide pathway in hepatic tissue following warm liver ischemia reperfusion (IR) injury. Excessive ceramide accumulation is known to potentiate apoptotic stimuli and a link between apoptosis and endoplasmic reticulum (ER) stress has been established in hepatic IR injury. Thus, this study determined the role of selective N-SMase inhibition on ER stress and apoptotic markers in a rat model of liver IR injury.

**Methods:** Selective N-SMase inhibitor was administered via intraperitoneal injections. Liver IR injury was created by clamping blood vessels supplying the median and left lateral hepatic lobes for 60 min, followed by 60 min reperfusion. Levels of sphingmyelin and ceramide in liver tissue were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS).

**Results:** Spingomyelin levels were significantly increased in all IR groups compared to controls. Treatment with a specific N-SMase inhibitor significantly decreased all measured ceramides in IR injury. A significant increase was observed in ER stress markers C/EBP-homologous protein (CHOP) and 78 kDa glucose-regulated protein (GRP78) in IR injury, which was not significantly altered by N-SMase inhibition. Inhibition of N-SMase caused a significant reduction in phospho-NF-kB levels, hepatic TUNEL staining, cytosolic cytochrome c and caspase-3, -8 and -9 activities which were significantly increased in IR injury.

**Conclusion:** Data herein confirm the role of ceramide in increased apoptotic cell death and highlight the protective effect of N-SMase inhibition in down-regulation of apoptotic stimuli responses occurring in hepatic IR injury.

## B-005

### Comparison of Two Multispecies Hematology Analyzers Used in Nonclinical Drug Safety Studies: Sysmex XT-2000iV vs Siemens Advia 120

C. Bull[1], M. Bierau gel[2]. *[1]Allergan, LLC, Irvine, CA, [2]Scripps Health, San Diego, CA*

**Background**

Complete blood counts and white blood cell differentials are standard panels used in the assessment of drug safety. This evaluation was conducted to compare the Sysmex XT-2000iV automated hematology analyzer to the Siemens Advia 120.

**Methods**

Analytical performance of intra-run imprecision and linearity were assessed using quality control material. Blood specimens from laboratory animals originally collected for nonclinical drug discovery and development studies, (Sprague-Dawley Rats n=58, Dogs n=32, NZW Rabbits n=19, Cynos n=45), were analyzed side by side for routine hematology parameters on both platforms. Correlation data generated from these same sample analyses were evaluated using regression statistics and percent bias.

**Results**

Analytical performance data from assessments of within-run imprecision and linearity were comparable between platforms. Regression values were considered satisfactory ($R \geq 0.90$) across all species analyzed, with exceptions that were primarily believed to be related to minor methodology differences. Percent bias data was generally within ±5%, yet the bias of some parameters (e.g. RETIC%), were greater and attributable to inherently low numeric result values.

**Conclusions**

Comparison of these systems revealed some differences in results, but none were considered significant enough to interfere with the interpretation of nonclinical study data. Our data demonstrated that the analytical performance results of the two platforms, including assessments of intra-run imprecision, linearity, and correlation, were satisfactory. To fully validate a comparison of these two platforms a greater number of sample analyses would be needed. The Sysmex XT2000iV had comparable performance and acceptable correlation to the Siemens Advia 120 when used to support nonclinical studies.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

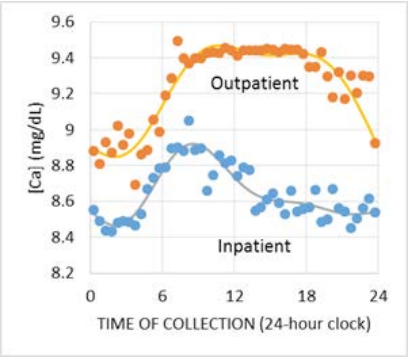
Automation/Computer Applications

## B-006

### Cyclical time-of-collection variation of calcium results for inpatients and outpatients at a university hospital: implications for patient-based quality control

Y. C. Ziemba, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

**BACKGROUND**: Patient-based quality control (PBQC) is typically based on running averages (RA), which may be affected by regular time-of-day (TOD) variation of results. This variation may occur due to multiple factors, including the mix of inpatient (IP) and outpatient (OP) results, and the possibility of physiological TOD variation. As calcium ([Ca]) is known to have physiological TOD variation, our objective was to examine whether this variation was evident in laboratory results for [Ca] based on time of collection (TOC), for IP and OP samples. **METHODS**: A six-month database (Mar-Aug 2016) of laboratory results for [Ca] was extracted from the laboratory information system to include TOC. Exclusions were patients <1 year of age, and results other than first-or-only results for a given patient within a given day. Average [Ca] was calculated for 30-minute bins within a 24-hour clock-time cycle (0000-2400) for IP and OP populations. **RESULTS**: After exclusions, the database contained 70,275 results (41.4% IP: median age 62, 52.5% male, average [Ca]=8.6+/-0.83 mg/dL, median 8.6 mg/dL; 58.6% OP: median age 61, 46.7% male, average [Ca]=9.4+/-0.58 mg/dL, median 9.4 mg/dL; reference range for [Ca] = 8.5-10.2 mg/dL). Results for average [Ca] vs. TOC are shown in Figure (CV = 7%-10% for all points). For both IP and OP, a clear TOD pattern was evident, in which both nadir and apex occurred in AM (0000-1200). Apex for IP (delta from nadir = approximately 0.4 mg/dL) was transient in comparison to apex for OP (delta from nadir = approximately 0.6 mg/dL), for which high [Ca] was sustained much longer. **CONCLUSIONS**: TOD variation was evident in laboratory results for [Ca] vs. TOC. TOC patterns were distinct for IP and OP. Results suggest that accounting for TOC variation in [Ca] might be useful in PBQC.




## B-007

### SMART CARD AND INTEGRATION WITH EMR

V. Hampapur. *Tata Memorial Centre, Navi Mumbai, India*

**Objective**

Tata Memorial Hospital **(TMH)** is a Comprehensive Care Centre for Cancer with a standing of 7 decades. Patients from all over India and some from neighboring countries choose to travel to Mumbai (Bombay) to take at our centre. Given the Geographical constraints TMH has adopted Information Technology to reach out to it's patients in distant communities.

TMH has a home grown Electronic Medical Record System the contents of which is shared with patients and providers over the Hospital Wide Intranet and globally through the website. The hospital has paperless and filmless operations since 2013 which enables real time exchange of information and continuum of care. Paper Records of yester years are scanned, archived and is part of the EMR.

In spite of the above automation and due to a heavy patient load queues for services in the hospital had resulted in patient inconvenience and dissatisfaction. It was in this context an innovative step was taken to adapt Smart Card Technology to address issues pertaining to health care services and business rules of the Institution.

**Planning & Implementation**

Every patient is issued a Photo ID Smart Card(SC) at the time of Registration with our Institution. The SC is personalized by recording a Personalized Information Number (PIN). The SM has an inbuilt chip which contains unique details of the patients for retrieval at all points of service.

Hospital Staff access information and provide services using Smart Card Readers. This helps in identifying patients and prevention of transcription errors. Patients authenticate all financial transactions by using their PIN. Short Messaging Service (SMS) is used to notify patients about debits to their Accounts and also when reports are finalized. Information Kiosks placed at strategic locations allow patients to access their EMR using their SC.

**Results**

The implementation of the above system was an exercise in change management involving training of all the stake holders and this study was undertaken to assess the impact of these changes on Turn Around Time (TAT), Patient Safety, Transparency and Audit Tracking.

The TAT for requisitioning of diagnostic services has reduced by 56% due to end to end automation. Similar reduction in Transaction time is seen in dispensing of drugs in the pharmacy based on electronic prescriptions.

The no of Transcription errors due to manual entry resulting in repeat tests has been eliminated in toto which is a significant measure of Patient Safety.

The patients and providers have instant access to the EMR and are notified through SMS as and when service provision is completed.

The no of Service transactions have increased from 593/day (2013) to 1545/day in 2015 a jump of nearly 160%. Similarly the Dispensary transactions have increased from 1522/day in 2013 to 1910 a jump of 25.42%.

**Needless to say the perception of care has improved significantly.**

## B-008

### Improving Laboratory Process with Total Laboratory Automation

H. E. Yu, J. Olson. *Geisinger Health System, Danville, PA*

**Background**: In recent years there have been tremendous advancements in laboratory automation systems, and an explosion in the number of options available for laboratories. In the past, total automation systems were offered only by third party vendors who specialize in automation. Today, many vendors who specialize in analytics also offer state-of-the-art options for total lab automation.

**Methods**: Our laboratory has moved from having an automation system for only the chemistry testing to one of the newly available total laboratory systems that connected our preanalytic systems, hematology, coagulation, chemistry, and sample storage unit. To determine whether there is efficiency gain and workflow improvement, we quantified various matrix before and after the implementation of total laboratory automation system.

**Results**: Our data show that the implementation of total laboratory automation system results in 73% less of discreet processing steps of specimen handling, even when starting from a partially automated laboratory. Consolidation of testing resulted in reduction in testing footprint and reduced 2.5 testing personnel. It also results in 82% reduction in hands-on time associated with add-on process. Even with the combination of both STAT and outreach work on the testing system, turnaround time remains consistent.

**Conclusions**: Implementation of total lab automation system can transform laboratory process and efficiency. Careful planning and optimization is required to bring this change to the laboratory.

## B-009

### Maximizing Laboratory Efficiency with Total Laboratory Automation System and Middleware Solution The University Malaya Medical Centre (UMMC) Experience

Y. Y. Chew, A. C. Yeoh, G. S. Vong, P. J. Megat Yunos, N. Idris, R. Ngabedan, F. A. Jamaluddin, P. Sthaneswar. *University of Malaya Medical Center, Kuala Lumpur, Malaysia*

**Background:** University Malaya Medical Centre (UMMC) is the largest teaching hospital in Malaysia. With 1100 beds, UMMC provides a full range of clinical services, medical education, training, and research. The Division of Laboratory Medicines (LMD) provides routine and specialized laboratory diagnostic services to internal and external clients. LMD faced challenges in continuing to provide efficient, quality testing services with limited staff. LMD went through a few phases of evolution, from compartmentalization to consolidation with laboratory automation installed in 2014. In 2016, LMD upgraded its facility to Siemens Aptio® Total Laboratory Automation (TLA) Solution. The goals for this new solution are improved operational workflow and increased productivity and quality.

**Method:** Aptio® Automation is configured based on Lean concepts to ensure efficient workflow and space utilization. 2 ADVIA Centaur® XPT Immunoassay Systems, 1 ADVIA® Chemistry XPT System and 2 ADVIA® 2400 Chemistry System are connected to 22.5 meters Aptio® high-speed track. Complete pre- and post-analytical modules further enhance the lab's speed and efficiency by automating non-value added steps. This including rack input for fast sample loading, input/ output with designated output lanes for sorting and archiving sample tubes, 3 centrifuges, refrigerated storage & retrieval module automates storage retrieval & disposal and desealer provides automatic tube desealing for rerun, reflex, and add-on testing. Integration of Aptio® TLA and the CentraLink® data management system includes robotic capabilities and rules that enable automatic tube routing without manual intervention. A few performance metrics are used to monitor workflow improvement, operational improvement and patient care enhancement after TLA. These including mean and median TAT (from the time the test order is downloaded into the CentraLink® data management system to delivery of the result to the LIS) for routine and STAT samples and LMD's service-quality promise of 95% of routine sample results within 2 hours and 90% of STAT sample results within 1 hour.

**Results:** After 8 months of Aptio® TLA operation, LMD experienced 25% improvement with mean TAT for all samples has reduced to 36 minutes, with a median of 32 minutes (versus 48 minutes and 39 minutes respectively pre-TLA). Average 98.4% routine sample results were reported within 1.5 hours and 97.1% of STAT sample results were reported within 1 hour. Consistency of TAT gave LMD the confident to redefine their service-quality promises. Their previous goals were to deliver 95% of routine sample results within 2.5 hours and 85% of STAT sample results within 1 hour. The new goals now are to deliver 95% of routine sample results within 1.5 hours and 95% of STAT sample results within 1 hour.

**Conclusion:** Since implementing Aptio® TLA, LMD has achieved predictable turnaround times for both routine and STAT. The evolution represented a great milestone and achievement for LMD, from a compartmentalized laboratory to consolidation of the clinical chemistry and immunoassay departments and recent Aptio® TLA. The journey has just begun. LMD will continue to focus on improvements and to set higher performance metrics for new heights and, ultimately, for better patient care.

## B-010

### Data mining for establishing limits for release of results by auto-verification—study of the parameters of the biochemical renal panel in cancer patients

S. Chakraborty. *Tata Medical Center, Kolkata, India*

**Background:** Setting limits for result release by auto-verification (review limits) is a challenging process and there are no clear guidelines. The review limits (RL) should be sufficiently wide to let moderately deranged results to pass automatically, yet retain clinically significant results for manual verification. The use of linearity of the assays to calculate the RL, commonly recommended in literature, often results in very wide intervals which perforce have to be reduced by subjective decision of the biochemist. Moreover, this method does not take into account the statistical distribution of the analytes in the population served by the hospital. We describe a novel approach using data mining to calculate the RL in ambulatory cancer patients who are not in need of emergency care or from inpatients during their first day of admission. A study with serum renal panel tests (sodium, potassium, blood urea nitrogen (BUN) and creatinine) is presented.

**Methods:** The renal panel data was obtained for 2014- 2015 (18-60 years). Only one result per patient was included. The internal quality controls and the proficiency test programs had a stable performance for the said period. The patients were categorized into outpatients and inpatients. Gender partition was done for serum BUN and creatinine. Data were trimmed by removal of the overtly diseased population and by the use of interquartile rules. Data mining was performed using the Bhattacharya method on the 'R' statistical platform. The Bhattacharya method can identify hidden Gaussian distribution in a dataset. The review limits were set at ± three standard deviations (3SD) from the population's mean. The obtained RLs were compared to linearity based limits. The average number of results auto-verified were expressed as a percentage for a period of two months.

**Results:** The RLs were derived from 6996, 6917, 2993, and 2679 patients for serum sodium, potassium, creatinine, and BUN respectively. The review intervals derived by data mining followed by linearity based intervals given in brackets in ambulatory outpatients are as follows: Serum sodium: 133-148 (33-196) mmol/L, serum potassium: 3.1-6.1 (1.65-9.20) mmol/L, serum creatinine(male): 0.58-1.36(0.48-7.5) mg/dL, serum creatinine (female): 0.41-1.02 (0.35-7.38) mg/dL, serum BUN (male): 1-19 (6-67) mg/dL, serum BUN (female): 1-17(5-66) mg/dL.

The review intervals in inpatients are as follows: Serum sodium: 125-147 mmol/L, serum potassium: 2.9-5.6 mmol/L, serum creatinine(male): 0.37-1.87 mg/dL, serum creatinine (female): 0.41-1.02 mg/dL, serum BUN (male): 1-20 mg/dL, serum BUN(female): 1-19 mg/dL. The linearity based RLs remain the same for inpatients. About 65% of the renal panel were auto-verified using the derived RLs.

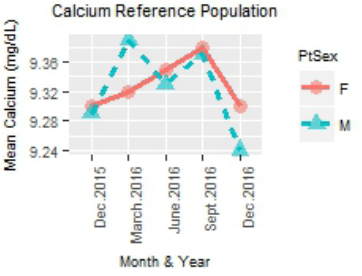
**Conclusion:** A population-based RL has been developed which can successfully verify a good proportion of the results during the first visit of the patient in our cancer hospital. RLs set at 3SD from the population means of each analyte provide a strong clinical basis for their calculation as compared to the static linearity based RLs. The linearity derived intervals on the other hand are very wide and may even be outside survival limits.

## B-011

### Data mining and reference interval studies indicate total calcium seasonal variation

A. B. Muenzenmeyer, E. Z. Reineks. *Cleveland Clinic, Cleveland, OH*

**Background:** Total Calcium's reference range was updated in September 2016 to 8.6-10.0 mg/dL on the basis of a reference range establishment performed using historical data from December 2015. Prior to September 2016, the range was 8.5-10.5 mg/dL. Although the abnormal flag frequency was expected to increase with this change, the observed increase was higher than expected. The goal of this study was to determine whether seasonal variation was a factor that could account for the unexpected rate of high abnormal flags. **Methods:** We used data mining and business intelligence software to establish Calcium reference ranges for following timeframes: March 2016, June 2016, September 2016 and December 2016. This study utilized Altosoft (*Kofax*, Irvine, CA), business intelligence software to readily identify suitable existing patient samples for results in our laboratory information system (*Sunquest*, Tucson, AZ). Data was exported into Excel (*Microsoft*, Redmond, WA) and filtered according to the December 2015 pre-defined criteria. The datasets were evaluated using an EP Evaluator® (*Data Innovations*, South Burlington, VT). Descriptive statistics were determined for all of the datasets, including subsets of male and female partitioned data. **Results:** The number of Calcium results for each study period ranged from N=408 to 551. For all healthy subjects, lower reference ranges were established from 8.5 to 8.7 mg/dL and upper ranges from 10.0 to 10.2 mg/dL. Total Calcium reference range will be updated to 8.5-10.2 mg/dL. **Conclusions:** Changes in Total Calcium concentration have a seasonal variation for populations served by our laboratory, which is important to address when establishing population based reference ranges. We excluded the possibility of instrument drift/analytical variation based on the observed differences between male and female populations. Data mining makes this type of evaluation feasible, both in terms of time and affordability.



## B-012

### A strategy to decrease the manual microscopic examination by introduction of the automated urinary sediment analyzer

J. Kim, E. Cho, K. Park, H. Hong, E. Shin, W. Lee, S. Chun, W. Min. *Asan Medical Center, Seoul, Korea, Republic of*

**Background:** Routine urinalysis consists of an assessment of physicochemical characteristics and manual microscopic examination (MME). MME is labor-intensive, time-consuming, and imprecise. The introduction of automated urinalysis systems based on flow cytometry or image-based analysis could eliminate or replace MME, improving the turnaround time. The aim of this study was to evaluate workflow processes when introducing automated urine sediment analyzers in terms of reduction in the requirement for MME.

**Methods:** We compared the two automated urine analyzers, the UF-1000i (Sysmex Corporation, Kobe, Japan) based on flow cytometry, and the cobas 6500 urine analyzer (Roche Diagnostics International, Rotkreuz, Switzerland) based on sediment microscopy. A total of 1055 and 1119 urine samples from inpatients and outpatients, respectively, were tested between June 14 and June 29, 2016. All samples were analyzed using both the UF-1000i and the cobas 6500. MME was performed according to the following criteria: With regard to RBC and WBC tests, when mismatch was discovered from the results of urine strip test and automated analyzers; when flag signs of automated analyzers occurred. Urinalysis was performed with four workflow processes including solitary assay of each automated analyzer and combined assay of the two analyzers. We compared the reduction rate of MME between the four workflow processes. When using cobas 6500, MME was performed after conducting the image review.

**Results:** In the case of UF-1000i single-use, MME was conducted on 361(34.2%) specimens of inpatients and 188(16.8%) specimens of outpatients. In the case of the cobas 6500 single-use, the image review were performed on 446(42.3%) specimens of inpatients and 211(18.9%) specimens of outpatients, and then MME for 165(15.6%) specimens of inpatients and 41(3.7%) specimens of outpatients was carried out. When using the Cobas 6500 after using the UF-1000i first, 361(34.2%) specimens of inpatients and 188(16.8%) specimens of outpatients underwent the image review, and then 119(11.3%) specimens of inpatients and 30(2.7%) specimens of outpatients, respectively, underwent the MME. When using UF-1000i after using the Cobas 6500 first, MME was conducted on 119(11.3%) specimens of inpatients and 30(2.7%) specimens of outpatients. When automated urine sediment analyzers were used, the rate of MME was reduced by 22.9% in the inpatient specimens and 14.1% in the outpatient specimens.

**Conclusion:** By introducing the automated urine sediment analyzers, the rate of MME was reduced, especially when combining the two automated analyzers which have different method principles. The reason for the difference in the rate of MME reduction by four workflow processes using the automated urine sediment analyzers would be elucidated according to the specimen characteristics.

## B-013

### Identification of steps necessary for general implementation of moving average for continuous QC in medical laboratories

H. H. van Rossum[1], H. Kemperman[2]. *[1]NKI-AvL, Amsterdam, Netherlands, [2]UMC Utrecht, Utrecht, Netherlands*

**Background:** Moving average (MA) can be used for continuous analytical quality control. Though MA has been described decades ago, general implementation in clinical chemistry laboratories has failed. We addressed several issues that we considered to be important to support a more general implementation of MA as continuous QC instrument in medical laboratories.

**Methods:** A MA optimization method described by our group (1,2) was used to generate optimal MA procedures that were implemented for continuous analytical quality control in daily practice(3). During the various phases of MA implementation, issues that potentially complicated the MA implementation and application were identified. Furthermore a MA-alarm case, describing a temporary sodium ion selective electrode (ISE) failure, was used to demonstrate the value of MA.

**Results:** The first step to support clinical laboratories to obtain and use optimal and validated MA for continuous QC is to make newly developed MA optimization methods commercially available for clinical laboratories. Secondly, improvements in MA management software are required to allow optimal support of MA management on clinical laboratories. These include continuous generation of MA values, adequate continuous alarming, MA resetting, exclusion of samples and presentation of MA in an accuracy plot. Finally, laboratory management issues were identified that included development of a clear protocol how to handle MA alarms and training of technicians.

**Conclusion:** The issues we encountered during implementation and application of MA illustrate the need to make newly developed MA optimization methods available for clinical laboratories and for improvements in the available MA management software. This should allow a more general implementation of continuous QC by MA on medical laboratories.

**References**

1) van Rossum HH, Kemperman H. A method for optimization and validation of moving average as continuous analytical quality control instrument demonstrated for creatinine. Clin Chim Acta 2016;457:1-7.

2) van Rossum HH, Kemperman H. Optimization and validation of moving average quality control procedures using bias detection curves and moving average validation charts. Clin Chem Lab Med 2017; 55(2): 218-224

3) van Rossum HH, Kemperman H. Implementation and application of moving average as continuous analytical quality control instrument demonstrated for 24 routine chemistry assays. Clin Chem Lab Med 2017, in press

## B-014

### Comparative analisys of two methods for determination of urine strips

D. González Benito, R. Escobar Conesa, V. García Moreira, A. Llorente Torres, E. Fernández Rodríguez. *Hospital Universitario de Cabueñes, Gijón, Spain*

Background: Urine analysis is a basic and very useful test to assess the existence, severity and development of kidney and urinary tract diseases. It consists in a macroscopic examination, a physical-chemical test, a microscopic examination of sediment and a urine culture if necessary. The objective of this study is to compare the results of the automatic analyzer Aution Max AX 4030 (recently implanted in our emergency laboratory), with the results of the semi-automatic analyzer Aution Eleven AE-4020 (to replace) for glucose, protein, blood, nitrite and leukocytes parameters, and to check the matching between these results from both analyzers.

Methods: A total of 150 received samples of isolated urination in our emergency laboratory during 3 consecutive days were included for a complete analysis. The samples were processed at the time of reception. For the physicochemical study, they were introduced in two Analyzers. The Aution Sticks 10EA test strips were used. Both analyzers employed the same methodology (double wavelength photometric reflection), the same ranges of analysis for the different parameters and the same test strips. Undoubtedly, the most significant difference between both instruments was the automaticity that typically characterized the Aution Max analyzer.

Results: Because is a semi-quantitative analysis, we calculated the Cohen's kappa coefficient (*k*), the 95% confidence interval (*95% CI)* and the concordance of the results of the two scanners for each parameter. We noted that there is a very good concordance for proteins (0.877*k*, 0.810-0.944 95% *CI)* and nitrites (1.000 k 1.000-1.000 95%*CI*), good for glucose (0.630 *k,* 0.430-0.831 95% *CI*) and blood (0.759 *k,* 0.664-0.855 95% *CI)* and a moderate for leukocytes (0.525 *k,* 0.403-0.647 95% *CI*).

Conclusions: With this study, we evidence that Aution Max Analyzer is more accurate than Aution Eleven, because it considerably reduces errors, especially taking of aliquot. The Aution Eleven process is manual and depends on the manipulator (aliquot amount, time while the Strip is dampened, and time that it takes to analyze), while Aution Max being automatic taking of the aliquot is constant.

## B-015

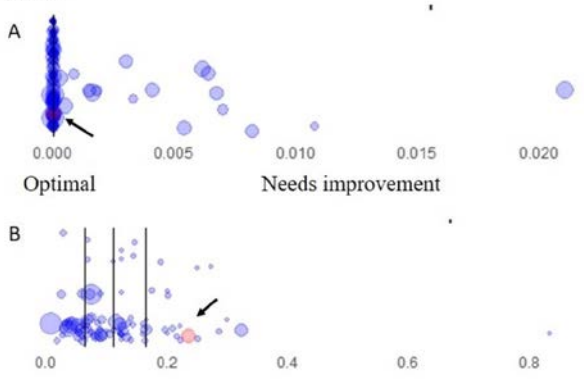
### Prioritizing local quality improvement projects using national laboratory and clinical data

K. Lankachandra[1], A. Cheng[2], R. Johnson[3], S. Sahil[2], M. A. Hoffman[4]. *[1]Truman Medical Center, University of Missouri Kansas City, Kansas City, MO, [2]University of Missouri Kansas City, Kansas City, MO, [3]Truman Medical Center, Kansas City, MO, [4]Childrens Mercy Hospital, Kansas City, MO*

**Background:** Quality improvement (QI) projects, informed by diagnostic laboratory and clinical data, are important for healthcare operations. By improving workflow in the lab, reducing errors, optimizing test utilization and enhancing provider application of lab test results, QI projects can reduce costs and improve patient outcomes, safety and satisfaction. Prioritizing QI project candidates can be difficult. Our objective is

to apply a de-identified national data laboratory and clinical data warehouse to guide QI project prioritization decisions. **Methods:** We utilized Cerner Health Facts ™ (HF), a national data warehouse populated with de-identified data extracted from electronic health records, to develop a methodology to assist Truman Medical Center (TMC), Kansas City, MO, in prioritizing QI projects. The version of HF utilized in this project includes data from 63 million unique patients, 863 healthcare facilities and 4.3 billion laboratory results from 2000 until December, 2015. We identified QI project candidates, parsed the projects into discrete machine readable parameters utilizing standardized medical terminologies and queried the HF data. HF contributor sites meeting the inclusion criteria were stratified for each QI candidate project. **Results:** For one project we evaluated antibiotic prescribing discord relative to antibiotic resistance findings, in this case oxacillin prescriptions for encounters with oxacillin-resistant *Staphylococcus aureus*. TMC ranked in the top group of the 174 facilities meeting the inclusion criteria (Figure 1A), with zero contraindicated orders. For another project candidate, the objective was a low frequency of hemoglobin A1c orders for patients with sickle cell disease. TMC patients with a sickle cell disease diagnosis were tested for hemoglobin A1c for diagnosis and management of diabetes mellitus more frequently than most of the 86 sites meeting the inclusion threshold (Figure 1B). **Conclusion:** Based on these findings, TMC should consider the sickle cell project as a higher priority QI project.

Figure 1: Health Facts facilities (circles, scaled by number of beds) grouped by QI project candidate, TMC facilities in red, with arrows. Y axis height is arbitrary. A – % oxacillin prescriptions for encounters with oxacillin resistant *Staphylococcus aureus*. B –Hemoglobin A1c orders / patients with sickle cell disease.




## B-016

### Multiple Approaches to the Improvement of the Turnaround Time in a Large Core Laboratory

A. Lou, M. Elnenaei, I. Sadek, S. Thompson, B. Crocker, B. Nassar. *Nova Scotia Health Authority, Dalhousie University, Halifax, NS, Canada*

**Background:** Core laboratory (CL), as a new business model, facilitates consolidation and integration of laboratory services to enhance efficiency and reduce costs. This study evaluates the impact of total automation system (TLA), electric track vehicle (ETV) system and auto-verification of results on overall turnaround time (PR-TAT=Phlebotomy to Reporting TAT) within a core laboratory setting. **Methods:** Mean, median and outlier percentages (OP) for PR- TAT were compared for pre- and post- CL eras using five representative tests based on different request priorities. Comparison studies were also carried out on the intra-laboratory TAT (IR-TAT= in-lab to reporting) and delivery-TAT (PI-TAT= phlebotomy to reporting) to reflect the efficiency of the TLA and ETV systems respectively. **Results:** For all the STAT requests, the median PR-TAT did not show significant differences between the pre- and post- CL periods for both potassium and urea tests when samples were centrifuged off-line; however, the median PR-TATs for both CBC and PT in the post- CL period were longer by 12min and 19min respectively when compared to the pre- CL period. Comparing the goal of PR-TAT for the STAT requests, the outlier percentages (OP) exceeding 60min were 26%, 57%, 64%, and 66% for CBC, potassium, urea and PT testing respectively in the post- CL period, which were all higher than those from the pre- CL era. Median PR-TATs for the urgent samples were 91mins, 106mins and 111mins for CBC, PT, and both urea and potassium with an average reduction of 16% across all the analytes. Median PR-TAT for the routine samples was curtailed by 51%, 50%, 49%, 34% and 22% for urea, potassium, TSH, CBC and PT respectively. The shorter PR-TAT was attributed to a significant reduction of IR-TAT by 68% and 73% for urgent and routine CBC as well as 52% and 37% for urgent and routine PT respectively. However, the median PI-TAT was delayed by an average of 14min for urgent samples but there was no significant difference for routine samples when the ETV was used. Application of various auto-verification rules shortened the median IR-TATs for both potassium and urea tests. Median IR-TATs were significantly reduced for both potassium and urea tests by an average of 6 min and 4 min for STAT and Urgent requests respectively. The median IR-TATs were slightly decreased for both routine tests, however, the differences were not significant. In addition, there was a significant reduction of the OP exceeding 60 min for both tests from all priority requests that ranged from 38- 77%. **Conclusions:** TLA and auto-verification rules help to efficiently manage substantial volumes of urgent and routine patient samples, evidenced by significant improvement in median PR-TATs and OP-TAT. However, the ETV application as it stands shows a negative impact on the PR-TAT.

## B-017

### Analytical evaluation of soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) on Brahms Kryptor automated immunoassay for the diagnosis of preeclampsia

S. Chan[1], S. Rana[1], S. Chintala[1], S. Salahuddin[2], A. Karumanchi[2], K. T. J. Yeo[1]. *[1]The University of Chicago, Chicago, IL, [2]Beth Israel Deaconess Medical Center, Boston, MA*

**Background:** Preeclampsia is one of the leading hypertensive disorders in pregnant women worldwide. It is estimated to cause more than 60,000 deaths per year. This disorder is induced by placental and maternal vascular dysfunction, which affects both maternal and neonatal health. Current diagnosis of preeclampsia in the U.S. is based on non-specific tests such as new onset of hypertension and proteinuria after 20 weeks of gestation. More recently, two emerging biomarkers anti-angiogenic factor soluble fms-like tyrosine kinase 1 (sFlt-1) and pro-angiogenic factor placental growth factor (PlGF), have been demonstrated to be involved in the pathophysiology of preeclampsia. Studies have shown that the usefulness of sFlt-1/PlGF ratio in confirming or ruling out suspected preeclampsia cases. Thus wider availability of the automated measurement of sFlt-1 and PlGF has the potential of improving the clinical management of preeclampsia.

**Methods:** This study examined $K_2$-EDTA plasma samples from 50 patients on Brahms Kryptor, an automated immunoassay platform. QC materials were used to access intra- and inter-precision of the assay. Lower limit of quantitation and interference studies were determined using pooled patient plasma.

**Results:** The sFlt-1 and PlGF assays demonstrated an analytical measuring range of 90-69,000 pg/mL and 11-7000 pg/mL, respectively ($r^2$>0.99). Lower limit of quantitation (20% CV) was interpolated to be 35 pg/mL for sFlt-1 and 10 pg/mL for PlGF. Total precision for both assay displayed CVs of <10%. Interference studies showed that both assays were not significantly affected by hemolysis up to an H-index of 1100 for sFlt-1 and 300 for PlGF; L- and I- index of 800 and 80 respectively for both assays. Passing-Bablok method comparison with an identical platform from another institution showed the equation y=0.9939x-37.15 with an overall bias of -90.35 for sFlt-1; and y= 0.8936x+2.562, with an overall bias of -39.644 for PlGF. The larger differences in overall bias based on individual tests however was greatly improved when a ratio of sFlt/PlGF is taken into account for method comparison, yielding an equation of y=1.05x+0.02, with an overall bias of 0.84.

**Conclusion:** The pre-eclamptic biomarkers, sFlt-1 and PlGF assayed on the Brahms Kryptor platform, demonstrate good analytical performance and are acceptable for clinical studies defining the role of these biomarkers for preeclampsia.

## B-018

### Analytical Evaluation of Clinical Chemistry Analyser Advia Chemistry XPT

Y. Y. Chew, A. C. Yeoh, G. S. Vong, P. J. Megat Yunos, N. Idris, R. Ngabedan, F. A. Jamaluddin, P. Sthaneswar. *University Malaya Medical Center, Kuala Lumpur, Malaysia*

Background

The Advia Chemistry XPT (Siemens Healthcare) is an automated clinical chemistry analyser that using the principle of spectrophotometric, immunoturbidimetric and ion-selective electrode measurement. Verification of method performance on this analyser and the reagents provided were carried out before approved for daily routine operation. The evaluation was performed according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI).

Materials and Methods:

The evaluation consisted of determination of imprecision based on CLSI EP15-A2 protocol; CLSI EP06-A2 protocol was used for linearity study and method comparison was carry out in between Advia Chemistry XPT with our current clinical chemistry analyser, Advia 2400 (Siemens Healthcare) based on CLSI EP09-A2 protocol. Method verification data was analysed used statistical software, Analysed-It.

Results

The results showed within-laboratory imprecision and total imprecision were within acceptable limit for all analytes. It was demonstrated that the analytical system operates within the claimed linearity ranges as stated in information for use (IFU). Intercept and slope met the preferences of Deming Fit regression for all the analytes as show in Table 1.

Conclusions

Advia Chemistry XPT shows acceptable precision for all analytes that evaluated. The analyser is fully comparable with Advia 2400 clinical chemisty system. We conclude that Advia Chemistry XPT demonstrates good performance capabilities, making this analyser suitable for a medium-to-high volume laboratory.

Table 1

| Analyte | Unit | Imprecision study | | Method comparison | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Within-run imprecision (%) | Total Imprecision (%) | Deming Regression | | | | |
| | | | | Sample Range | | Slope | Intercept | $R^2$ |
| Acetominophen | umol/L | 0.40 | 2.00 | 29.0 | 1380 | 1.00 | 7.03 | 0.9988 |
| Albumin (BCP) | g/L | 0.70 | 1.30 | 12.7 | 82.10 | 0.97 | 0.81 | 0.9983 |
| ALP_2C | U/L | 0.40 | 0.60 | 18.0 | 982 | 0.98 | 2.72 | 0.9996 |
| ALT | U/L | 0.70 | 2.20 | 11.0 | 988.85 | 1.03 | -1.86 | 0.9997 |
| Ammonia | umol/L | 4.00 | 8.10 | 7.0 | 750 | 0.97 | 2.38 | 0.9969 |
| Amylase | U/L | 0.80 | 0.90 | 3.5 | 1418.50 | 1.04 | -1.63 | 0.9997 |
| AST | U/L | 1.00 | 1.40 | 12.0 | 984 | 1.02 | 0.06 | 0.9997 |
| Calcium | mmol/L | 0.60 | 0.60 | 0.7 | 3.61 | 1.04 | -0.04 | 0.9950 |
| Cholesterol | mmol/L | 0.40 | 0.80 | 1.0 | 17.16 | 0.97 | 0.14 | 0.9985 |
| Creatine Kinase | U/L | 0.70 | 0.70 | 4.0 | 1340 | 0.97 | 2.39 | 0.9996 |
| Chloride | mmol/L | 0.40 | 0.60 | 21.0 | 195 | 1.00 | 0.54 | 0.9996 |
| CO2_C | mmol/L | 0.70 | 1.50 | 11.4 | 38.20 | 1.01 | -0.28 | 0.9958 |
| Creatinine | umol/L | 0.60 | 0.60 | 15.5 | 2125.57 | 0.98 | 2.70 | 0.9998 |
| DBIL | umol/L | 0.00 | 0.00 | 2.0 | 250 | 0.95 | -0.11 | 0.9999 |
| DHDL | mmol/L | 0.30 | 0.60 | 0.3 | 2.81 | 1.03 | -0.10 | 0.9875 |
| GGT | U/L | 0.90 | 1.80 | 8.0 | 1128 | 1.03 | -1.14 | 0.9995 |
| Glucose | mmol/L | 0.00 | 1.00 | 0.5 | 38.20 | 0.96 | 0.02 | 0.9996 |
| Iron | umol/L | 0.30 | 0.40 | 1.6 | 178.50 | 1.01 | -0.42 | 0.9994 |
| Lactate | mmol/L | 0.40 | 0.90 | 0.4 | 7.60 | 1.01 | 0.07 | 0.9989 |
| LDH | U/L | 0.30 | 0.60 | 28.3 | 688.91 | 1.00 | 4.16 | 0.9974 |
| Magnesium | mmol/L | 0.60 | 1.00 | 0.3 | 2.05 | 0.95 | 0.04 | 0.9987 |
| Phosphate | mmol/L | 0.80 | 0.90 | 0.2 | 6.40 | 1.00 | 0.03 | 0.9996 |
| Potassium | mmol/L | 0.00 | 1.40 | 1.1 | 10.20 | 0.99 | 0.10 | 0.9979 |
| Protein (CSF) | mg/L | 0.50 | 0.60 | 150.0 | 1141 | 1.00 | -15.33 | 0.9988 |
| Protein (Urine) | mg/L | 0.30 | 1.20 | 58.0 | 1252 | 1.01 | -11.98 | 0.9969 |
| Protein (total -Serum) | g/L | 0.00 | 0.00 | 27.5 | 120 | 1.01 | -1.28 | 0.9984 |
| Salicylate | mmol/L | 4.30 | 7.00 | 0.2 | 6.98 | 1.03 | -0.03 | 0.9993 |
| Sodium | mmol/L | 0.20 | 0.70 | 102.0 | 201 | 0.98 | 3.38 | 0.9986 |
| TBIL | umol/L | 0.50 | 3.10 | 4.0 | 558 | 0.97 | -0.24 | 0.9996 |
| TG | mmol/L | 0.00 | 0.00 | 0.5 | 6.10 | 0.99 | 0.03 | 0.9995 |
| Transferrin | g/L | 0.00 | 0.00 | 0.2 | 4.15 | 1.00 | -0.02 | 0.9952 |
| Urea | mmol/L | 1.00 | 1.20 | 2.4 | 53.60 | 0.96 | 0.10 | 0.9996 |
| Uric Acid | umol/L | 0.20 | 0.70 | 63.1 | 1122.44 | 0.97 | -0.35 | 0.9986 |

## B-019

### An Abbott Alinity ci-series Instrument system familiarization study

E. Hamdi[1], S. Bouhadiba[1], J. Rogers[2], G. Gomez[2], J. Laplanche[1]. *[1]Département de Biochimie et Biologie Moléculaire, Hôpital lariboisière, Assistance Publique Hôpitaux de Paris, (APHP), Paris, France, [2]Abbott laboratories, Abbott Diagnostics Division, IL USA, IL*

**Background:** We performed a familiarization study of a manufacturing prototype of the Alinity ci ® clinical chemistry (c) and immunoassay (i) instrument systems (Abbott, IL, USA). The Alinity ci-series is a fully automated analyzer allowing random and continuous access as well as priority and automated retest for both clinical chemistry and immunoassays tests.

**Method:** Calibration and quality controls including automated quality controls (QC) processing and QC analysis were assessed. The performance of selected assays (seven clinical chemistry parameters: ICT, calcium, glucose, creatinine and urea, and two immunoassays: TSH and hsTn-I) were evaluated through precision on QC, linearity using provided standards, and limits of blank (LoB), detection (LoD) and quantitation (LoQ), based on CLSI guidelines. Method comparison was performed using the ARCHITECT instrument system C8000 and i2000 system. Throughput and internal controls stability on board were also evaluated. Performance analyses were completed using quality control materials from the manufacturer and biological samples from our institution. Statistical analyses were performed using PC SAS 9.3.

**Results:** The coefficients of variation (CVs) for within-run precision for clinical chemistry tests ranged from 0.5 to 2% and were of 1.77% for TSH and 3.35 % for hsTn-I. The CVs for day-to-day precision for clinical chemistry tests ranged from <1 to 2% and were of 1.2 % for TSH and 1.94 % for hsTn-I. Linearity testing verified the assay linearity claims for all parameters (r = 0.999). Comparison studies showed good correlations with ARCHITECT instruments (r ranging from 0.974 to 1). All estimates of LoB, LoD and LoQ met the manufacturer's claim. Throughput study revealed 1204 tests/hour and 167 tests/hour capability for clinical chemistry assays and immunoassays, respectively. On board stability of chemistry QC was in the range announced by the manufacturer.

**Conclusion:** Taken together, the familiarization study performed on a selected set of parameters tested on a manufacturing prototype of the Alinity ci-series analyzer revealed satisfactory analytical performances, allied to simplified maintenance procedures, software easy-to-use, shortened hands-on time and overall system reliability.

## B-020

### Method verification fully automated with R and LYX

C. T. Nakas, G. M. Fiedler, A. B. Leichtle. *University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland*

Background

Method verification is an essential control step for the introduction of new commercial tests or test generations in accredited laboratories. Especially during platform changes, a large number of methods has to be verified at once – a cumbersome, repetitive task generating formalized reports for the quality management. Applying R and LYX, two freely available open source tools, the report generation can be fully automated, yielding highly standardized verification reports within seconds.

Methods

Raw data is obtained by the lab technicians in a formalized Excel™ sheet, containing two columns for the method comparison data (old vs. new), three for the QC levels, three for the intra-day data of the three QC levels, three for the between-day data of the three QC levels, and several columns for data that is inserted in the report text (e.g. method name, platform, matrix, etc.). For the report generation we use LYX, a free TEX processor, which is able to call R and execute R code in "chunks" defined via the R package "knitr". The standard TEX code covers the scaffold of the report, that is replenished by data and results drawn from the excel input files via R chunks. The method description from the vendor is attached to provide all supplementary information necessary for the final clearance of the document. LYX generates a clickable pdf form, which is commented by the lab supervisor and electronically signed.

Results

Providing the verification raw data in a standardized Excel™ form is a fast and simple procedure for the lab technicians, that requires no programming or statistical skills.

The files are saved in a specified directory and LYX is pointed at the filename. Processing takes about 20 seconds, and a PDF form is generated, containing the raw data table, a data summary, the intra-day and between-day CVs, standard deviation, bias, measurement uncertainty, correlation testing, Passing-Bablok regression, and Bland-Altman plots. Dropdown menus are automatically filled, e.g. for the selection of old or new reference ranges. The form also contains a comment field for the final verification clearance.

Discussion

Following an update of our clinical chemistry platform we verified 92 methods without substantial problems (besides minor formatting issues). During re-accreditation, the reports were reviewed and considered as *exemplary* and *comprehensive*. The ease-of use, the speed, the high degree of formalization and standardization as well as the use of free and open software are the major advantages that easily outweigh the efforts necessary to build the process. Automation of verification report generation with LYX and R is a rapid and comfortable way to generate high-quality reports for accreditation and regulatory authorities.

## B-021

### Daily variation in thyroid function test results: A data mining approach

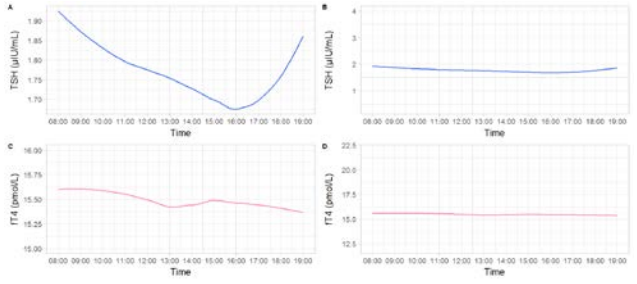
D. I. Topcu, M. S. Gungoren, C. Züngün. *Duzen Laboratories Group, Ankara, Turkey*

**Background:** Thyroid function tests are useful diagnostic tools for patient management. For more accurate clinical assessment, any factor causing variation of results should be recognized and avoided. One of the most considerable source of variation is the diurnal pattern of TSH secretion. Prospective studies to determine biological variation values are challenging to design and conduct in daily practice. Recently, data science applications allow laboratories to investigate retrospective data efficiently. The aim of this study is to demonstrate the distribution patterns of TSH and fT4 results timewise by data mining approach.

**Methods:** 5-year laboratory records including TSH and fT4 results of patients from 18 to 65 years of age were obtained from laboratory information system (LIS). Initial number of records was 61,229. As information of thyroid medications and diagnosis were not commonly available for all records, individuals with recurrent TFT reports were considered as follow-up patients and not included (n=34,465). Also, patient records including any outlier results of TSH and fT4 tests were excluded by utilizing Tukey method. Final number of patient records included was. 27,393. For each test, all test results distributed within daytime (08.00-20.00) were evaluated according to their sampling times as 1-hour intervals. All statistical analyses were performed with R 3.3.2 (R Working Group, Vienna, Austria).

**Results:** Results were given in Figure 1. Median of TSH results varied from 1.66 to 1.95 µIU/mL whereas median of fT4 results varied from 15.3 to 15.6 pmol/L.

**Conclusion:** According to our results, we can conclude that serum TSH levels vary considerably while same amount of variation cannot be observed in fT4 levels. This kind of information can be easily obtained by data mining applications for any other analyte.

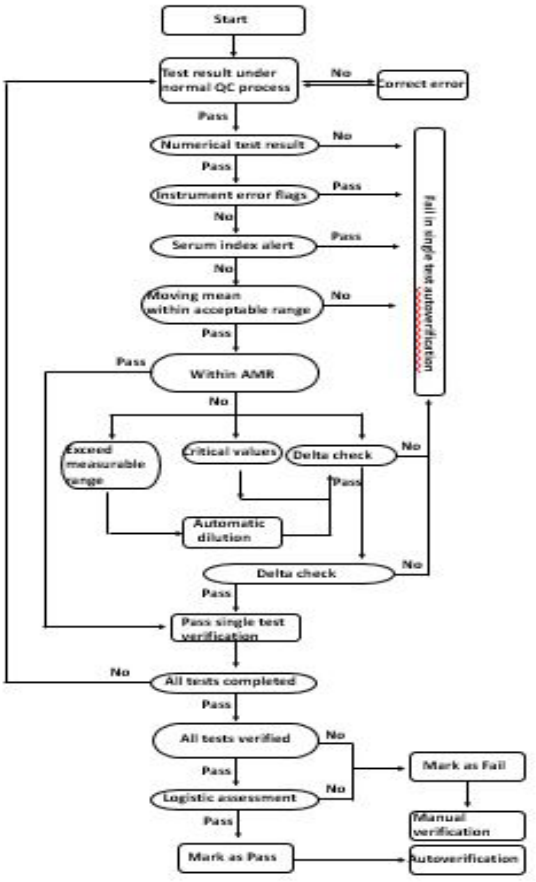



## B-022

### Establishment and Application of an Autoverification System for Chemistry and Immunoassay Tests

D. Wen[1], W. Wang[1], Y. Zheng[2], H. Dai[2], Y. Fan[1], S. Xu[1], J. Xie[2], J. Ge[2]. *[1]Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China, [2]Siemens Healthcare Diagnostics, Shanghai, China*

**Background:** Autoverification is a process of using computer-based rules to verify clinical test results. We aimed to establish an autoverification system for clinical chemistry and immunoassay tests, thereby shortening the turnaround time (TAT). **Methods:** Seven categories of 472 verification rules covering 63 routine chemistry and 26 chemiluminescence tests were encoded using the autoverification functionality of the CentraLink® Data Management System on the Aptio® Automation platform (Siemens Healthcare Diagnostics Inc.). **Results:** We setup and tested the autoverification system from August 2015 to April 2016. In total, the system ran 4,496,425 tests on 366,180 chemistry specimens. The autoverification rate increased from 53.4 to 87%. Average TAT for verification decreased by 97.7%. The system ran 410,040 tests on 160,119 chemiluminescence specimens. The autoverification rate increased from 40.2 to 89%. Average TAT decreased by 77.4%. From May 2016 to January 2017 (when autoverification was operational), compared with the same period in 2014 (when manual verification was employed), the following changes were observed: a) Volume of routine chemistry tests increased by 46.4%; b) Median TAT for tests decreased by 41.9%; c) Median TAT for critical values decreased by 50.5%; d) Rates of tests that didn't go through autoverification were 88.2% for NS(Normal Severity), 6.05% for SS(Sample Status), 2.40% for DS(Delta Check Severity), 2.00% for LS(Logical Assessment Standard), 0.97% for IS(Instrument Severity), and 0.43% for CS(Clinical Diagnostic Standard); e) Rates of abnormal specimen status identified by various modules of Aptio Automation were 7.13‰ for jaundice, 5.39‰ for blood lipids, 2.20‰ for hemolysis, 0.17‰ for barcode error, and 0.15‰ for insufficiency; f) Error rate decreased by 93.3%; and g) patient and staff satisfaction increased by 85%. **Conclusion:** Our results indicate that using the CentraLink Data Management System to perform autoverification can decrease TAT, minimize the error rate and reduce the pressure of performing manual verification.



## B-023

**Anthropometric Measurements in Field Surveys.**

E. K. Togbe, M. F. Annani-Akollor, B. A. Eghan. *Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

**Introduction:** Muscle and fat constitute the soft tissues that vary most with protein depletion in disease or excessive or deficient intakes of energy. Gold standard methods of measuring muscle and adiposity, such as dual X-ray absorptiometry (DXA), Magnetic Resonance Imaging (MRI) and Bioelectrical Impedance Analysis (BIA) may not be feasible in large field studies. In clinical practice and epidemiological surveys anthropometric measurements represent simple, convenient, rapid, noninvasive and inexpensive means to assess body composition measurements. Manual anthropometric measurements, such as of body fat, muscle and skinfold determinations, may, however, be accomplished by using inexpensive portable equipment. **Objective**: In this protocol we undertook manual anthropometric measurements as compared with a mechanized instrument Omron® Body Composition Monitor (Model HBF-500), which employs Bioelectrical Impedance Analysis (BIA), of body fat and muscle to find out the suitability of the manual methods. **Methods:** The exercise was performed on 405 volunteers of mean age 58.5 years in Kumasi, Ghana. The parameters measured manually were obtained as: Total Upper arm Area, TUA = $C^2/4\pi$; Upper arm Fat Estimate, UFE = C x TS/2; Upper Arm Muscle area Estimate, UME = TUA - UFE, where C is mid - upper arm circumference and TS is suprailiac triceps skinfold thickness. TS was measured with skinfold caliper (Holtain, UK) to the nearest 1 mm and C was measured with a nonstretch Zerfas insertion tape to the nearest 1mm. PERUME was obtained as UME/TUA%, TUA was represented as Total arm Fat and muscle and UFE represented subcutaneous fat whilst UME and PERUME were measures of skeletal muscle. OFAT and OMUSC were values for Total fat and muscle respectively generated by and Omron® Body Composition Monitor. UME and PERUME were each compared with OMUSC and TUA and UFE were each compared with OFAT. To demonstrate accuracy of these measurements, we employed Student's t test to compare mechanized anthropometric measurements with manual. To address reliability, we evaluated intra-rater technical error of measurement as a quality control procedure conducted on all participants. Pregnant women, children and people who did not meet manufacturer's criteria were excluded in Omron® Body Composition Monitor measurements. All other measurements were done according to the manufacturers' instructions and WHO standard criteria by a well-trained health worker. **Results:** UME versus OMUSC, PERUME versus OMUSC, TUA versus OFAT, $p < 0.001$ for each pair. **Conclusion:** Manual methods promise to be suitable replacement for expensive and automated devices for body composition measurements in epidemiological studies.

## B-024

**Changes in Laboratory Testing Turnaround Times at a Major Academic Medical Center After Converting to a Total Laboratory Automation System for Chemistry and Hematology**

M. R. McGill, L. Ashby, C. S. Eby, A. M. Gronowski, M. G. Scott. *Washington University School of Medicine, St. Louis, MO*

Background: Laboratory turnaround time (TAT) has a major impact on patient care. Longer TATs can delay decision making and prolong hospitalization. In April 2016, our large academic medical center (1300 beds) became one of the first two hospitals in the country to implement the Roche 8100 total laboratory automation (TLA) system, with integrated chemistry and hematology analyzers. Previously, we had a partially automated laboratory with separate spaces for chemistry and hematology. Improvement of TATs was a major goal of this conversion.

Objective: To determine the effect of implementing a TLA system on TAT in a major hospital laboratory.

Method: Median and 90$^{th}$ percentile TATs for surrogate tests of the basic (BMP) and complete (CMP) metabolic panels (sodium and protein, respectively), and complete blood count (CBC) (platelets), as well as cardiac troponin I (cTnI), from one month before and six months after the conversion were compared.

Results: Mean and 90$^{th}$ percentile TATs fluctuated and even increased at times in the first months following the conversion due to mechanical, work flow and software issues. However, after working with the manufacturer to make hardware and software adjustments, TATs markedly improved. Median and 90$^{th}$ percentile TATs for BMPs decreased 38% and 34%, respectively. Similarly, median and 90$^{th}$ percentile TATs for CMPs decreased 35% and 27%, respectively. More modest decreases were observed for CBC TATs (Median: 23%, 90$^{th}$ percentile: 10%). Finally, decreases were also observed for troponin (Median: 23%, 90$^{th}$ percentile: 11%), despite the fact that it is measured on an off-line analyzer after processing on the 8100. The latter suggests that automation of processing alone can appreciably decrease TAT.

Table 1. Turnaround times before and after TLA implementation.

| Test | Median (min) | | 90$^{th}$ percentile (min) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| BMP | 45 | 28 | 71 | 47 |
| CMP | 46 | 30 | 75 | 55 |
| CBC | 13 | 10 | 49 | 44 |
| Troponin I | 43 | 37 | 65 | 58 |

Conclusions: Our data demonstrate that adoption of a TLA system can significantly reduce TATs for both common and critical laboratory tests but that close interactions with manufacturers is critical to optimizing the advantages of TLA.

## B-025

**Modular automation for immunoassays in the clinical laboratory**

S. N. Kairs, R. B. Carino, M. Jennings, R. Dillon, B. E. Wilcox. *Applied Proteomics, San Diego, CA*

**Background:** Pre-analytical sample processing of specimens for immunoassay involves several repetitive manual tasks including sample dilution to the minimum required dilution (MRD), generating calibrator curves and delivering diluted samples to assay plates. Variability is introduced by pipetting technique and operator and throughput is limited. Laboratory automation was introduced to minimize the variability of these pre-analytical steps, identify and track samples within the workflow, and increase throughput via the parallel preparation of up to eight (8) 96-well plates for each multi-plexed immunoassay. A modular design was used to increase the overall throughput, with the flexibility to support the development process and transfer to the clinical laboratory.

**Method:** The pre-analytical sample processing pipeline is comprised of two Tecan EVO automated liquid handling systems (ALHS). A standardized plate map is applied to all assays, allowing for the development of common modules for sample arraying, diluting, and plating as well as calibrator curve generation and process control specimen handling. A shared sample source plate is used for all assay dilutions, reducing sample loss to the well dead volume and overall processing time. Samples are introduced to the pipeline pre-arrayed in the source plate or in barcoded test tubes. Sample identification is tracked throughout the process via barcoded labware and tools within Evoware, and reported to text files for the LIMS system. After plating, the assay operator controls the timing of incubations and subsequent process steps, reducing ALHS downtime and allowing the laboratory to maximize platform use.

Individual process modules were transferred to the intended ALHS platform and verified by comparing endpoint assay measurements with those produced by the manual laboratory process.

**Results:** Before implementing the ALHS platform, the laboratory processed up to two immunoassay plates per operator per run by a fully manual process. At capacity, the ALHS platform handles the pre-analytical tasks of calibrator curve generation, sample dilution and duplicate plating of up to 296 samples across five immunoassay panels, each with an MRD between 1:4 and 1:300,000. The processing time for 296 pre-arrayed samples is approximately five hours and results in eight (8) 96-well assay plates for each immunoassay panel, for a total of forty (40) plates. Arraying samples from source tubes adds approximately 1.5 hours to the overall runtime.

Calibrator curves prepared by the ALHS were comparable to manual preparation when evaluated for percent recovery and signal-based coefficient of variance (%CV) of each calibrator point. Signal-to-noise improved for the tested calibrator curves at all MRDs. Calibrator curves were plated by the ALHS and manually on separate assay plates. Similar replicate %CV and intra-plate %CV were obtained for each calibrator point by both methods. Sample dilution was tested at each MRD with ten plasma samples with four parallel preparations each. Replicate and intra-plate concentration-based %CV for samples diluted by the ALHS platform were equivalent to manual dilutions.

**Conclusion:** A four-fold improvement in batch capacity was achieved with comparable assay performance by transferring key pre-analytical tasks to an automated platform. Sample identification is tracked throughout the workflow via barcoded labware and scanning devices.

## B-026

### Nova tecnologia para otimização de produção

D. Felix, L. Lopes. *Laboratorio Sabin, Brasilia, Brazil*

**Background:** Technological innovations are increasingly present in clinical analysis laboratories, providing great progress in production and safety processes. With this in mind, we believe it is essential to search for more agile and efficient solutions, maintaining the quality of results. For this, the immunology sector requested the acquisition of a new set of equipments, which made possible a series of improvements in the production of FAN and DNA exams. Thus, the objective of this work was to execute performance comparisons of equipment 1 (AP-16 IF Plus) in relation to equipment 2 (Sprinter XL) and to compare titration configurations in order to optimize the productive capacity of the ANF (antinuclear factor) and Anti-dsDNA tests. **Methods:** The evaluation method adopted was to compare the performance of equipment 1 with equipment 2. Subsequently, a new set of equipments were installed that offer a microscope capable of scanning the image of the slides. At the end of the study, the procedures and workflow were reformulated in order to make better use of the new technology.**Results:** As an initial result, the equipment replacement increased the processing capacity from 142 samples to 198 samples in addition to a 25% reduction in preparation time of the slides in the screening step. The scanning of the images of the slides expedited the reading process in computers; it also increased the traceability and safety of the process. With the reformulation of the procedures and the fact that the new technology stores the images in a database we have obtained a reduction in primary titration repetition, which resulted in an increase of up to 50% in the slides´ dilution capacity during the titration step. As a final result, there was a 40% reduction in the processing time of each sample, an increase of capacity in the titration step, and also a greater traceability and safety in the overall production. **Conclusion:** The process automation of ANF (antinuclear factor) and Anti-dsDNA tests improved the production of the immunology sector, however, only the implantation of the new technology did not result in the maximum optimization of the processes, whereas a more refine evaluation of the proposed set and production flow was necessary in order to reach the optimal configuration for screening and titration of the exams. We were able to prevent a underutilization of the mechanism by 65%, bringing a real gain in productivity by 33% of sequential dilution capacity.

## B-027

### Accurate Detection of ANCA and Endpoint Titer Using Single Well Titer on NOVA View® Automated Fluorescence Microscope

C. Auza, J. DeAnda, E. Aron, M. Gonzalez, C. Bentow, M. Mahler. *Inova Diagnostics, San Diego, CA*

**Objective:** Automation of indirect immunofluorescent (IIF) assays has the potential to improve reproducibility and eliminate subjectivity of anti-neutrophil cytoplasmic antibodies (ANCA) testing, while improving workflow and efficiency. The purpose of this study was to compare the performance of NOVA View®, a computer-aided automated fluorescence microscope to that of the traditional manual method for ANCA detection and endpoint titer, using a clinically and analytically characterized cohort of samples from patients with ANCA-associated vasculitis (AAV) and controls. **Methods:** The study included 653 samples for positive agreement from patients with AAV (n=185), relevant disease controls (n=409), and anti-MPO and anti-PR3 positive samples, previously characterized by ELISA (n=59). All samples were tested on both NOVA Lite® DAPI ANCA (formalin) and NOVA Lite® DAPI ANCA (ethanol) kits (Inova Diagnostics, San Diego, USA). The study for endpoint titer was performed on 24 anti-MPO (P-ANCA) and 23 anti-PR3 (C-ANCA) positive samples. Slides were analyzed and interpreted by NOVA View. Subsequently, a trained technologist interpreted the digital images from the NOVA View computer monitor, and also read the slides with a manual fluorescence microscope. NOVA View software generated results and digital image interpretation results were compared to those obtained with manual microscopy, and with each other. **Results:** In the clinical cohort population, NOVA View digital image reading, manual reading, and NOVA View® output results showed a high level of agreement. Based on 47 samples, 76.6% of SWT results were within ± 1 dilution step of that of the manual titer, and 91.5% were within ± 1 dilution step of that of the digital titer, and 97.7% of SWT results were within ± 2 dilution steps of that of both the manual titer and digital titer.

**Conclusion:** This study demonstrates that the new ANCA module on the NOVA View automated system generates results including endpoint titer that are equivalent to ANCA testing by manual microscopy. NOVA View is an attractive option for labs who want to automate and streamline the reading and interpretation of the traditional fluorescent microscopy.

## B-028

### Early development of DxONE Insights, a tool to enhance data driven decision making to improve laboratory efficiency

J. P. Ferguson. *Adventist Health, Portland, OR*

**Background:** As hospital networks face today's paradox of declining reimbursements and increased emphasis on patient satisfaction, it is difficult to find the balance between reducing costs and maintaining quality of service. We must continually seek improvement opportunities that reduce cost without compromising quality. Adventist Health laboratory network was a pilot site for a new clinical informatics tool: DxONE Insights from Beckman Coulter.

**Objective:** Evaluate DxONE Insights in its ability to provide important data to guide labs to improve overall efficiency. Some of the applications we evaluated were reagent waste reduction; testing consolidation; appropriate assignment of low volume testing to Adventist Regional Core Lab or Reference Lab; and better alignment of staffing to the workload.

**Methods:** Eighteen Adventist Health Hospitals with Beckman Coulter DxI and DxC instruments equipped with PROService Remote monitoring were provided access to their instrument data via the DxONE Insights pilot tool. After training on the tool, lab directors were challenged to evaluate their lab's data in key areas using the DxONE Insights pilot tool: reagent efficiency, instrument to instrument test menu comparison, cartridge utilization, and staffing efficiency. Recommended testing changes were coordinated with the Medical Director and Medical Staff to gain alignment.

**Results:** One laboratory used the Sample Count Report and made an adjustment to better align staffing to testing volumes. Several laboratories noted that low volume assays were loaded on two analyzers, and consolidated testing to a single analyzer (reducing the need for additional QC and calibration testing). Low volume tests were identified and some were moved to a centralized regional lab and other critical tests were identified to be run with QC on an as needed basis vs. routinely. Overall reagent usage is being monitored with an intent to improve efficiency; it was 78.5% in Q4 2016 & 74.5% in the current quarter.

**Conclusions:** The DxONE Insights pilot tool has become a catalyst to change our view of the business of laboratory testing. The visibility provided with the DxONE Insights tool has helped Adventist Health Laboratories involved in the pilot program reduce waste in their processes and increase efficiency. This leads to decreased costs, without sacrificing the quality of patient care. Routine use of this tool will provide a method of sustainment of the improvements gained and opportunity for future improvement opportunities. The data allowed us to collaborate with lab staff, and with the Medical Directors and Medical Staff to ensure that essential tests have remained available without compromise to patient care.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Electrolytes/Blood Gas/Metabolites

## B-029

### Comparison of Electrolyte Measurement Using Laboratory Autoanalyzer and Blood Gas Analyzer

T. Adilistya[1], N. Indrasari[2]. *[1]Dr. Kanujoso Djatiwibowo Hospital, Balikpapan, Indonesia, [2]Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

**Background:** In many hospital laboratories, electrolyte values are measured both by laboratory autoanalyzers (AA) and blood gas analyzers (BGA). The BGA gives faster results than the AA but the AA is very widely used, especially in hospital's central laboratory because it uses venous blood and thus, can be done with other clinical chemistry tests. The study aimed to evaluate whether sodium and potassium ion concentrations measured using an AA and a BGA were equivalent and comparable.

**Methods:** We retrospectively studied outpatient and hospitalized patients between January and December 2014. Of 89,749 samples, we identified 2,104 samples where electrolytes were measured using an AA (Cobas, Roche Diagnostics, Mannheim, Germany) and a BGA (pHOx Stat Profile Plus L, Nova Biomedical, Waltham MA, USA). Statistical analysis to compare the data included Spearman's correlation and Bland-Altman plot.

**Results:** The median sodium concentrations of AA and BGA were 140 (91-190) mmol/L and 137 (93-192) mmol/L, respectively ($p<0.001$). The median potassium levels of AA and BGA were 4.33 (1.29-16.22) mmol/L and 4.00 (1.26-16.14) mmol/L, respectively ($p<0.001$). Bland-Altman plot showed mean difference of sodium and potassium of 3.9152 and 0.3307, respectively.

**Conclusion:** We conclude that the AA and the BGA do not yield equivalent sodium and potassium results. Although the mean difference between the results of two assays was within the range given by the US CLIA 1988 guidelines, a difference of 0.3307 mmol/L in potassium level is clinically relevant when intra-individual variation is considered. For electrolyte measurement in clinical practice, we suggest not to use the AA and the BGA interchangeably.

## B-030

### Performance of the EKF Diagnostics, Stanbio β-Hydroxybutyrate LiquiColor® Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System

G. Snodgrass[1], K. Ackles[1], A. Blanco[2], A. Versaggi[1]. *[1]Ortho Clinical Diagnostics, Rochester, NY, [2]EKF Diagnostics, Boerne, TX*

The EKF Diagnostics, Stanbio β-hydroxybutyrate LiquiColor assay quantitatively determines the concentration of β-hydroxybutyrate in serum or plasma. β-hydroxybutyrate, acetoacetate and acetone are three ketogenic byproducts from the metabolism of fatty acids. The byproducts are typically present in low concentration; however in disease state conditions such as diabetic ketoacidosis the levels are elevated, with β-hydroxybutyrate being present in the highest concentration. The traditional method for detecting the ketogenic byproducts is the qualitative nitroprusside test which detects only the ketone bodies acetoacetate and acetone, but fails to detect the ketoacid, β-hydroxybutyrate, the most prominent ketone body. The mechanism for the Stanbio β-hydroxybutyrate LiquiColor assay is as follows: β-hydroxybutyrate in the presence of NAD is converted to acetoacetate and NADH by β-hydroxybutyrate dehydrogenase. NADH produced by this reaction reacts with 3-p-nitrophenyl-2-p-iodophenyl-5-phenylterazolium chloride (INT) in the presence of diaphorase to generate a colorimetric signal at 510nm. We have assessed the performance of the Stanbio β-hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. The assay was run on the VITROS MicroTip assay processing side of the MicroImmunoassay Center using 4.0uL of patient sample and the two Stanbio LiquiColor reagents. Endpoint absorbance measurements were taken at 510nm and converted to a concentration using a linear calibration model. The absorbance is directly proportional to the concentration of β-hydroxybutyrate in the patient specimen. We evaluated the accuracy of 105 serum and plasma samples (0.05 - 12.56 mmol/L) on the VITROS 4600 and VITROS 5600 Systems compared to the Stanbio SIRRUS Clinical Chemistry Analyzer. The VITROS 4600 and VITROS 5600 Systems showed excellent correlation with the SIRRUS Analyzer. VITROS 4600 System = 0.9542 * SIRRUS - 0.0123; (r) = 0.995. VITROS 5600 System = 0.97 * SIRRUS - 0.0227; (r) = 0.997. A 28-day precision study conducted on the VITROS 4600 and VITROS 5600 Systems showed excellent precision. Mean β-hydroxybutyrate concentrations of 0.191 mmol/L and 4.224 mmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 2.58% and 0.88% respectively, for the VITROS 4600 System and 1.06% and 0.87% respectively, for the VITROS 5600 System. The Limit of Detection (LoD) for the VITROS 4600 and VITROS 5600 Systems is 0.02 mmol/L based on 120 determinations with 2 low-level samples. The Limit of Blank (LoB) is 0.01 mmol/L based on 120 determinations with 2 blank samples. The VITROS 4600 System and VITROS 5600 System results for an endogenous interferent panel evaluated at a β-hydroxybutyrate concentration of 1.50 mmol/L showed no interference to hemolysis (600 mg/dL), conjugated and unconjugated bilirubin (40 mg/dL), and triglycerides (Intralipid,1600 mg/dL). The β-hydroxybutyrate LiquiColor assay run on the VITROS 4600 and VITROS 5600 Systems exhibited excellent correlation with the SIRRUS Clinical Chemistry Analyzer, excellent precision and low end sensitivity. In addition, the assay was free from interference by endogenous substances at a clinically relevant β-hydroxybutyrate concentration.

## B-031

### $P_{50}$ calculation using IFCC approved equation (Hill's equation); a first report in Korea.

S. Shin[1], Y. Yoon[2]. *[1]Seoul Metropolitan Dongbu Hospital, Seoul, Korea, Republic of, [2]Soonchunhyang University Cheonan Hospital, Cheonan, Korea, Republic of*

**Background:** High oxygen-affinity hemoglobin (Hb) variants and 2,3-diphosphoglycerate (2,3-DPG) deficiency are one of the causes of congenital(familial) erythrocytosis. High oxygen-affinity Hb variants/2,3-DPG deficiency result in low tissue oxygen tension and a left shift of the oxygen dissociation curve, with reduction of the $P_{50}$ (the oxygen tension at which hemoglobin is 50% saturated). Therefore $P_{50}$ is included in diagnostic strategies for erythrocytosis. The acquired secondary (pulmonary, renal, cardiac etc.) and acquired primary erythrocytosis (Polycythemia vera, *JAK2* mutation with low serum EPO level) are excluded. Low serum EPO level with negative *JAK2* mutation is suggestive of primary familial congenital polycythemia (PFCP). In cases of normal or high serum EPO level, $P_{50}$ is recommended to rule out high oxygen-affinity Hb variants and 2,3-DPG deficiency. According to this diagnostic strategies, we found a first Korean case of high oxygen-affinity Hb variant (Hb Heathrow) with low $P_{50}$ (14mmHg). To the best of knowledge, there have been no studies for $P_{50}$ in Korea until now. **Methods:** In this study, we established the $P_{50}$ reference range using International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) approved equation (Hill's equation, using single venous/arterial blood). We selected the 227 blood gas analysis results (oxygen saturation ranged 40 to 90%, Hb<16mg/dL).

**Results:** Total 143 male and 84 female were enrolled. The mean white blood cell, platelet, Hb levels were followings; $8.0\times10^3$/uL, $239\times10^3$/uL, 12.7mg/dL, respectively. The mean±SD and reference range (2.5%-97.5%) of $P_{50}$ were 27.3±2.25mmHg and 23.0-31.2mmHg respectively.

**Conclusion:** Some previous studies reported $P_{50}$ reference range as 22.6-29.4mmHg. And they suggested that high-affinity Hb variant or 2,3-DPG deficiency should be suspected if the $P_{50}$ level is <20-22.6mmHg. Hill's equation is simple and approved method for $P_{50}$. Only single venous or arterial blood sample and blood gas analyzer is required to obtain $P_{50}$. Our study will provide the useful tool for work-up of erythrocytosis.

## B-032

### Performance of GEM Premier 5000 at High Altitude

M. DeAbreu, S. Mansouri, N. Raymond. *Instrumentation Laboratory, Bedford, MA*

**Background:** Traditional blood gas analyzers are calibrated with humidified gas mixtures and require correction for barometric pressure. However, cartridge-based systems measuring blood gases typically feature solutions pre-equilibrated with specific gas tensions and sealed in gas-impermeable bags. Barometric pressure correction is not performed on these systems, based on the assumption that ambient pressure has no influence.

**Methods:** In this study, we compared pH, $pO_2$ and $pCO_2$ performance on the GEM Premier 5000 analyzer with Intelligent Quality Management 2 (iQM2) at different altitudes. Two levels of internal Process Control Solutions (PCSs) D and E and five levels of external linearity controls, were analyzed and compared at two elevations. In addition, six levels of tonometered whole blood were run at low altitude (near sea level) and seven levels were run at high altitude. The low elevation test was performed at Instrumentation Laboratory (IL) facilities, Bedford, MA (elevation = 135 ft/41m). The high elevation test was performed at St. Vincent's Hospital, Leadville, CO (elevation = 10,152 ft/3,094 m).

**Results:** The bias between high and low altitude results for pH and blood gases in both aqueous controls and tonometered whole blood samples was within total allowable error specifications (TEa) for all levels tested. Whole blood results at Medical Decision Levels (MDLs) were calculated from a linear regression against a reference method (tonometry for gases and GEM Premier 3000 for pH) and compared at both altitudes as shown in Table 1. Aqueous controls at high altitude compared to aqueous controls at low altitude are also shown in Table 1.

Table 1: Whole blood and aqueous results at High vs. Low Altitude:

| Sample | pH 3,094m | pH 41m | pH bias (±0.04) | $pO_2$ (mmHg) 3,094m | $pO_2$ (mmHg) 41m | $pO_2$ bias (±9 or 10%) | $pCO_2$ (mmHg) 3,094m | $pCO_2$ (mmHg) 41m | $pCO_2$ bias (±5 or 8%) |
|---|---|---|---|---|---|---|---|---|---|
| WB-MDL1 | 7.30 | 7.30 | 0.001 | 35 | 31 | 4.4 | 37 | 36 | 0.3 |
| WB-MDL2 | 7.35 | 7.34 | 0.002 | 50 | 45 | 4.8 | 52 | 51 | 1.3 |
| WB-MDL3 | 7.44 | 7.44 | 0.004 | 65 | 60 | 5.1 | 72 | 70 | 3.9% |
| QC-L1 | 6.62 | 6.63 | -0.008 | 31 | 35 | -4.0 | 132 | 129 | 2.0% |
| QC-L3 | 7.22 | 7.22 | 0.002 | 90 | 91 | -0.4 | 67 | 65 | 2.4% |
| QC-L5 | 7.73 | 7.73 | 0.003 | 565 | 556 | 1.7% | 18 | 18 | 0.2 |
| PCS-D | 7.35 | 7.35 | 0.000 | 64 | 67 | -2.9 | 24 | 24 | 0.0 |
| PCS-E | 7.21 | 7.21 | -0.005 | 104 | 105 | -1.1% | 67 | 68 | -0.8% |

**Conclusion:** Based on the results obtained in this study, gas tensions in sealed PCS bags on the GEM Premier 5000 cartridge PAK demonstrated analytical stability, independent of altitude.

## B-033

### Surrogate Prognostic Marker for Diabetic Nephropathy by NMR-based Urine Metabolomics

C. Lin[1], H. Yang[2], C. Chou[1], I. Chen[1], S. Chen[1], D. Chiu[3]. *[1]Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]Yuanpei University of Medical Technology, HsinChu, Taiwan, [3]Chang Gung University, Taoyuan, Taiwan*

**Background:** Chronic kidney disease (CKD) is a major health issue worldwide. Patients with varying stages of CKD have high risks for end-stage renal disease (ESRD), cardiovascular disorders and death. Diabetic nephropathy is the leading cause of CKD, which is one of the most significant long-term complications in terms of morbidity and mortality for patients with diabetes. Early diagnosis and treatment are important in preventing the progression of CKD. Urine metabolites can offer direct insights into the pathophysiology of kidney. Elucidation of characteristic metabolic alterations during diabetic nephropathy progression is critical to identify potential markers and therapeutic targets. The purpose of this study is to investigate the change of metabolites at different stage of CKD in diabetic patients and apply these metabolites as potentially predictive surrogate markers for the detection of early kidney deterioration.

**Methods:** Patients from various stage of CKD referred to a medical center for diabetes monitoring were recruited with informed consent from September 2013 to September 2015. Urine samples were collected and metabolites were assessed by using $^1$H nuclear magnetic resonance (NMR) spectroscopy coupled with multivariate statistical analysis. Metabolites were identified and quantified using the 600 MHz Library within Chenomx NM suite 7.5 professional software.

**Results:** A total of 77 participants (42 males and 35 females) were separated into five CKD stages according to the K/DOQI guideline. After creatinine normalization, 15 metabolites had significant differences in the advanced CKD stage comparing to the early CKD stage including 1-methylnicotinamide, 3-hydroxyisovalerate, Alanine, Choline, Dimethylamine, Formate, Hippurate, Isobutyrate, Lactate, Myo-inositol, N-phenylacetylglycine, O-acetylcarnitine Trigonellin, Tyrosine, and Valine.

**Conclusion:** We have analyzed urine metabolomes by NMR metabolomics from patients at different stages of CKD to find association between the consecration of metabolites and the degeneration of renal function. These metabolomic-based prognostic urine markers may improve CKD management with the potential of predicting the risk of rapid progression to ESRD and can be potential therapeutic targets.

## B-034

### Determination of serum calcium levels by $^{42}$Ca isotope dilution inductively coupled plasma mass spectrometry

B. Han, M. Ge, H. Zhao, Y. Yam, J. Zeng, T. Zhang, W. Zhou, J. Zhang, J. Wang, C. Zhang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:** Serum calcium level is an important clinical index that reflects pathophysiological states. However, detection accuracy in laboratory tests is not ideal; as such, an improved reference method is needed.

**Methods:** We developed a candidate reference method for measuring serum calcium levels by isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), using $^{42}$Ca as the enriched isotope. Serum was digested with 69% ultrapure nitric acid & diluted to a suitable concentration. The $^{44}$Ca/$^{42}$Ca ratio was detected in $H_2$ mode; spike concentration was calibrated by reverse IDMS using Standard Reference Material (SRM) 3109a as a standard; sample concentration was measured by a bracketing procedure on Agilent 700X ICP mass spectrometer. We compared the performance of ID ICP-MS with those of three other reference methods using the same serum & aqueous samples. Among them, a total of 46 different serum samples were analyzed by both the aluminum internal standard ICP-MS & ID ICP-MS method in our laboratory, while 16 samples including nine serum & seven aqueous samples were tested in various laboratories using two-way ID ICP-MS, ion chromatography method along with our ID ICP-MS method.

**Results:** The relative expanded uncertainty of the sample concentration was 0.40% (k = 2). The range of repeatability (within-run precision), intermediate precision (between-run precision) & intra-laboratory precision were 0.12%-0.19%, 0.07%-0.09%, & 0.16%-0.17% respectively, for two serum samples with high or low concentration. SRM909bI, SRM909bII, SRM909c & GBW09152 were found to be within the certified value interval, with mean relative bias values of 0.29%, 0.02%, 0.10%, 0.19% & intra-laboratory CV of 0.18%, 0.18%, 0.11%, 0.16%, respectively. The range of recovery was 99.87%-100.37%. Methods comparison results showed ID ICP-MS method had a good comparability with three other reference methods with correlation coefficient of 0.99983, 0.9932 & 0.9796. Aqueous samples results shows it has a better performance with lower bias & imprecision due to its low interference including isobar, polyatomic ions (oxide, chloride, hydride, & argon compounds) & doubly charged ions.

**Conclusion:** New ID ICP-MS is a simple, precise & accurate candidate reference method for serum calcium measurement.

## B-035

### Effects of in vitro exposure of static magnetic field on human blood electrolyte levels

S. K. Datta[1], P. Parashar[2], K. K. Deepak[1], S. Anand[2], A. K. Mukhopadhyay[1]. *[1]All India Institute of Medical Sciences, New Delhi, India, [2]Indian Institute of Technology, New Delhi, India*

**Background:** Major concerns exist regarding safety of static magnetic fields (SMF) and their effects on physiology. These are sometimes attributed to changes in cell membrane property. The authors hypothesize that any global effect on membrane physiology is likely to impair the electrolyte transport across the membrane. Here, the authors have tried to experimentally evaluate the effect of SMF on membrane physiology in vitro.

**Methods:** In this study, 6 ml of blood was collected in heparinised vials from 30 young (18 to 25 yrs) healthy volunteers after informed consent and institutional ethical clearance. Each sample was divided into 6 equal parts (1ml each) in 3 pairs. One aliquot from each pair was then exposed for 20 minutes to SMF strengths of 500Gauss (G). SMF was generated by a coil electromagnet between 2 soft iron cores and the control samples were shielded by placing them in an iron box. After exposure, all samples were immediately estimated for electrolytes: sodium ($Na^+$), potassium ($K^+$), ionic calcium ($Ca^{++}$) and chloride ($Cl^-$) by Combiline blood gas and electrolyte analyzer (manufactured by Eschweiler, Germany). The process was repeated for the other sample pairs with SMF exposure of 5000G and 1Tesla (1Tesla = 10000G). Paired shielded controls were used each time to eliminate the effect of the time lapse on electrolyte estimation. Results were analyzed using paired 't' test. $p<0.05$ was taken to be significant.

**Results:** The results are depicted in Table 1. Statistically significant changes in the ionic concentrations between cases and controls were not observed for any level of SMF exposure.

**Conclusions:** This indicates that SMF of 500 G, 5000 G and 1 Tesla do not result in any significant change in the membrane permeability of RBCs. This probably excludes the chances of any global effect of SMFs on cell membrane physiology.

Blood electrolyte levels at different Static Magnetic Fields strength

| | 500 Gauss SMF | | 5000 Gauss SMF | | 1 Tesla SMF | |
|---|---|---|---|---|---|---|
| **Electrolytes** | **Controls** Mean ±SD | **Cases** Mean ±SD | **Controls** Mean ±SD | **Cases** Mean ±SD | **Controls** Mean ±SD | **Cases** Mean ±SD |
| Potassium $K^+$ (mmol/L) | 4.49 ± 0.45 | 4.48 ± 0.45 | 4.49 ± 0.39 | 4.51 ± 0.63 | 4.52 ± 0.45 | 4.45 ± 0.43 |
| Sodium $Na^+$ (mmol/L) | 143.15 ± 1.87 | 143.12 ± 1.83 | 142.65 ± 2.74 | 142.86 ± 2.50 | 142.67 ± 2.79 | 143.09 ± 2.41 |
| Ionic Calcium $Ca^{++}$ (mmol/L) | 1.22 ± 0.05 | 1.22 ± 0.05 | 1.23 ± 0.06 | 1.23 ± 0.06 | 1.25 ± 0.07 | 1.25 ± 0.05 |
| Chloride $Cl^-$ (mmol/L) | 110.56 ± 5.00 | 110.20 ± 4.64 | 110.19 ± 4.71 | 110.09 ± 4.66 | 110.46 ± 4.39 | 110.37 ± 4.83 |

## B-036

### Unstable Trends in Metabolites Predict Mortality Within 48 Hours Among Long Term Hospitalized Patients

A. Momeni Boroujeni, A. Zuretti. *SUNY Downstate Medical Center, Brooklyn, NY*

**Background:** Patients who are hospitalized for an extensive period are at risk for adverse outcomes and are likely to have a high mortality rate. These patients often rapidly deteriorate leading to death, with cause of death attributable to different factors like sepsis and end organ damage. To date, there has been limited study of end of life laboratory trends. In this study, we aim to evaluate end of life laboratory values time trends among deceased long term inpatients.

**Methods:** Time stamped laboratory data for deceased adult inpatients who had died in hospital from all-cause mortality between January 2014 and December 2016 was extracted. All adult deceased patients who were hospitalized for at least one week were included in the study. Time stamp data was reformatted to show time to death (in hours) and data was averaged for each reformatted time stamp. The time series of averages as well as lower and upper bounds of confidence interval were evaluated using ARIMA and Mann-Kendall trend test. Recent unstable trend was evaluated by comparing variations of the observed values from the fitted ARIMA model.

**Results:** Laboratory results from 110 patients were evaluated. The average length of hospitalization prior to death was 12.3 days. Significant time trends that were observed included increases in BUN, AST and ALT implying Mann-Kendall trend test showed that there is an upward trend in BUN and liver function tests indicative of end organ damage. Segmentation of the data showed that in the last two days of life the slope of the fitted curve significantly increases and Pettitt's homogeneity test shows a shift in upper bound values in the last 48 hours of life. Sodium, chloride and potassium levels are stationary over time, however, in the last 48 hours of life the metabolites become unstable with values varying from the expected value by more than one standard deviation.

**Conclusion:** Our results suggest that in the last 48 hours of life in chronically morbid hospitalized patients an alteration of the physiologic state of the patient occurs which manifests as subtle changes in metabolite levels especially when compared to the long-term result trends of the patient. Perhaps early detection of these changes can allow for timely interventions for the patients.

## B-037

### Verifying Biological Reference Intervals of venous blood gases of High endurance Athletes - Biological variation on athletes BIOVAth Study

J. Diaz - Garzon, J. Sanguino, P. Fernandez-Calle, P. Oliver, A. Buno. *Hospital Universitario La Paz, Madrid, Spain*

**Background:**A special physical condition can modify the reference intervals (RI) of certain biological magnitudes and could lead to misinterpretation of laboratory results. The aim of this study was to verify the biological reference intervals established in our laboratory for blood gas parameters in a high endurance athlete's population. **Methods:** 29 athletes including runners and triathletes (15 males and 14 females) aged from 18 to 53 were included. All of them were advised to not perform exercise in the previous 12 hours. Venous blood drawn was performed after a fasting overnight, between 8:00-10:00 by the same phlebotomist using syringes safePICO (Radiometer). pH, pO2, pCO2, sodium, potassium, chloride, ionized calcium, bicarbonate, lactate, base excess, anion Gap, glucose, hemoglobin, carboxyhemoglobin and methemoglobin were measured immediately after blood collection in ABL90 Flex (Radiometer). The RIs were taken from healthy Spanish subjects study and verified in our general healthy population. A statistical analysis was performed to verify the RIs in this specific group of subjects following the CLSI EP-28 A3 protocol. **Results** All results fit a normal distribution model and no outliers were found. **Conclusion:** pO2, pCO2, chloride, ionized calcium, bicarbonate, lactate, base excess and anion Gap reference intervals could not be verified in athlete's population. The values outside the IR could be explained by the increase of the metabolic activity related to physical condition. Specific Reference intervals should be estimated in these subjects in order to get a correct interpretation of laboratory testing results.

Results: *(-) Below RI; (+) Above RI

| Analyte | Units | RI | % Outside Limits | p-Value | Verified |
|---|---|---|---|---|---|
| pH | mmHg | 7.33-7.43 | 7 | 0.659 | Yes |
| pO2 | mmHg | 30-50 | 78 (-) | <0.0001 | No |
| pCO2 | mmHg | 38-50 | 64 (+) | <0.0001 | No |
| Sodium | mEq/L | 136-146 | 0 | 0.108 | Yes |
| Potassium | mEq/L | 3.5-5.0 | 4 | 0.905 | Yes |
| Chloride | mEq/L | 98-106 | 42 (+) | <0.0001 | No |
| Ionized Calcium | mg/dL | 1.0-1.2 | 36(+) | <0.0001 | No |
| Bicarbonate | mg/dL | 23-27 | 81 (+) | <0.0001 | No |
| Lactate | mg/dL | 0.5-1.6 | 17 (+) | 0.0011 | No |
| Base Excess Female, Male | - | (-3.4-1.4), (-2.7-2.5) | 67, 79 (+) | <0.0001 | No |
| Anion Gap | - | 8-16 | 28 (-) | <0.0001 | No |
| Glucose | mg/dL | 70-105 | 0 | 0.1075 | Yes |
| Hemoglobine Females, Males | mg/dL | (12.0-16.0),(13.5-17.5) | 0 | 0.429, 0.476 | Yes |
| Methemoglobine | mg/dL | 0.0-1.5 | 0 | 0.102 | Yes |

## B-038

### Abbott Alinity c Sigma Metrics and Precision Profiles for Na, Cl, and K

S. Westgard[1], S. Schneider[2], V. Petrides[2], M. Berman[2], A. Orzechowski[2]. *[1]Westgard QC Inc, Madison, WI, [2]Abbott, Abbott Park, IL*

**Introduction:** Assay performance is dependent on the accuracy and precision of a given method. These attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for 3 assays – Sodium, Chloride and Potassium – tested on the Alinity c-series. Additionally, precision profile charts were created for each assay to compare the precision performance of the assays tested using the Alinity c-series and the ARCHITECT *c* system.

**Methods:** A sigma value was estimated for each assay and was plotted on a method decision chart. The sigma value was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*, where the CLIA TEa value was used as the total allowable error. A precision study was conducted at Abbott on each assay using the Alinity c-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, >100 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity c-series and ARCHITECT *c*8000 systems. The mean concentration of the Alinity c-series results were regressed versus the mean ARCHITECT *c*8000 results and a weighted Deming regression model was fit. Using the regression model, the %bias was estimated at a medical decision point. A precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity c-series and the ARCHITECT *c*8000 system, where the ARCHITECT *c* system within-laboratory %CV and mean concentration values were obtained from the assay package inserts.

**Results:** The method decision chart showed that Sodium had at least 4 sigma performance and Chloride and Potassium had at least 5 sigma performance at or

near the medical decision point. The precision profile charts of the within-laboratory %CV results for the Alinity c-series overlaid with the ARCHITECT *c* system showed similar performance for each assay.

**Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. Chloride and Potassium had sigma values greater than 5, and Sodium had a sigma value greater than 4. The precision performance on the Alinity c-series and ARCHITECT *c* systems was comparable for the three assays. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

## B-039

### Glucose Assay Performance on Abbott's next-generation immunochemistry analyzer (Alinity c)

M. Berman, S. Syed. *Abbott Laboratories, Abbott Park, IL*

**Objective:** To evaluate the analytical performance of the Glucose assay for measurement in human serum/plasma, urine and cerebrospinal fluid (CSF) on Alinity c, clinical chemistry analyzer using photometric technology. The Alinity c instrument is a high throughput instrument for up to 900 tests per hour. The sample is dispensed into a cuvette followed by reagent. The contents are mixed and incubated allowing for the reaction to occur. Absorbance readings of the sample are taken at regular intervals throughout the process at a primary and a secondary wavelength. Data reduction generates a calculated absorbance based on the reaction mode of the assay (end point) and measures the calculated absorbance using a calibration curve to generate a result.

**Methods**: Key performance testing including tube type, precision, linearity, sample dilution and method comparison were assessed per CLSI protocols. The assay measuring interval was defined by the range which acceptable performance for bias, imprecision and linearity was met. Impact of common interferences was assessed at low and high analyte concentrations. Sample dilution studies were performed to assess performance between the Alinity c and ARCHITECT c systems. Reference interval verification was performed using healthy subjects.

**Results:** Total imprecision, linearity, and defined measuring intervals are shown for the Glucose assay in the table below. Results versus an on-market comparator assay demonstrated a slope 0.99 - 1.00 and r = 1.00. The assay was not affected by endogenous or exogenous interferents.

| Assay | Total %CV | Linearity | Measuring Interval |
|---|---|---|---|
| Glucose Serum | ≤ 5 | 0 - 828 mgl/dL | 5 - 800 mgl/dL |
| Glucose Urine | ≤ 6 | 0 - 843 mgl/dL | 1 - 800 mgl/dL |
| Glucose CSF | ≤ 5 | 0 - 843 mgl/dL | 1 - 800 mgl/dL |

**Conclusion:** The Alinity Glucose assay demonstrated excellent precision, linearity, dilution and correlation with an on-market comparator assay. The Glucose assay also showed minimal interference from endogenous serum interferents (hemolysis, lipemia, bilirubin), exogenous urine interferents, and exogenous drug interferents.

## B-040

### Validating Lipaemic Interference on Roche Cobas 501 for common analytes

J. Tan, S. Cosio, A. Omar, M. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

**Background:** Lipaemic interference is the second most common pre-analytical artefact after hemolysis encountered by clinical laboratories. Lipaemia affects the reliability of results through spectral interference, particularly for methods which employ spectrometric absorbances at lower wavelengths [such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin], or methods which create volume displacement (eg indirect potentiometry such as sodium and potassium). Manufacturers have incorporated serum indices in automated biochemistry analyzers to determine lipaemia interference. Individual laboratories should validate these cut-off values and implement a workflow to manage lipaemic specimens.

**Objective:** We aim to validate the manufacturer's threshold for common analytes on the Cobas c501 analyser (Roche Diagnostics, Basel, Switzerland). We also aim to determine the effectiveness of high speed centrifugation for lipaemic specimens.

**Methods:** Fifteen levels of lipaemia were obtained by spiking varying amount of SMOFlipid (Fresenius Kabi, Germany) to pooled patient serum. For each of the 15 levels and baseline pooled serum, sodium, potassium, alanine aminotransferase, aspartate aminotransferase, and albumin were analysed on Cobas c501 analyser (Roche Diagnostics) in triplicates. Lipaemic index (L-index), a measure of lipid turbidity, was obtained via spectrometry on the c501 analyser. Means and coefficients of variation were determined for each level. Controls for each level of lipaemia were produced by spiking deionized water and were analyzed in triplicates. To assess the effectiveness of high speed centrifugation in removing lipaemia, 5 levels of lipaemic samples underwent high speed centrifugation at 21000G for 60 minutes. With the lipid faction removed, the aqueous phase was analysed in duplicates and the mean obtained. The results were compared to the baseline pooled serum sample.
**Results:** Our results were largely in agreement with the manufacturer's inserts. At up to an L-index of 1079, sodium showed good recovery of 97.2% (difference of 4 mmol/L from baseline, 139 mmol/L vs baseline 143 mmol/L) and potassium had good recovery of 97.4% (difference of 0.1 mmol/L from baseline, 3.8 mmol/L vs baseline 3.9 mmol/L). AST and ALT had recovery of 87.2% and 81.6% respectively at an L-index of 158, consistent with the manufacturer's claim of interference threshold at L-index of 150. Albumin had a recovery ratio of 95.1% at an L-index of 626, consistent with manufacturer's claim of L-index threshold at 550. Upon high-speed centrifugation, L-index dropped from 426.5, 823.5, 1212.5, 1551.5, 1952 to 19.5, 24, 29.5, 35.5 and 41.5 respectively. The 5 levels of analytes that underwent high speed centrifugation showed good recovery (97%-107%) compared to baseline pooled serum values. Prior to centrifugation, recovery were poor from 17% to 111%, with worse results for AST and ALT. **Conclusion:** Our study shows that lipaemic thresholds for the common biochemical analytes of sodium, potassium, aspartate aminotransferase, alanine aminotransferase and albumin on the Roche c501 analyser follow manufacturer's claims. For laboratories that do not have an ultracentrifuge in view of space and financial constraint, high speed centrifuge at 60 min 21 000G facilitates the removal of lipids.

## B-041

### Dilute and shoot FI-MS-MS method for quantifying Glucocholic acid in bile using standard addition

R. Kakarla[1], R. Voggu[1], J. Donaldson[2], B. Guo[1]. *[1]Cleveland State University, Cleveland, OH, [2]Mississippi State University, Starkville, MS*

**Background**

Bile acids not only aid in digestion of dietary fat and cholesterol homeostasis but also play crucial role in pathological conditions of liver, gall bladder and small intestine including cancer. Hence quantitation of bile acids in various fluid compartments might be useful in detecting cancer at early stages. Though most methods reported quantify bile acids in serum, plasma and feces, very few quantify bile acids in bile. In our current work, we chose Glycocholic acid (GCA) as a model bile acid molecule to develop a simple and fast flow-injection MS/MS quantification method. In order to ensure minimize matrix effect, the sample has been diluted and quantified using a standard addition-internal standard technique instead of using a traditional internal standard-calibration curve.

**Methods**

Briefly each bile sample was first diluted by 4000 times with methanol and standard solutions are added into the aliquots of diluted bile along with internal standard. A 10 µL of resulted sample was directly flow-injected without any chromatographic separation into the ESI source of a triple-quadrupole mass spectrometer using 90% methanol at 0.3 mL/min for 2.5 minutes. Detection was carried out in negative MRM mode with the transitions, 464.1 ® 74 and 401.2 ® 249.1 for GCA and internal standard respectively. Linearity was achieved in the range of 12.5 to 200 ng/mL. The method was also validated for LLOQ, matrix effect, accuracy, inter and intra-day precision.

**Results**

*Linearity, calibration and LLOQ:* The standard addition curve constructed from the calibrators demonstrated a correlation coefficient of 0.99. The method was found to be linear in the range of 12.5-200 ng/mL and the %CV for the LLOQ was less than 15%.

*Accuracy and precision:* Accuracy, Intra and inter-day precision were determined using three QCs. The % relative error (% RE) of intra and inter assays was 7.38-14.88 and -12.03-14.80 respectively. The % relative standard deviation (% RSD) of intra and inter-assays ranged from 4.00-11.60.

*Matrix effect:* The matrix effects determined by comparing the peak area ratios of GCA and IS in diluted bile and solvent. The mean matrix effect ranged from 87.35 to 94.78 and % RSD was 4.05-9.97which were within the limits.

**Conclusion**

In conclusion, a greatly simplified MS/MS method has been developed and validated for the quantification of a model bile acid, GCA in bile. With the advantages of high

throughput, and low cost, the method is especially beneficial in a clinical laboratory setting. To the best of our knowledge, this is the first flow injection method that takes advantage of standard addition-internal standard strategy. The method has been validated according to FDA guidelines. This method can also be modified to facilitate individual or simultaneous quantification of other bile acids in bile.

## B-042

### Accuracy of glucose measurements by two popular blood gas analyzers as assessed by error grid, ISO and FDA criteria

J. Jung[1], G. S. Cembrowski[2], E. Xu[3], T. Curic[4], H. Sadrzadeh[4]. *[1]University of Alberta, Edmonton, AB, Canada, [2]Alberta Health Services, Edmonton, AB, Canada, [3]University of Manitoba, Winnipeg, MB, Canada, [4]Calgary Laboratory Services, Calgary, AB, Canada*

**Objective:** We used retrospective comparison of central laboratory (CL) glucose results to evaluate the glucose accuracy of two point of care blood gas systems, the GEM 4000 and the Radiometer ABL 800, housed in, or just outside intensive care units (ICU) in two university hospitals.

**Materials and Methods:** A laboratory data repository provided arterial glucoses generated by two GEM 4000s on ICU patients in 2012-2013 at Calgary's Foothills University Hospital and glucoses produced by the central laboratory (CL) Roche Cobas 8000-C702 modules. Another repository provided arterial glucoses produced by two Radiometer ABL 800 systems in 2012-2013 on University of Alberta Hospital ICU patients as well as glucose results produced by the CL Beckman DxC. Point of care and CL glucoses were compared if the interval between their analyses was under 30 minutes. The agreements between the CL and the point of care glucoses were assessed with the 2000 Parkes Error Grid, the 2014 Klonoff Surveillance Error Grid, the 2013 ISO 15197 limits and the 2014 FDA draft guidance limits.

**Results:** The Table summarizes our findings. There are many more GEM than Radiometer CL comparisons. The Radiometer and GEM usual and hypoglycemic mean glucoses are roughly equivalent. The Radiometer demonstrates a positive bias compared to the CL glucose. There is little difference in the accuracy assessments with the Parkes error grid and surveillance error grid showing very limited outlier distributions and both demonstrating acceptable ISO 15197 agreement, but demonstrating unacceptable FDA performance.

**Conclusions:** The use of this retrospective approach probably cannot adequately assess the FDA's 10% or 7 mg/dL accuracy requirements as glycolysis in the CL specimen will invalidate the assessment. Delayed analysis and resultant glycolysis of the CL glucoses at the Radiometer site may explain the positive Radiometer glucose bias.

| | Radiometer | GEM |
|---|---|---|
| Total | 2786 | 15217 |
| Bias (mean) mg/dL | 5.1 (145) | -0.27 (153) |
| hypoglycemic (N) | 43 | 396 |
| hypoglycemic bias | -4.1 (65) | -9 (64) |
| Parke's Error Grid Analysis % Risk Zones E, D, C, B, A | 0; 0; 0; 2; 98 | 0; 0; 0; 0.5; 99.5 |
| Surveillance Error Grid Analysis % Risk Zones H, G, F, E, | 0; 0; 0; 0; 0.1; 0.5; 1.77; 97.76 | 0; 0; 0; 0.1; 0.1; 0.3; 1.3; 98 |
| ISO 2013 | | |
| >100 mg/dL (within +/- 15%) | 2511 (98.1%) | 12347 (96.22%) |
| <100 mg/dL (within +/- 15mg/dL) | 275 (95.3%) | 2870 (95.1%) |
| FDA | | |
| >73 mg/dL (within +/- 10%) | 2743 (93.7%)* | 14821 (91.2%)* |
| <73 mg/dL (within +/- 7 mg/dL) | 43 (69.8%)* *<95 | 396 (70.2%)* *<95 |

## B-043

### Analysis of clinical phenotype of of compound heterozygotes of Hb NewYork and β-thalassemia

M. Suo[1], D. WEN[1], X. Liu[2], Z. Dong[1]. *[1]Division of Clinical Laboratory, Zhongshan Hospital of Sun Yat-sen University, zhongshan, China, [2]Division of Clinical Laboratory, Zhongshan Hospital of Sun Yat-sen University, Zhongshan, China*

**Background:** Hb New York has been noted to be a relatively common variant in Southern China. A few cases of double heterozygosity of β-thalassemia and Hb New York had been mentioned, while the spectrum of its clinical manifestations was reported consistent withβ-thalassemia trait. But cases in this study showed different conditions.

**Methods:** Peripheral blood samples from 2 patients with double heterozygosity of Hb NewYork and β-thalassemia and 10 Hb New York heterozygous cases were collected. The investigation of deafness heredity was made in a family by the consent of the family members. RBC analysis, serum bilirubin detection and hemoglobin electrophoresis were performed.Genotypes of α and β-globin were also analyzed.

**Results:** The father of the proband 1 and the 10 sporadic cases were Hb NewYork carriers.All of them were asymptomatic and had normal hematological parameters except for an abnormal hemoglobin band detected on hemoglobin electrophoresis. The mother and brother of the proband 1 were β-thalassemia mutation CD41/42 alone, the hemoglobins of them were 109g/L and 118g/L, respectively. Mean corpuscular volume of red cell was smaller than that of normal sample and the results of serum bilirubin detection were in the normal reference ranges. The proband 1 who maintained a stable hemoglobin level at 8 g/L was a compound heterozygote for Hb NewYork and β-thalassemia mutation CD41/42, characterized by low pigment-small-cell anemia. Proband 2 was a compound heterozygote for Hb NewYork and β-thalassemia mutation IVSII-654, whose hemoglobin was 143 g/L and mean corpuscular volume of red cells was smaller . Both of heterozygotes had different levels of hemolysis, and the results of blood bilirubin detection were 37.8μmol/L and 81.2μmol/L, respectively, which were out of normal reference ranges. Results of hemoglobin electrophoresis showed that Hb A2, Hb F increased and Hb A were not detected.Abnormal hemoglobin bands Hb NewYork were also detected.

**Conclusion**: Carriers of Hb NewYork alone are asymptomatic. but double heterozygosity of Hb New York and β0 codons 41/42 has clinical symptoms such as anemia and hemolysis, which is more serious than that of CD41/42 alone. Double heterozygosity of Hb New York and β0 IVSII-654 also has symptom of hemolysis.Therefore,couples with one carrying Hb NewYork and another carrying a β-thatassemia mutation need to be notified that it would be a problem.

## B-044

### Evaluation of Nova Biomedical Clot Catchers for Analysis of Blood Gas Samples on the Radiometer ABL90

R. J. Scott, A. M. Wockenfus, J. M. Barney, B. S. Karon, L. J. Donato. *Mayo Clinic, Rochester, MN*

**Background:** The Radiometer ABL90 (Radiometer Medical ApS, Brønshøj, Denmark) blood gas analyzer can be used to analyze neonatal and other low volume blood gas samples (0.5mL in 3.0mL blood gas syringe (Smiths Medical, Keene, NH)). Shortly after implementation, we began to experience a high number of calibration error flags, consumable replacements, and extended downtime. Representatives from Radiometer suggested that silicone, micro-clots or other substances in low volume blood gas specimens could cause damage to internal components and recommended use of a clot catcher before sample analysis to reduce instrument errors and downtime.

**Methods:** The number of calibration and analyzer errors, consumable replacements, and analyzer downtime were recorded for 3 months using our standard low volume blood gas collection protocol. For three months we trialed the use of Nova Biomedical Stat Profile Critical Care Express syringe clot catchers (Nova Biomedical, Waltham MA) for all heparinized whole blood specimens analyzed on the ABL90. To ensure a clean and optimally-operating analyzer before beginning the clot catcher trial, preventative maintenance was performed, new tubing was installed, and fresh consumables were loaded. We then compared the number of calibration and analyzer errors, consumable replacements, and analyzer downtime in the three months after implementing clot catchers to the three months prior to the use of clot catchers.

**Results:** Calibration errors decreased 23% (n= 26 pre-clot catcher versus n= 20 post-clot catcher). Sensor cassette maintenance interruptions, indicating that the analyzer cannot perform calibration, were eliminated with the use of clot catchers (n= 21 pre-clot catcher versus n= 0 post-clot catcher). The number of sensor cassettes and solution packs required during the pre- and post-clot catcher periods decreased 50% and 35%, respectively (16 cassettes and 17 packs pre-clot catcher versus 8 cassettes and 11 packs post-clot catcher). Lastly, analyzer downtime was significantly reduced by 92% (416 hours pre-clot catcher versus 32 hours post-clot catcher).

**Conclusion:** The use of clot catchers for blood gas analysis on the Radiometer ABL90 greatly reduced the number of error codes, consumable replacements, and downtime. Future studies are planned using clot catchers for additional sample types prone to micro-clot and sample debris issues such as cord blood and pleural fluid.

## B-045

### Validation of spinal fluid lactate measurements and effect of HIL interferences using the Abbott ARCHITECT plasma lactic acid assay

M. L. Parker[1], P. Martens[2], P. M. Yip[1]. *[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Department of Clinical Biochemistry, University Health Network, Toronto, ON, Canada*

**Background:** Differentiation between viral and bacterial causes of meningitis is critical because aseptic meningitis is usually less severe and resolves on its own, while bacterial meningitis can be life threatening and requires immediate medical attention. Lactate measurements on spinal fluid (SF) specimens can be a key marker with elevations above 3.0 mmol/L being suggestive of bacterial meningitis. However, the Abbott ARCHITECT® assay is cleared for use with plasma but not SF specimens. **Objectives:** To validate the analytical performance of the ARCHITECT Lactic Acid assay (#9P18) on SF specimens, and to expand the limited information on the effects of common interferences due to hemolysis, icterus, and lipemia (HIL) on the performance of this assay in plasma specimens. **Methods:** A new ARCHITECT lactic acid reagent was released in June 2016 to replace the previous assay (#9D89). The old and new reagents were compared using 60 SF specimens. Imprecision of the new assay was determined using two levels of Bio-Rad Liquichek™ Spinal Fluid Quality Control measured in duplicate, two times per day for 11 days (n = 44). Linearity was assessed by mixing high and low patient SF specimens. Accuracy and dilution recovery were evaluated by spiking with sodium lactate. The effects of HIL interference on lactate quantitation were studied on plasma specimens at 1.0 or 4.0 mmol/L lactate by spiking with red cell lysate, unconjugated or conjugated bilirubin, or Intralipid® (triglycerides). Data were processed using EP Evaluator® Release 7. **Results:** The two assays correlated well over a range of lactate concentrations from 0.82 to 5.94 mmol/L, with a slight positive bias for the new reagent (slope = 1.03; intercept = -0.03; $R^2$ = 0.997; mean bias = +0.02 mmol/L). Total imprecision at 2.03 and 3.99 mmol/L lactate was 1.0% and 0.9% CV, respectively. Linearity was confirmed from 0.7 to 8.8 mmol/L lactate, and dilution recovery was 93 to 110%. Accuracy ranged from 95 to 99.8% recovery at 1.0, 3.0, and 7.0 mmol/L lactate. However, automatic on-board dilution above the analytical measurement range (13.3 mmol/L) resulted in 90% recovery at 16.0 mmol/L. Significant positive interference (>10% error) from hemoglobin occurred at 1.2 and 4.6 g/L for the 1.0 mmol/L and 4.0 mmol/L lactate specimens, respectively. At the corresponding lactate levels, conjugated bilirubin showed significant negative interference at 69 and 232 µmol/L, while unconjugated bilirubin did not reveal a significant negative bias until concentrations were 177 and 360 µmol/L, respectively. Triglycerides up to 47 mmol/L did not significantly interfere at 4.0 mmol/L lactate, but showed a positive bias above 44 mmol/L at 1.0 mmol/L lactate. **Conclusion:** The new Abbott ARCHITECT Lactic Acid assay correlates well to the predecessor using SF specimens. The assay meets performance goals for precision, linearity, and dilution recovery in SF specimens, but is susceptible to moderate levels of hemolysis and icterus in plasma specimens. Overall, these data support the ARCHITECT Lactic Acid assay for the testing of lactate in spinal fluid for clinical purposes.

## B-046

### Comparison of Relative Frequency, Magnitude and Etiology of Zero/Negative Anion Gaps of two Popular Blood Gas Analyzers

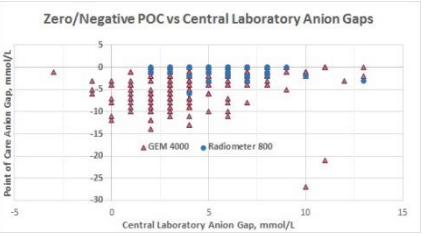
E. Xu[1], H. Sadrzadeh[2], T. Curic[2], G. S. Cembrowski[3]. *[1]University of Manitoba, Winnipeg, MB, Canada, [2]Calgary Laboratory Services, Calgary, AB, Canada, [3]Alberta Health Services, Edmonton, AB, Canada*

**Background:** Negative anion gaps occur infrequently and are often attributed to laboratory error. Probably the most common cause of negative anion gaps is random error affecting the measurement of sodium, chloride, and/or bicarbonate generating pseudohyponatremia, pseudohyperchloremia, pseudohyperbicarbonatemia, or a combination of these measurement errors. As zero/negative anion gaps can indicate significant altered pathophysiology, it is important that they be investigated. We used data mining to correlate zero/negative anion gaps discovered by point of care blood gas analysis to concurrent electrolyte measurements performed in the central laboratory.

**Methods:** A laboratory data repository provided arterial blood gas, electrolyte and metabolite results generated by two GEM 4000s on ICU patients in 2012-2013 at Calgary's Foothills University Hospital as well as electrolyte results produced by the Roche Cobas 8000-C702 modules. Another repository provided similar results generated by two Radiometer ABL 800 systems on ICU patients in 2012-2013 at University of Alberta Hospital as well as electrolyte results produced by the Beckman DxC. Point of care and central laboratory electrolytes were correlated If the interval between their testing was under 30 minutes.

**Results:** The Figure shows the distribution of the zero/negative anion gaps compared to the central laboratory. Of 7586 GEM gaps, 348 were zero/negative with the average Na, Cl, HCO3 deviating by -1.5, +2.5 and +3.9 mmol/L, respectively. Of the 2985 Radiometer gaps, 73 were low with the Na, Cl, HCO3 deviating by -1.6, +3.5, and +1.2 mmol/L, respectively. The relative incidence of zero/negative gaps is higher for the GEM compared to the Radiometer ($p<0.001$)

**Conclusion:** The GEM 4000 tends to produce larger and more artefactual zero and negative gaps than the Radiometer with these gaps being associated with positive shifts in HCO3.




## B-047

### Increased disialotransferrin evident with chronic alcohol consumption

D. Truong[1], B. Wan[2], M. Knauer[2], D. Bailey[3], L. MacNeil[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada, [3]Dynacare, London, ON, Canada*

**Background:** Single biomarkers for alcohol abuse screening provide insufficient sensitivity and specificity. Multiple analytes including carbohydrate deficient transferrin (CDT), GGT, and AST: ALT ratio are needed to avoid misinterpretation. CDT is an abnormal transferrin glycoform known to arise following heavy alcohol consumption (> 60 g of ethanol per day for at least two weeks). Tetrasialotransferrin is the predominant fraction in healthy people, and the increased presence of tri-, di-, mono- or asialotransferrin alludes to abnormal glycan metabolism. Typically, di- and asialotransferrin are influenced by heavy alcohol consumption, whereas tri- and monosialotransferrin appear to be unaffected by heavy alcohol use. %CDT is the percentage of altered transferrin relative to total transferrin and can be measured by immunoassays, although the specific isoform being measured remains unclear. High performance liquid chromatography (HPLC) is a more specific method and allows for quantification of each transferrin glycoform. The purpose of this study was to compare CDT immunoassay and HPLC measurements to determine which transferrin glycoform has the greatest influence on the CDT immunoassay results. Furthermore, HPLC analysis was performed on patient samples with measured ethanol concentrations to determine the effect of acute elevations in ethanol on transferrin glycoform profiles.

**Design and Methods:** 21 apparently healthy patient samples submitted for alcohol abuse screening were analyzed for CDT and transferrin by particle-enhanced immunonephelometric and immunonephelometric assay, respectively, on a Siemens BN ProSpec (Siemens Healthcare Limited). %CDT was calculated as [CDT (mg/L)/ Transferrin (g/L)]/10. GGT, ALT, AST were analyzed on the Roche Modular (Roche Diagnostics). Transferrin glycoform profiles were analyzed by HPLC on an Agilent 1200 using an anion exchange column and UV-Vis detector (GE Healthcare). Prior to HPLC analysis, patient samples were iron-saturated with ferric nitrilotriacetic acid (FeNTA) and lipoproteins precipitated. Additionally, 12 patient samples with elevated ethanol concentrations were analyzed by HPLC to determine whether acute elevations in ethanol concentrations affected transferrin glycoform profiles.

**Results:** %disialotransferrin fraction on HPLC analysis correlated highly with %CDT ($R^2$ = 0.9829), whereas %trisialotransferrin fraction demonstrated poor correlation with %CDT ($R^2$ = 0.1064). Only 2 and 4 of 21 patient samples had detectable mono- or asialotransferrin, respectively. Overall, this suggests that the CDT immunoassay detects disialotransferrin. A high concordance of abnormal results was observed between %disialotransferrin fraction on HPLC and %CDT. Of 12 patients with %CDT above the normal reference range (%CDT ≤ 2.5%), 11 also had %disialotransferrin above the normal reference range (%disialotransferrin < 1.7%). No significant correlation was evident between %disialotransferrin fraction and GGT or AST:ALT ratio ($R^2 \leq 0.001$ and $R^2 \leq 0.002$, respectively). HPLC analysis of patient samples with elevated ethanol concentrations demonstrated poor correlation between %disialotransferrin and ethanol concentration ($R^2$ = 0.08), suggesting that acute heavy alcohol consumption does not induce rapid changes to transferrin glycoform profiles.

**Conclusion:** The CDT immunoassay appears to detect disialotransferrin, and a significant increase in %disialotransferrin on HPLC is observed in patients suspected of chronic alcohol abuse. The lack of correlation between ethanol concentration and %disialotransferrin suggests that changes in transferrin glycoform profile are observed in chronic and not acute alcohol abusers.

## B-048

### Evaluation of Candidate Serum/Artificial Serum Low Level Creatinine Reference Materials by Routine Clinical Assays in an Interlaboratory Study

J. E. Camara. *NIST, Gaithersburg, MD*

**Background:** The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials® (SRMs) intended for use as accuracy controls in the analysis of clinical samples. While these materials are typically composed of pooled donor samples, NIST has begun to investigate the use of artificial matrices as bases or diluents for the preparation of a new generation of reference materials. NIST currently sells SRM 967a Creatinine in Frozen Human Serum, which provides two levels of creatinine in serum at adult normal and high levels to support clinical measurements for assessment of kidney disease. Pediatric ranges for serum creatinine are significantly lower than the adult normal range. Therefore, the current SRM 967a does not support the accurate measurement of creatinine in the range necessary for measuring serum creatinine and screening for kidney disease in the pediatric population, which is a concern that has been voiced in recent years by the National Kidney Disease Education Program (NKDEP) of the National Institute of Diabetes and Digestive and Kidney Diseases-National Institutes of Health. As it is not feasible to obtain large volumes of pediatric serum, NIST has begun to investigate the use of artificial serum matrices for a next-generation material with a target value of 4 µg/g (0.4 mg/dL) creatinine.**Methods:** NIST obtained SeraFlx BIOMATRIX and SeraFlx LCMSMS artificial serum from Cerilliant. In addition, a pre-market SigMatrix Ultra Serum Diluent was provided by MilliporeSigma. Based on previous studies at NIST, all these artificial serum matrices performed satisfactorily when analyzed by isotope-dilution liquid chromatography mass spectrometry (ID-LC-MS). To determine if these materials would be fit-for-purpose in routine clinical creatinine assays, NIST organized a interlaboratory study with manufacturers and clinical laboratories. NIST prepared six candidate materials with combinations of the artificial and normal human serum or pure creatinine. Creatinine values were determined by a NIST ID-LC-MS Reference Measurement Procedure (RMP) and ranged from 2.1 µg/g (0.22 mg/dL) to 5.1 µg/g (0.52 mg/dL). Candidate materials and SRM 967a were shipped to ten laboratories and analyzed in triplicate for creatinine using a total of eleven Jaffe-based and twelve enzymatic-based assays.**Results:**For SRM 967a Level 2, all assays reported the certified value [(3.877 ± 0.082) mg/dL]. However, several Jaffe and enzymatic values were outside the certified range [(0.847 ± 0.018) mg/dL] for SRM 967a Level 1. Candidate mixtures containing either SeraFlx BIOMATRIX or SeraFlx LCMSMS resulted in errors for several Jaffe and enzymatic assays, possibly due to turbidity caused by undissolved components, and displayed a wide range of % bias to the NIST RMP value (-163 %, +78 %). The narrowest range of % bias was observed in the material containing normal serum diluted with SigMatrix Ultra (-13 %, +32 %). **Conclusion:** Additional feedback from stakeholders regarding the results of the study will be required to determine if any of these materials are fit-for-purpose with routine methods or if specific materials are required for Jaffe versus enzymatic methods to improve measurements of creatinine in low level serum samples.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Infectious Disease

## B-049

### biochemical Effects of Ethanolic Roots Extracts of *Uvaria charmae* on liver of Albino wistar rats infected with *Staphylococus aureus*

I. I. J. Joseph, F. v. victor. *university of calabar, calabar, Nigeria*

**Background:** Man has utilized different medicinal plants, one of which is Uvaria charmae in the treatment of *Staphylococcus aureus* Infection.This work was carried out to determine the biochemical effects on liver enzymes aspartate aminotransferase ALT, alanine aminotransferase AST, alkaline phosphatase ALP of Swiss albino Wister rats infected with *Staphylococcus aureus* and treated with *Uvaria charmae*

**Methods:** Twenty five (25) adult Swiss albino Wister rats weighing 170g-220g were used. The control group (group 1) was administered feed and water, Group 2 was infected with *Staphylococcus aureus* only, treatment group (group 3) was infected with *Staphylococcus aureus* and treated with *Uvaria charmae* extract for 10 days, comparison group (group 4) was infected with S. aureus and treated with Vancomycin for 10days, Group 5 was administered with uvaria charmae extract only for 10 days

**Results:** In analysis, AST levels were elevated in group3 (170.25±12.764), group 4 (120.00±7.071), group5 (179.00±3.742) as compared with the control group (46.00±5.657). This was statistically significant (P=0.001). Alanine aminotransferase (ALT) levels were elevated in group3 (174.75±3.745), group4 (104.50±3.775), group5 (111.25±5.377) as compared with the control group (36.50±2.121) and was statistically significant (P = 0.001). Alkaline phosphatase (ALP) was elevated in group3 (303.75±5.315), group4 (263.50±39.854), group5 (325.25±8.139) as compared to the control group (155.50±9.192). This was statistically significant (P =0.001). The Tukey post hoc test shows in AST, there was statistical difference between group2 and control (P =0.001), group3 (P =0.0001) and group4 (P = 0.015). For ALT levels, there was statistical significant difference between group2 and control (P =0.000), group3 (P =0.000), group4 (P =0.006) and group5 (P = 0.001). For ALP levels, there was statistically significant difference between group2 and group5 (P =0.007), group5 and group4 (P =0.009)

**Conclusion:** Hyper production of this enzymes could constitute a threat to the life of cells that are dependent on a variety of the phosphate esters for vital life processes (Butterworth & Moss, 2002) . This pattern of increase of ALT, AST and ALP observed in this study are biochemical indicators of liver cytolysis suggesting that the extracts may have adverse effect on the liver especially on continuous usage. AST and ALT occupy a central position in amino acid metabolism of the tissues and thus alteration in their activity by excessive hyper production affect normal growth of the tissues (Pantosi et al., 1997).Thus suggesting that *Uvaria charmae* may be unsafe even though utilized in commercially sold herbal drug like Ruzu bitters.

## B-050

### Detection of biofilm in *Staphylococcus aureus* isolated from wound infection and its association with antimicrobial resistance

P. Neopane[1], H. P. Nepal[2], R. Gautam[3], R. Shrestha[4], B. R. Adhikari[1], Y. Abiko[1]. *[1]Health Sciences University of Hokkaido, Hokkaido, Japan, [2]Trinity School of Medicine, Kingstown, Saint Vincent and the Grenadines, [3]Chitwan Medical college and Teaching Hospital, Bharatpur, Nepal, [4]Hokkaido University, Hokkaido, Japan*

**Background:** *Staphylococcus aureus* including methicillin-resistant *staphylococcus aureus* (MRSA) has the propensity to form biofilms, and cause significant mortality and morbidity in the patients with wounds. The aim of this study was to determine the prevalence of biofilm formation by *S. aureus* isolated from wound infection, and to evaluate its antimicrobial resistance pattern. **Methods:** A total of 43 clinical isolates of *S. aureus* were isolated from 100 pus samples using standard microbiological techniques. Biofilm formation in these isolates was detected by tissue culture plate method and tube adherence method. Antimicrobial susceptibility test was performed using the modified Kirby-Bauer disk diffusion method as per CLSI guidelines. MRSA was detected using the Cefoxitin disk test.

**Results:** Biofilm formation was observed in 30 (69.7%) and 28 (65.1%) isolates of *S. aureus* via TCP and TM methods, respectively. Biofilm producing *S. aureus* exhibited a higher incidence of antimicrobial resistance when compared with the biofilm non-producers ($p < 0.05$). Importantly, 86.7% of biofilm producing *S. aureus* were multidrug resistance (MDR), whereas all the biofilm non-producers were non-MDR ($p < 0.05$). Large proportions (43.3%) of biofilm producers were identified as MRSA; however, none of the biofilm non- producers were found to be MRSA ($p < 0.05$). **Conclusion:** This is the first study to determine the prevalence of biofilm forming *S. aureus,* including MRSA, isolated from wound infections in Nepal. The occurrence of antimicrobial resistance was higher in the biofilm producers than in the non-producers. Therefore, we recommend screening for the detection of biofilm formation and monitoring the antimicrobial resistance profiles of *S. aureus,* which may help in formulating effective antimicrobial strategies when dealing with wound infections.

## B-051

### Frequency of false positive IgM anti-hepatitis B core antibody results

M. Smith[1], R. Schifman[2], D. Luevano[3]. *[1]University of Arizona, Tucson, AZ, [2]Southern AZ VA Healthcare System, Tucson, AZ, [3]Southern Arizona VA Healthcare System, Tucson, AZ*

**Background:** IgM antibody to hepatitis B virus core antigen (IgM anti-HBc) is present in patients with acute hepatitis B virus (HBV) infection or with reactivation of disease in chronic carriers. Total anti-HBc (both IgM and IgG) antibodies are detected in patients with either chronic HBV infection, past infection with immunity, or acute infection. Furthermore, false positives are well documented and especially common in populations with low prevalence of HBV infection. However, the frequency of false positive IgM anti-HBc results is unknown. The objective of this study was to evaluate the rate of false positive IgM anti-HBc results in a large veteran population.

**Methods:** A retrospective observational study covering a 5 year period through October 2016 was conducted with information extracted from the Veterans Affairs corporate data warehouse. Data collected included all anti-HBc IgM results in which total anti-HBc was also tested within the previous 7 days. Positive IgM anti-HBc results were classified as true positives if total anti-HBc results were positive or as false positives if total anti-HBc results were negative.

**Results:** Results from 52,426 paired IgM and total anti-HBc tests among 44,377 patients from 110 facilities were analyzed. Within this group, the median number of paired tests per facility was 154.5 with 10$^{th}$ to 90$^{th}$ percentile range of 22.5 to 1105.5. Total anti-HBc was positive in 28,810 (55%). The median rate of positive IgM anti-HBc tests per facility was 1.2% with 10$^{th}$ to 90$^{th}$ percentile range of 0.0% to 4.1%. Overall, a total of 662 (1.3%) IgM anti-HBc results were positive among 551 patients of which 59 (8.2%) were classified as false positives (negative total anti-HBc) among 50 patients. False positive IgM anti-HBc results persisted from 3 to 330 days in 7 patients retested 2 to 3 times.

**Conclusion:** This observational study provides evidence that positive IgM anti-HBc measurements are sometimes erroneous. While it is possible that some discrepant results could have been due to false negative total anti-HBc measurements, this has been rarely reported. In contrast, false positive total anti-HBc results are well documented. Therefore, it is likely that this would also occur with IgM anti-HBc measurements. Other causes contributing to discordant results such as mislabeling or clerical errors could not be evaluated, but would only marginally impact false positive rates. False positive IgM anti-HBc results might also be due to greater method sensitivity than total anti-HBc measurements. However this explanation is also unlikely due the persistence of false positive IgM anti-HBc results observed upon retesting days to months later. In conclusion, these results suggest that positive IgM anti-HBc results should be cautiously interpreted unless reflex testing with total anti-HBc is performed for confirmation. Alternatively, when acute HBV infection is suspected, total anti-HBc could be tested first and if positive followed by IgM anti-HBc measurement. This latter testing algorithm might be preferred since a negative total anti-HBc result would help to simultaneously exclude the diagnosis of acute as well as past or chronic HBV infection.

## B-052

### Comparison of Two Assays for Measurement of Quantitative Hepatitis B Surface Antigen in Patients with Chronic Hepatitis B

T. Adilistya[1], N. Indrasari[2]. *[1]Dr. Kanujoso Djatiwibowo Hospital, Balikpapan, Indonesia, [2]Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia*

**Background:** Serum hepatitis B surface antigen (HBsAg) levels correlate with hepatitis B virus intrahepatic covalently closed circular (ccc) DNA and may be useful to predict treatment response. Currently two commercial platforms are available for HBsAg quantification in clinical practice: the Roche HBsAg QT and Architect HBsAg Quant. This study aimed to compare the results of both assays.

**Methods:** HBsAg levels were measured in 50 serum samples from chronic hepatitis B patients and subsequently analyzed for quantitative HBsAg levels using both assays.

**Results:** Correlation between results obtained from the Roche and Architect platforms was high ($r=0.989$, $p<0.05$). By Bland-Altman analysis, agreement between the two assays was close (mean difference between Roche and Architect: 0.001 log IU/mL; limit of agreement: -0.20 log IU/mL [95% CI: -0.25 - -0.15) to 0.21 log IU/mL (95% CI: 0.15-0.26). Two (4%) samples were not consistent (difference: 0.42 and 0.30 log IU/mL). We hypothesize that this discrepancy is caused by HBsAg mutants.

**Conclusion:** There is a high correlation and close agreement between quantitative HBsAg measurement using the Roche and Architect platforms. Clinical prediction rules derived from data from one platform can be applied to another. Both assays can be used interchangeably in clinical practice.

## B-053

### Transcriptional profile of macrophage infected with different violent Mycobacterium tuberculosis strains by RNA-seq

H. Xu, F. Li, J. Luo, M. Chen. *Third military medical university, Chongqing, China*

**Background:** Tuberculosis caused by *Mycobacterium tuberculosis* (MTB) remains a significant public health problem, which leads to 2 billion individuals and approximately 10 million new infections every year throughout the world. With the continually emerged multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, a comprehensive understanding of anti-TB immunity in the host is urgently needed. Transcriptomic analysis has potential to greatly increase our understanding of MTB infection, which reveals gene expression and the complex network of gene regulations at transcriptional level. However, the transcriptomic change of macrophage infected with different virulent MTB strains still poorly understood. Here, we studied the transcriptional profile of macrophage infected with H37Rv and H37Ra using RNA-Sequencing(RNA-seq).

**Methods:** THP-1 cells were primed with PMA for 24 hours. Three groups were set, H37Rv infection group, H37Ra infection group and control group. Each group was treated for 1 hour, 4 hours, 12 hours, 24 hours and 48 hours. Then cells were harvested for RNA isolation. The sequencing library were prepared and sequenced in half lane in flowcell of Illumina Hiseq 3000 high throughput sequencer. Differentially expressed genes were identified using the edgeR program.

**Results:** Top four pathways involved in differentially expressed genes between H37Rv and H37Ra groups for 4h infection were: lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism and cell death and survival signaling. Top four pathways between H37Rv and H37Ra groups for 24h infection were: cell cycle, DNA replication, recombination, and repair, cell death and survival signaling. Only 1 pathway were common between 4h and 24h MTB infection.

**Conclusion:**Our study revealed the transcriptomic change of macrophage infected with different virulent MTB strains, which could help to gain a better understanding of the regulation system in MTB infection immunity.

## B-054

### Detection Of ESBL, MBL, & Ampc Producing Bacterial Isolates Causing Nosocomial Urinary Tract Infections With Special Reference To Biofilm Formation

R. Chalese[1], S. K. Mishra[1], B. P. Rijal[1], M. Sigdel[2], L. B. Sapkota[3], S. Neupane[4]. *[1]Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal, [2]Manipal College of Medical Sciences, Pokhara, Nepal, [3]Chitwan Medical College, Chitwan, Nepal, [4]Universal College of Medical Sciences, Bhairahawa, Nepal*

**Background:** Nosocomial infection is a global problem with multi facet outcomes. At present, the emergence of resistance to antimicrobial agents is a global public health problem which is well pronounced in developing countries. Microbial bio films pose great threat for patients requiring indwelling medical devices (IMDs) as it is difficult to remove them. It is, therefore, crucial to follow an appropriate method for the detection of bio films. **Methods:** The aim of this study was to determine the prevalence of bacteria causing nosocomial urinary tract infections & their antimicrobial resistance pattern with special reference to biofilm formation among the patients admitted at Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The study was conducted during a period of February to July 2015. Six hundred thirty five clinical specimens; mid-stream & catheterized urine were subjected for bacterial culture and their antibiotic sensitivity test & test for the detection of biofilm formation was performed by following standard methods.

**Results:** Of the 635 specimens studied, 89 bacteria & two Candida spp. were isolated from 91specimens. The most common isolates (N=41, 46.1%) were *E coli*, (N=14, 15.7%) were *E faecalis*, (N=13, 14.6%) were *P aeruginosa*. In-vitro antibiotic susceptibility tests revealed that the Gram-negative bacilli were sensitive to Carbapenem, Polymyxin B and Colistin Sulphate while the Gram-positive cocci were sensitive to Tigecycline, Teicoplanin and Vancomycin. Tissue culture plate method detected 53 (59.5%) biofilm producers and 36 (40.5 %) biofilm non-producers. The most common Gram negative isolates producing biofilm (n=45) were, *Escherichia coli* (31 %) *Klebsiella pneumoniae* (13.3%), & *Pseudomonas aeruginosa* (29%), and biofilm producing Gram-positive organism (n = 8) was *Enterococcus faecalis* (57%). Among the Gram negative biofilm producers (N=45), 44 (97.7%) were found to be multidrug resistant. 23 (51.1%) were ESBL producers, 13 (28.8%) MBL producers, 4 (8.8%) AmpC producers & 1 (2.2%) ESBL & AmpC co-producer. Among biofilm non producers (N=30), 30 (100%) were found to be MDR, 18 (60%) ESBL producers, 4 (13.3%) MBL producers, 1 (3.3%) AmpC producer & 1 (3.3%) ESBL & AmpC co-producer. **Conclusion:** The findings showed that biofilm producers were more multidrug resistant and strong beta lactamases producers as compared to the non-producers leading to resistance to the commonly prescribed antimicrobial agents & difficulty in treatment of the infections. Necessary measures should therefore be taken to detect biofilm producers in the lab & to reduce the rate of biofilm formation in the clinical settings.

## B-055

### CD4+ T-lymphocytes counts in children with Human immunodeficiency virus infection at the Paediatric Department of Korle Bu Teaching Hospital in Ghana

C. E. Lekpor[1], W. Ababio[2], F. A. Botchway[1]. *[1]Korle-Bu Teaching Hospital/ University of Ghana/Department of Pathology, Accra, Ghana, [2]KorleBu Teaching Hospital/ University of Ghana, Accra, Ghana*

**Background:** The high cost of CD4 count estimation in resource-limited countries is a major challenge in initiating patients on highly active antiretroviral therapy (HAART). Since laboratory assessments of HIV-infected patients by flow cytometric methods are expensive and unavailable in resource-limited countries, total lymphocyte count by haematology cell counter is supposed to be a suitable surrogate marker to initiate and monitor course of the disease in these patients. The aim of this study was to evaluate the utility of total lymphocyte count as a surrogate marker for CD4 count in HIV-infected patients. **Methods**: In a prospective study 98 HIV-positive children between the ages of 5-16 years were evaluated for total and CD4 lymphocyte count. For correlation between CD4 count and total lymphocyte count, haemoglobin and haematocrit we defined cut-off values as 200 cell/µl, 1200 cell/µl, 12 gr/dl and 30%, respectively, and compared CD4 count with each parameter separately. Positive predictive value, negative predictive value, sensitivity and specificity of varying total lymphocyte count cutoffs were computed for CD4 count ≤ 200 cell/µl and ≤ 350 cell/µl. **Results**: Strong degree of correlation was noted between CD4 and total lymphocyte count (r: 0.590, $P < 0.001$). Mean and standard deviation of total lymphocyte count, haemoglobin and haematocrit in relation to CD4 count were

calculated which indicated significant correlation between these variables. Kappa coefficient for agreement was also calculated which showed fair correlation between CD4 200 cell/µl and total lymphocyte count 1200 cell/µl (0.35). **Conclusion**: This study reveals that despite low sensitivity and specificity of total lymphocyte count as a surrogate marker for CD4, total lymphocyte count is of great importance and benefit in resource-limited settings.

## B-056

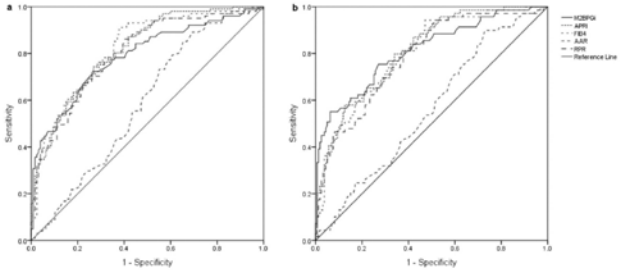
### Predicting liver fibrosis staging using noninvasive biomarker M2BPGi in patients with hepatitis B infection

S. Feng[1], B. Wei[1], E. Chen[2], D. Li[1], T. Wang[1], Y. Gou[1], T. Yang[1], D. Zhang[2], H. Tang[2], C. Tao[1]. *[1]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China, [2]Center of Infectious Diseases, West China Hospital of Sichuan University, Chengdu, China*

**Background:** Liver fibrosis is the common consequence of chronic hepatitis B virus infection, leading to the formation of cirrhosis. Accurate assessment of liver fibrosis is specifically essential for the management of patients with liver cirrhosis accompanied by distortion of hepatic vasculature. Currently liver biopsy is widely used, but it has many limitations, for instance, subjectivity in reporting, high costs, risks of bleeding and pneumothorax, and discomfort to patients. Thus, a novel serum glycobiomarker, M2BPGi, has been newly developed. The present study aimed to investigate the diagnostic performance of M2BPGi in HBV infected patients by comparing with other noninvasive methods such as AST-to-platelet ratio (APRI), FIB-4, AST to ALT ratio (AAR), and RDW to platelet ratio (RPR), in order to assess the utility of M2BPGi as a liver fibrosis stages monitoring tool.

**Methods and results:** Serum samples were collected from 228 HBV infected patients. Liver fibrosis stages of all patients were diagnosed using FibroScan, with 127 patients (55.7%) had fibrosis of F0-1, 32 patients (14.04%) of F2-3, and 69 patients (30.29%) of F4. Median M2BPGi values in each fibrosis stage were: 0.88 cut-off index (COI) in F0-1, 1.165 COI in F2-3, and 1.92 COI in F4, respectively. Furthermore, as for diagnosing significant fibrosis (≥F2), the areas under the receiver operating characteristic curve (AUC) of M2BPGi (0.788) was comparable to FIB-4 (AUC=0.820), APRI (AUC=0.817) and RPR (AUC=0.799), but significantly superior to AAR (AUC=0.585) (Figure a). In addition, M2BPGi yielded the highest AUC of cirrhosis (≥F4) (AUC=0.811) compared with APRI (AUC=0.809), FIB-4 (AUC=0.799), AAR (AUC=0.560) and RPR (AUC=0.786) (Figure b).

**Conclusion:** An increasing trend in M2BPGi levels associated with the progression of liver fibrosis in HBV infected patients was observed. M2BPGi can be served as a potential noninvasive glycobiomarker to assess the stage of liver fibrosis, especially for patients with F4 HBV fibrosis.




## B-057

### Evaluation of the Bio-Rad Geenius HIV 1/2 Confirmation Assay as an Alternative to Western Blot for visa screening protocol in UAE

s. Atef. *National Reference Laboratory, Abu Dhabi, United Arab Emirates*

**Background:** For several decades, western blot ( WB )has been the gold standard as a confirmation assay after reactive enzyme immune assay(EIA) for human immunodeficiency virus(HIV) antibody. In April 2014, updated recommendations for diagnosis of HIV infection by the US Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) suggest a new diagnostic algorithm.Aim of work: to evaluated the Geenius HIV1/2 as a rapid, simple, and reliable alternative to WB for visa screening program in UAE. **Methods:** This study was conducted at National Reference Laboratory, Abu Dhabi ,UAE. Forty-nine samples were selected based on the HIV infection status of the patients, defined as positive (confirmed HIV-positive by WB) or negative (screening test negative). All serum specimens were initially determined by the 4th generation HIV antigen/antibody assay (Elecsys® HIV combi PT 4th Gen,Roche ), and when reactive, we performed duplicated tests. All serum specimens were tested by the Geenius HIV1/2. WB was performed for all reactive samples. According to the new recommendation, sera with reactive HIV antigen/antibody assay but nonreactive or indeterminate Geenius HIV1/2 results should be further tested with HIV-1 nucleic acid amplification test (NAT) **Results:** In our study we consider WB as a gold standard method for evaluating the Geenius HIV 1 /2 differentiation assay. As according to the new algorithm HIV-1 NAT is included as a next step in differentiation assay. Overall sensitivity and specificity of the Geenius HIV 1/2 based on WB were (100% and 97.2% ) respectively. Our results showed a high agreement between WB and Geenius assay for both HIV-negative individuals and HIV- positive infection. **Conclusion:** In view of previous results we recommend the use of genius HIV1/2 assay instead of WB for visa screening program in UAE.HIV-1 NAT should be performed in negative or indeterminate specimens by the Geenius HIV1/2 to detect acute HIV infection as recommended in new HIV testing algorithms.

## B-058

### Quantitative Detection on Serological Markers for Hepatitis B Virus Infection by Chemiluminescence Immunoassay

K. Lian, Y. Li, H. Huang, L. Chang, H. Xu, X. Chen. *Suzhou Hybiome Biomedical Engineering Co.Ltd., Suzhou, China*

**Abstract**

**Purpose:** Hepatitis B virus (HBV) infection has been a worldwide health problem nowadays and quantitative detection of its serological markers allows identifying or accessing HBV infection phases, monitoring antiviral therapy and may help elucidating the natural course of HBV infection. Thus, the main aim of the present study was to evaluate quantitative performance of Hybiome CLIA detection reagents for HBV serological markers on HYBIOME AE-240 platform.

**Methods:** Quantitative CLIA reagents for HBV serological markers manufactured by HYBIOME were evaluated on HYBIOME AE-240. CLIA reagents for HBsAg and anti-HBs of Company A on ARCHITECT i2000sr and time-resolved fluorescence immunoassay (TRFIA) reagents for HBeAg, anti-HBe and anti-HBc produced by Company F on TALENT-II were used as the reference reagents respectively. Comparison experiment about each marker quantitative detection reagent was performed in three different hospitals. Specimens (1245, 1230, 1087, 1112 and 1112 for HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) from different donors were collected. The comparison study was approved by Ethic Committee of each hospital and was compliance with Guidance of *in vitro* Diagnostic Reagent for Clinical Study (China).

**Results:** Measured values of HYBIOME reagents for HBV serological marker detection had good linear correlation with the theoretical values in the linearity range of HYBIOME HBV reagents ((0.1~150)IU/mL for HBsAg, (7~500)mIU/mL for Anti-HBs, (0.5~8)IU/mL for Anti-HBc, (0.5~50)PEIU/mL for HBeAg, (0.25~4) PEIU/mL for Anti-HBe), and the linear correlation coefficients were all greater than 0.9900($r$>0.9900). Positive, negative and total coincidence rates of Hybiome assay kits with their respective reference reagents were really high in clinic (99.33%, 99.37% and 99.36% for HBsAg; 98.57%, 96.28% and 97.97% for anti-HBs; 98.67%, 98.28% and 98.57% for anti-HBc; 100.00%, 100.00% and 100.00% for HBeAg; 98.85%, 98.77% and 98.80% for anti-HBe). In addition, clinical test results of HYBIOME reagents and the reference reagents showed good correlation, correlation coefficient $r$ was 0.9917, 0.9912, 0.9869, 0.9910 and 0.9906 for serum HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc detection.

**Conclusions:** HYBIOME quantitative detection reagents for HBV serological markers, with HYBIOME CLIA platform AE-240, present an excellent quantitative analysis performance and a good correlation with reference reagents in clinic, which offers a rapid and accurate technology for HBV serological tests and may be useful for clinical surveillance and HBV infection research.

## B-059

### Study on resazurin based antimicrobial susceptibility test of *Staphylococcus aureus* using real-time thermocycler

S. Song, J. Shin, S. Yu, J. Lee, K. Jun, J. Shin, H. Kim, J. Lee, S. Oh. *Department of Laboratory medicine, Inje University College of Medicine, Busan, Korea, Republic of*

**Background:** Antimicrobial susceptibility testing is very important when it is sought to select appropriate antibiotics to treat bacterial infections and to monitor the spread of bacterial resistance. Early initiation of antibiotic therapy is directly associated with survival. Therefore, rapid antimicrobial susceptibility testing improves patient outcomes and reduces costs; the therapeutic period is brief and invasive procedures minimized. Many existing antimicrobial susceptibility tests (ASTs) (disk diffusion and commercial instrument-based methods) require ≥ 16-18 h. More rapid, accurate methods are required. Here, we developed a new experimental resazurin-based antimicrobial susceptibility test (Resazurin-AST) using cell viability indicator resazurin; we compared our results to those afforded by broth microdilution testing.

**Methods:** The reference strain *Staphylococcus aureus* ATCC 29213 and 43 *S.aureus* isolates from various specimens were included. Oxacillin and clindamycin (commonly used to treat *S.aureus*) were the antibiotics of interest. We used 20 and 40 μL volumes of all of bacterial suspension, antibiotic solution, and resazurin solution to ensure acceptance of growth rate. All strains were grown in real-time thermocycler; fluorescence values were measured. Growth inhibition gradients were established and their reproducibilities were verified employing the reference strain. These gradients were used to determine antimicrobial susceptibilities, and the results were compared to those of the broth microdilution method and VITEK®2. The strains were divided into three groups by their minimal inhibitory antibiotic concentrations (MICs) upon broth microdilution testing, and the growth inhibition gradients were analyzed.

**Results:** Of 44 strains including the reference strain, 21 were susceptible to oxacillin and clindamycin, 20 were resistant to both antibiotics, and 3 were oxacillin-resistant but clindamycin-susceptible. The acceptabilities of the growth rates when the volumes used were 20 and 40 μL were 67.6% and 90.0%, respectively. The growth inhibition gradients revealed by fluorescence measurements tended to increase over time in susceptible strains, and to decrease or increase slightly in resistant strains. Upon repeated testing of the reference strain, the growth inhibition gradient remained identical. When the test volumes were 20 μL and 40 μL, oxacillin-susceptible and -resistant strains were distinguishable at 100 min (cycle 20) or 80 min (cycle 16). All clindamycin-susceptible and -resistant strains were distinguishable at 65 min (cycle 13). The growth inhibition gradient of strains divided by MICs showed differences among susceptible group with low MIC value, susceptible group with high MIC value and resistant group of oxacillin. However, no obvious distinctions were evident within clindamycin-susceptible subgroups.

**Conclusion:** The Resazurin-AST for *S. aureus* yielded data matching those of the broth microdilution test. The test results were obtained within 2 h, thus, suggesting the application of the Resazurin-AST in clinical settings.

## B-060

### Comparison of the Traditional and Reverse Syphilis Screening Algorithms in Medical Health Checkups

E. Nah. *Korea Association of Heath Promotion, Seoul, Korea, Republic of*

**Background:** The syphilis diagnostic algorithms applied in different countries vary significantly according to the local syphilis epidemiology and other considerations, including the expected workload, need for automation in the laboratory and budget factors. This study compared the usefulness of the traditional and reverse syphilis diagnostic algorithms in health checkups.

**Methods**: In total, 1,000 blood samples were obtained from 908 men and 92 women during their regular health checkups. Traditional screening and reverse screening were applied to the same specimens using automatic rapid plasma regain (RPR) and *Treponema pallidum* latex agglutination (TPLA) tests, respectively. Second treponemal tests were performed using the chemiluminescent microparticle immunoassay (CMIA) in the reverse algorithm.

**Results:** Among the 1,000 samples tested, 68 cases (6.8%) were reactive in reverse screening (TPLA) compared to 11 cases (1.1%) in traditional screening (RPR). The corresponding κ value of the traditional algorithm compared with the reverse algorithm was 0.191 (95% confidence interval = 0.060–0.322), which indicates slight agreement between the traditional and reverse algorithms. The traditional algorithm missed 48 cases [TPLA(+)/RPR(-)/CMIA(+)]. The median cutoff index (COI) of TPLA was higher in CMIA-reactive cases than in CMIA-nonreactive cases (90.5 vs 12.5 U).

**Conclusions:** The reverse screening algorithm detected subjects with possible latent syphilis who could be given opportunities for evaluating syphilis infection in health checkups. The COI values of the initial TPLA test may be helpful in excluding false-positives in TPLA tests.

Table 1. Comparison of the traditional and the reverse algorithms

| Reverse algorithms | Traditional algorithms | | Total | Agreement (%) | | Kappa value (95% CI) |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| Positive | 6 | 48 | 54 | 11.1 | (6/54) | 0.191 |
| Negative | - | 946 | 946 | 100 | (946/946) | (0.060–0.322) |
| Total | 6 | 994 | 1,000 | 95.2 | (952/1,000) | |

Abbreviation: CI, confidence interval.

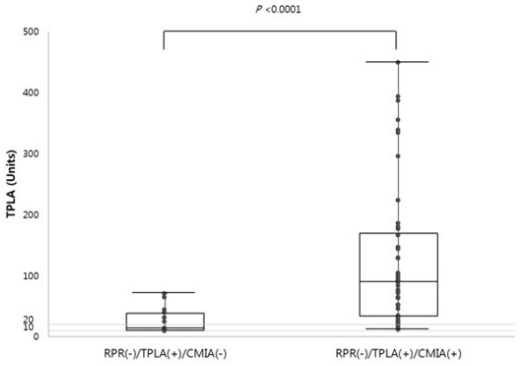



Fig 1. Comparison of the quantitative results of TPLA between CMIA-reactive and CMIA-nonreactive cases in the reverse algorithm.
Abbreviations: TPLA, *Treponema pallidum* latex agglutination; RPR, rapid plasma regain; CMIA, chemiluminescent microparticle immunoassay

## B-061

### The establishment of non-invasive diagnosis of liver fibrosis model in chronic hepatitis C in Chinese people

Y. Ren[1], Z. Wu[2], R. Chen[1]. *[1]Department of Laboratory Medicine, The Second Xiangya Hospital, Central South University, Changsha, China, [2]Department of Chest Surgery,the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, China*

**OBJECTIVES:** Noninvasive markers of liver fibrosis in patients with chronic hepatitis C are needed for predicting disease progression. We aimed to establish a non-invasive diagnostic model to predict liver fibrosis development in hepatitis C virus (HCV)-infected patients in China. **METHODS:** One hundred and seventy-five patients with chronic HCV infection without other potential risk factors who underwent liver biopsy and serological tests were enrolled; factors evaluated included age, sex, viral load, fibrosis stage, aspartate and alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, total protein, albumin, fasting glucose, total, bilirubin, direct bilirubin, total bile acids, creatinine, total cholesterol, triglycerides, platelet count, prothrombin time, thrombin time, activated partial thromboplastin time, hyaluronidase, type III collagen N-telopeptide, laminin, type IV collagen, anti-HCV IgG. For the formulation of diagnostic model, univariate logistic regression analysis was performed on variables between patients in the training set. Significant variables from the univariate analysis ($P < 0.05$) were then subjected to multivariate logistic regression analysis to identify independent factors associated with fibrosis. The fibrosis index derived from the training set was then applied to the validation set to test the predictive power of the selected model. Clinical data were compared with those for other noninvasive models (AST-to-platelet ratio (APRI), AST to ALT ratio (AAR), age-to-platelet ratio (API), cirrhosis discriminate score (CDS), FIB-4, and Forn's index) for estimating liver fibrosis using receiver operating characteristic (ROC) analysis. ROC curves were constructed to measure the diagnostic capacity of each test. **RESULTS:** The multivariate logistic regression analysis identified alanine aminotransferase (ALT), total bile acids (TBA) and hyaluronidase (HA) as independent risk factors for fibrosis. The model of ATH is consist of 3 indicators, ALT, TBA and HA, ATH= ln (ALT) × ln (TBA)× ln (HA). ATH was independently

associated with liver fibrosis stage as determined by liver biopsy($r$ = 0.716, $P$ <0. 01). The cutoff values of ATH for fibrosis stages ≥S2, ≥S3, and S4 were 3.71, 4.72, and 6.21, respectively. The area under the receiver operating characteristic curve values (AUROC) of ATH for significant fibrosis (≥S2), severe fibrosis (≥S3), and cirrhosis (S4), were 0.877, 0.897, and 0.841, respectively. The AUROC of ATH model was significantly higher than alone ALT, TBA, HA index in fibrosis stages of significant fibrosis, severe fibrosis, and cirrhosis, respectively ($P$<0.01). ATH values offered a superior AUROC curve for the diagnosis of significant fibrosis, severe fibrosis and cirrhosis compared with the APRI, CDS, API, AAR, FIB-4 and Forns Index. Compared with the other noninvasive models s and scoring systems, ATH was the most useful marker for differentiating between fibrosis stages. For predicting liver cirrhosis (S4), ATH model had the highest AUROC (AUCATH=0.841, $P$<0.001) and for predicting severe liver fibrosis (≥S3), ATH model had the highest AUROC (AUCATH=0.897, $P$<0.001) and for predicting significant liver fibrosis (≥S2), ATH model also had the highest AUROC (AUCATH=0.877, $P$<0.001). **CONCLUSION:** The ATH model may be a simple, reliable, and non-invasive method to evaluate liver fibrosis in HCV-infected patients in China without an unnecessary liver biopsy.

## B-062

### BK virus-IgG and BK virus-specific ELISPOT assay in healthy donors and pretransplant recipients

H. Bae[1], A. Choi[2], K. Park[1], J. Rye[1], H. Lee[3], E. Oh[1]. *[1]Catholic University of Korea, Seoul, Korea, Republic of, [2]Seoul St. Mary's Hospital, Catholic University of Korea, Seoul, Korea, Republic of, [3]Samkwang Medical Laboratories, Seoul, Korea, Republic of*

**Background:** BK virus (BKV) is widespread in the human population, but rarely induced apprarent disease in healthy individuals. However, the reactivation of BKV is major infectious complication in kidney transplant patients that can lead to graft loss. The aim of this study was to investigate the BK virus-specific cellular and humoral immunity in healthy contols and pretransplant chronic kidney disease (CKD) patients.

**Methods:**Peripheral blood mononuclear cells and sera were collected from 44 healthy individuals (M:F=23:21, age= 35.8±11.2) and 26 CKD patients (M:F=13:13, age=45.7±11.6). BKV specific IgG levels were measured using qualitative Human BK Virus IgG ELISA Kit (MyBioSource, USA). The IFN-γ ELISPOT assays were performed to measure BKV-specific T cells (spots per $3x10^5$ lymphocytes) following the stimulation with different BK virus antigens (Large T antigen (LT), Small T antigen(ST), VP1, VP2 and VP3 antigens).

**Results:** BKV-specific IgG was detected in 59.1% of healthy individuals and 57.7% of CKD patients (P=0.909). The CKD patients demonstrated significantly increased BKV-specific ELISPOT results compared to the healthy individuals (LT, P <0.001; ST, P=0.002; VP1, P=0.019; VP2, P=0.002; VP3, P=0.002) The positive BKV-ELISPOT results (the cut-off > 10 spots/$3x10^5$) were more frequent in CKD patients versus healthy controls (LT, 76.9% vs. 47.7%, P=0.017; ST, 73.1% vs. 29.5%, P<0.001; VP2, 69.2% vs. 25.0%, P<0.001; VP3, 73.1% vs. 31.8%, P<0.001). LT and VP1 antigens were the most immunogenic proteins showing significantly higher ELISPOT results in healthy individuals (p=0.007, 0.001, respectively). In addition, LT and VP1 antigens induced a wide range of ELISPOT results in CKD patients (0-833 and 0-717 spots/$3x10^5$, respectively). However, BKV-ELISPOT results were not different between individuals with BKV-IgG(+) and BKV-IgG (-) (P>0.05). In BKV-ELISPOT (+) patints, the responses to different BKV antigens were highly heterogeneous, and the positive ELISPOT reactions to all five BK antigens were more frequent in CKD patients compared to healthy controls (53.9% vs. 11.4%, P <0.001).

**Conclusion:** BKV-specific IFN-γ ELISPOT responses were not associated with BKV-IgG and presented the increased activity and wide spectrum of responses in CKD patients. It might be a useful tool to monitor the viral replication and to guide immunosuppression.

## B-063

### Evaluation of two commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV)

L. G. S. S. ASSUNÇÃO, L. Moutinho, B. Moura. *LABREDE, BELO HORIZONTE, Brazil*

**Background:** Antibodies against the capsid antigen IgG (VCA IgG) typically appear at the time of the onset of clinical symptoms of acute infection, and remain positive for life, whereas IgM antibodies (VCA IgM) usually appear at the same time as VCA IgG and disappear within a few weeks, although they may persist for several months. About 90% of adults throughout the world have antibodies against EBV. It is normally possible to distinguish acute from past infection associating VCA IgM and VCA IgG with EBV nuclear antigen (EBNA)-1 IgG. The presence of VCA IgM and VCA IgG without EBNA-1 IgG indicates acute infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection. The primary objective of this pilot study was to correlate the performance of Abbott-Architect® VCA IgG and VCA IgM against the same profile on Siemens- Immulite® 2000 systems. **Methods:** A total of 42 unselected serum samples, stored at -20°C, sent for EBV routine serological testing were assayed with ARCHITECT i2000 platform (USA) and Immulite 2000 (UK), both commercially chemiluminescent microparticle immunoassay. Data were submitted to EP evaluator® program for method comparison and statistical analysis. **Results:** The overall analytical performance assays was acceptable: 93,3% (82,1 to 97,7%) for EBV VCA-G, positive agreement 97,2% and negative agreement 77,8%, Cohen's Kappa 78,3%. EBV VCA-M qualitative method comparison also presented an overall agreement of 90,5% ( 77,9 to 96,2%), positive agreement 86,7% and negative agreement of 92,6%, Cohen's Kappa 79,3%. No sample of EBV VCA-M was excluded from data and "no agreement" was found in four samples, being two positive discordant and two negative discordant. Two samples of EBV VCA-G were negative and one sample positive with discordant results. **Conclusion:** Although there is good agreement between analytical platforms Siemens/Immulite® and Abbott-Architect, different results may be obtained. An isolated result of VCA IgM or VCA IgG should be cautiously interpreted when using only two parameters. Commercially available EBV nuclear antigen (EBNA)-1 IgG should be associated to distinguish acute and past infections in immunocompetent patients and pregnant women with symptons similar to cytomegalovirus.

## B-064

### Clinical benefits of the FilmArray GI Panel in an academic medical center

S. Beal[1], E. Tremblay[2], K. Rand[1]. *[1]University of Florida, Gainesville, FL, [2]UF Health Shands Hospital, Gainesville, FL*

Introduction

Diarrheal diseases are a major cause of emergency department (ED) visits and hospitalization. Conventional methods for identification of gastrointestinal pathogens are time consuming, expensive, and have limited sensitivity. Patients may not receive antibiotics in a timely manner, may undergo unnecessary diagnostic testing, and incur excessive healthcare costs. The aim of this study is to determine the clinical impact of a new comprehensive molecular panel, the FilmArray® Gastrointestinal (GI) Panel (BioFire Diagnostics, Salt Lake City, UT) which tests for nearly all known agents of infectious diarrhea in approximately one hour. It is hypothesized that use of the GI Panel resulted in shorter length of ED or hospital stay and more appropriate time in isolation. Also, we sought to determine if the GI Panel lessened the need for other, more invasive and expensive tests, such as abdominal imaging studies.

Methods

Following approval from the University of Florida Health Institutional Review Board (IRB), patients with stool cultures submitted between 6/1/16 and 12/31/16 that were processed via the BioFire GI Panel (n=123) were included as cases within this study. A historical control group (n=594) was obtained from the same time period a year prior to control for seasonality in GI illness, 6/1/15 to 12/31/15. Both the cases and controls were filtered to include only patients with a length of stay (LOS) that was 14 days or fewer.

Results

A total of 123 patients were tested on the GI Panel. The organisms detected were norovirus (n=10), *Salmonella* (n=7), *Shigella*/EIEC (n=2), *Campylobacter* (n=2), sapovirus (n=3), rotavirus (n=1), Giardia lamblia (n=2), Vibrio cholera (n=1), Cyclospora cayetanensis (n=1), Adenovirus (n=1), Enteroaggregative *E. coli* (n=1), Enteropathogenic *E. coli* (n=6), and Enterotoxogenic *E. coli* (n=1). These patients were compared with 594 historical controls who were tested using conventional stool test methods. 30/123 (24.4%) of cases were positive for at least one organism other than *C. difficile* compared with 41/594 (6.9%) of control patients. Two or more non-*C. difficile* organisms (co-infections) were identified in 4.9% (n=6) of patients who were tested on the GI Panel but no co-infections were found in our control population. Patients tested on the GI Panel had an average of 2.6 other infectious stool tests whereas the control population averaged 3.02 additional stool tests (p < 0.05). The number of abdominal radiologic exams was also slightly lower in the GI Panel group (0.35 vs 0.39) along with the length of stay following specimen submission (3.17 vs. 3.43 days); however, these differences were not significant. The average time from collection to result was 4.6 hours for patients tested by the GI Panel, compared with 54.75 hours for stool culture in the control population. Average days on antibiotics were 1.59 for the GI Panel group compared to 2.12 days in the control group (p<0.05).

Discussion

The FilmArray GI Panel has the potential to improve patient care by rapidly identifying a broad range of pathogens, reducing the need for other diagnostic tests, reducing unnecessary use of antibiotics, and leading to a reduction in hospital length of stay.

## B-065

### Prevalence of the infection of high-risk human papilloma virus among women in urban Changchun, northeast China: a 38260 female cases survey

G. Hu, Y. Wang, X. Pang, W. Miao, L. Li, Z. Wang, W. Xu. *Department of Laboratory Medicine, The First Hospital of Jilin University, Changchun, China*

**Background:** Persistent infection of high-risk human papilloma virus (HR-HPV) is associated with cervical cancer. HR-HPV testing as a screening tool for cervical cancer is considered to be one of the more sensitive methods of screening for cervical cancer.

**Objective:** To investigate the prevalence of the infection of high-risk human papilloma virus among women in urban Changchun China.

**Methods:** This retrospective study was conducted between May 2011 and May 2015 at the First Hospital of Jilin University in urban Changchun China. All the women involved in this study, including 35568 female outpatients and 2692 healthy women, attended routine gynecological examinations and detected for high-risk human papilloma virus (HR-HPV) with Hybrid Capture 2.

**Results:** The overall positive rate of HR-HPV testing is 22.17% (female outpatients) and 18.29% (routine gynecological examination women) in urban Changchun northeast China from May 2011 to May 2015. The survey reflected a decreasing trend in the positive rate of HR-HPV testing, even as the total number of the woman for HR-HPV testing and the HR-HPV positive cases increased. Positive rate of HR-HPV testing of female out-patients increased and decreased with the season, but the positive rate of HR-HPV testing of healthy women who were attending routine gynecological examinations did not change a lot. Positive rate of HR-HPV testing of female outpatients among different age groups had statistical difference with each other. ①The positive rate of HR-HPV testing in 0~20 age group (39.59%) is higher than other age groups (except 61~65 age group) which has statistical significance. ②The positive rate of HR-HPV testing in 21~25,56~60 and 61~65 age groups (28.11%, 28.28%, 30.29%, respectively) had no statistical difference with each other, but lower than 0~20 age group and higher than any other age groups from 21 to 55 years old with statistical significance.③The difference of the positive rate of HR-HPV testing among the six age groups from 21 to 55 years old has statistical significant.④The positive rate of women ≥66 years old has no statistical difference with other age groups (except 0~20 age group). Positive rate of HR-HPV testing of routine gynecological examination women had no statistical difference between different age groups. The most common age of female outpatients and healthy women tested for HR-HPV is 41~45 years (19.01% and 21.77%, respectively), followed by 36~40 years (16.96% and 19.24%, respectively).

**Conclusions:** In Changchun northeast China, the overall positive rate of HR-HPV testing (22.17% and 18.29%) rank above average. For female outpatients, the positive rate of HR-HPV testing is higher in winter (Dec. Jan. and Feb.). The peak of the positive rate of HR-HPV testing appeared in young women (≤25 years old) and older women (56~65 years old). For routine gynecological examination women, the positive rate of HR-HPV testing doesn't change with the season and had no statistical difference between different age groups. The most common age of women tested for HR-HPV is 41~45 years old, followed by 36~40 years old.

## B-066

### Reevaluation of enzyme linked fluorescent immunoassay comparing with pcr assay for detection of *Clostridium difficile* toxins

K. LEE[1], W. Kim[1], D. Kim[1], S. Kim[1], M. Chong[2]. *[1]Cheju Halla hospital, Jeju-self governing province, Korea, Republic of, [2]Cheju Halla college, Jeju-self governing province, Korea, Republic of*

**Background** : Enzyme immunoassay capable of detecting both toxin A and B of *Clostridium difficile (C. difficile)* is routinely tested in hospital for the diagnosis of *C. difficile* associated disease. Recently, PCR test for the gene of *C. difficile* toxin B in diarrhea stool specimen is becoming popular in the diagnosis of *C. difficile* associated disease. Therefore, we compared the results of two methods in detection of *C. difficile* toxins.

**Methods** : In total, 643 diarrhea samples that were simultaneously analyzed for toxin A and B by enzyme linked fluorescent immunoassay (VIDAS CDAB, Bio-Merieux sa, France) and for *C. difficile* toxin B by PCR using the Seeplex® Diarrhea ACE Detection kit (Seegene, Korea) in Cheju Halla Hospital from March 2015 to December 2016, Retrospectively we investigated the positive detection rates, concordance rate according to the level of relative fluorescence value in ELFA

**Results** :

The positive rate was 8.55% when we regard "equivocal" as "positive" in ELFA, 5.8% when we regard "equivocal" as "negative") in ELFA, 8.8% in PCR. The concordance rate between ELFA and PCR was 94.4%. Nineteen (3.3%, 19/588) samples that were negative in ELFA were positive in PCR. Nine (50%, 9/18) samples that were equivocal in ELFA were positive in PCR, Eight (21.2%, 8/37) samples that were positive in ELFA were negative in PCR.

In the test of enzyme linked fluorescent immunoassay, the cut-off level in Relative fluorescence value(RFV)

between "Negative" and "Equivocal" and between "Equivocal" and "positive" is 0.13, 0.34. ROC curve showed that the cut-off of 0.04 was 0.892 in Area under the curve (AUC) with 82.5 in sensitivity, 88.9 in specificity.

Positive rates of *C. difficile* by PCR is 1.9% under 0.04, 27.9% over 0.04 in RFV

**Conclusions** : The positive detection rates of PCR were higher than those of ELFA for the detection of *C. difficile* associated disease, hence the PCR assay for detection of toxin B is recommended especially in case EIA is negative. PCR test is more useful to detect the causative agents including *C. difficile* toxin B. Re-set of Cut off limit in RFV in ELFA should be re-set based on the result of PCR test for *C. difficile* toxins.

**Key Words** : *Clostridium difficile* toxin, PCR, enzyme linked fluorescent immunoassay

## B-067

### Evaluating A Novel Host-immune Based Assay For Distinguishing Bacterial From Various Viral Infections In Febrile Children

E. Eden[1], I. Srugo[2], A. Cohen[1], R. Navon[1], O. Boico[1], T. Gottlieb[1], E. Bamberger[1], M. Paz[1], L. Etshtein[1], L. Shani[1], M. Stein[3], A. Klein[3], K. Oved[1]. *[1]MeMed Diagnostics, Tirat Carmel, Israel, [2]Bnai Zion Medical Center, Haifa, Israel, [3]Hillel Yaffe Medical Center, Hadera, Israel*

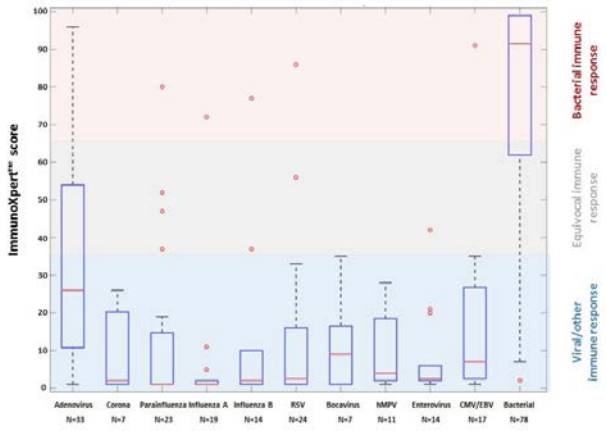
**Background:** A major challenge in effective management of febrile children is the clinical difficulty of distinguishing bacterial from viral infections. This uncertainty drives antibiotic misuse, hampering patient care and contributing to emergence of antibiotic resistance. ImmunoXpert™ is a novel assay that distinguishes bacterial from viral infections based on the serum levels of three host-proteins (TRAIL, IP-10, and CRP). Here we evaluated the assay's ability to assign correct infection classification (viral or bacterial) in children infected with ten different types of viral strains as well as in bacterially infected children.

**Methods:** We studied 233 febrile children aged 3 months to 18 years presenting at the emergency department. Infection etiology (78 bacterial, 155 viral) was determined by clinical adjudication of three physicians and microbiological confirmation of pathogenic viral strains using multiplex-PCR applied to nasal swabs (Seeplex-RV15). Based on the manufacturer's pre-determined cut-offs, ImmunoXpert generated one of three results: viral (score 0-35), equivocal (score 35- 65) or bacterial (score 65-100).

**Results:** ImmunoXpert correctly classified 90% of bacterial cases and 91% of viral cases, when compared to the expert panel diagnoses (13% of patients had an equivocal result; Figure 1). For coronavirus, bocavirus, human metapneumovirus, and enterovirus, the assay classified all patients correctly. In the case of adenovirus, which is known to trigger a bacterial-like inflammatory host response, the assay correctly classified 83% of the patients. In comparison, CRP (cut-off: 40 mg/l) correctly classified only 42% of adenovirus infections.

**Conclusion:** The host-immune based assay represents a promising new tool for aiding clinicians in determining infection etiology in febrile children. Importantly, it may assist in distinguishing between adenovirus and bacterial infections, which can be associated with similar clinical presentation.

**Figure 1. Box plots of ImmunoXpert™ scores of patients presenting with different infection types**
Blue boxes present first to third quartiles. Red line corresponds to group median. RSV - Respiratory syncytial virus; hMPV – human Metapneumovirus. Some patients presented with more than one viral strain




## B-068

### A Fully Automated Immunoassay for the Detection of Zika Virus Immunoglobulin M

T. Bunnell, F. Bonelli, S. Penaloza, L. Kelzenberg, D. Vaught, G. Olson, J. Wassenberg. *DiaSorin Inc., Stillwater, MN*

**Objective:** Zika virus is a mosquito-borne flavivirus in the family *Flaviviridae* and is closely related to dengue, yellow fever, and West Nile viruses. It is primarily transmitted by *Aedes* mosquitoes, which are found throughout the tropical and subtropical regions of over 100 countries.

During the first one to two weeks after onset of symptoms, Zika virus disease can be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) in the blood of symptomatic patients. Virus-specific IgM and neutralizing antibodies are typically present after the first week of illness and may be detectable for up to 12 weeks. Combined with patient demography and clinical findings, detection of IgM antibodies to Zika virus provides an essential tool for diagnosis and follow-up care of an acute or recent infection. The objective of this study is the development and validation of a rapid, reliable and accurate automated immunoassay for the detection of Zika virus IgM antibodies in human sera.

**Methods:** The LIAISON® XL Zika Capture IgM immunoassay is a chemiluminescent (CLIA) *in vitro* diagnostic designed for the LIAISON® XL automated analyzer platform, and is intended for the qualitative detection of Zika virus IgM antibodies in human sera. It is a two-step, antibody capture, sandwich assay that utilizes paramagnetic particles coated with monoclonal anti-immunoglobulin antibodies, followed by recombinant Zika virus non-structural protein 1 (NS1), labeled with a luminol-derived reporter molecule.

**Validation:** The LIAISON® XL Zika Capture IgM assay has been validated for precision, interference, and cross reactivity as well as clinical sensitivity and specificity. Intra and total assay imprecision are <9% and <14% respectively. The assay was evaluated for interference from endogenous substances including hemoglobin, serum albumin, bilirubin, triglycerides, cholesterol, HAMA and rheumatoid factor (RF). None of these compounds interfered in the assay. The assay was tested for cross reactivity to other related flaviviruses using samples that were positive for Dengue virus or West Nile Virus IgM, or from subjects who had been vaccinated for Yellow Fever. No cross reactivity was detected.

Clinical specificity was evaluated using 220 apparently healthy donors and 32 pregnant donors collected in the United States and presumed negative for Zika virus infection. The LIAISON assay was negative in 251 of the 252 samples, for a clinical specificity of 99.6%.

Clinical sensitivity was evaluated using serially collected samples from 56 symptomatic subjects (including 15 pregnant subjects) from the Dominican Republic found to be initially PCR positive for Zika virus. All subjects were detected by the LIAISON® assay as Zika IgM positive by the first draw after 8 days post-onset of symptoms, with detection in some subjects as early as 4 days and as late as 83 days post-symptom onset.

**Conclusions:** DiaSorin's new LIAISON® XL Zika Capture IgM immunoassay has excellent diagnostic sensitivity and specificity with a demonstrated lack of cross reactivity to related flaviviruses. The LIAISON® XL Zika Capture IgM immunoassay is the newest member of the DiaSorin infectious disease assay panel, providing continued assistance to clinicians for accurate detection of infectious agents.

## B-069

### Comparison of qSOFA (quick SOFA) Score, Presepsin, Procalcitonin and Lactate for Severity Assessment and Mortality Prediction in Patients with Initial Sepsis

E. Spanuth[1], H. Ebelt[2], B. Ivandic[3], R. Thomae[4], K. Werdan[2]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]Department of Medicine III, University Clinics Halle (Saale), Martin-Luther-University Halle-Wittenberg, Halle, Germany, [3]Department of Medicine III, University of Heidelberg; DIAneering – Diagnostics Engineering & Research, Heidelberg, Germany, [4]Mitsubishi Chemical Europe, Düsseldorf, Germany*

**Background**

The SOFA score is associated with an increased probability of mortality in sepsis. The Third International Consensus Definitions for Sepsis and Septic Shock defined the qSOFA score, which can be assessed at admission without laboratory tests.

**Objective**

To compare sepsis biomarkers with qSOFA for differentiation of sepsis, severe sepsis or septic shock and risk of mortality prediction.

**Methods**

66 Patients admitted with signs of sepsis were included. Severe sepsis and septic shock were defined according to current guidelines. qSOFA score was calculated from respiratory rate, GCS score and stystolic blood pressure using the recommended thresholds. Presepsin (PSEP) and procalcitonin (PCT) were determined using the POC assay PATHFAST Presepsin, LSI Medience Corporation and the BRAHMS luminescence immune assay.

**Results**

Discrimination between sepsis (n=30, mortality=6.6%) and severe sepsis or septic shock (n=36, mortality=36.1%) revealed AUC values of 0.621, 0.627, 0.731, 0.740 and 0.781 for lactate, PCT, qSOFA, PSEP and the combination qSOFA+PSEP, respectively. 15 patients died during hospitalization. AUC values of mortality prediction were 0.715, 0.558, 0.734, 0.758 and 0.803 for lactate, PCT, qSOFA, PSEP and qSOFA+PSEP, respectively. qSOFA scores ≥2 should identify greater risk of death or prolonged ICU stay. Discrimination between qSOFA <2 and ≥2 revealed AUC values of 0.756, 0.669 and 0.606 for PSEP, lactate and PCT.

Using the threshold ≥2 of qSOFA and ≥500 ng/L of PSEP, the combination qSOFA+PSEP detected 14 non-survivors (93%) and 33 (92%) patients of the high-risk group (n=36), whereas qSOFA alone detected only 10 non-survivors (67%) and 21 patients of the high-risk group (58%).

**Conclusion**

The results demonstrated that the qSOFA score is not a standalone criterion for risk stratification in sepsis at admission. Simultaneous assessment by combining qSOFA and PSEP improved the validity significantly. The POC assay PATHFAST Presepsin showed superior performance compared to lactate and PCT.

| Detection rate of non-survivors and severe sepsis or septic shock | | | | | |
|---|---|---|---|---|---|
| Marker | Criterion | Non-survivors Detection rate | Non-survivors AUC | Severe Sepsis/ shock Detection rate | Severe Sepsis/ Shock AUC |
| PCT | 2 µg/L | 47% | 0.558 | 20% | 0.627 |
| Lactate | 2 nmol/L | 75% | 0.715 | 53% | 0.621 |
| PSEP | 500 ng/L | 93% | 0.758 | 89% | 0.740 |
| qSOFA | 2 | 67% | 0.734 | 58% | 0.731 |
| qSOFA+PSEP | 2/500 ng/L | 93% | 0.803 | 92% | 0.781 |

## B-070

### Simultaneous detection of HIV- and hepatitis C- specific antibodies and hepatitis B surface antigen (HBsAg) by multiplex rapid diagnostic test

L. Belec, L. Robin, R. Mboumba Bouassa, S. Simon, M. Matta. *Université Paris V, Paris, France*

**Background:** Multiplex rapid diagnostic test (RDT) for HIV, HCV and HBV may be advantageous in maximizing resources as these diseases share common risk factors and transmission modes. Exacto® HIV/HCV/HBsAg (Biosynex, Strasbourg, France) consists in manually performed, visually interpreted, immunochromatographic RDT simultaneously detecting in 15 minutes HIV- and HCV- specific antibodies (Ab) and HBV surface antigen (HBsAg) in serum, plasma and whole blood (venipuncture and fingerstick).

**Methods:** Hospital-based cross-sectional study was conducted on prospective panel of 750 sera from adult inpatients of hôpital Europèen Georges Pompidou, Paris, including 250 sera positive for HIV-specific Ab, 250 for HCV-specific Ab, 250 for HBsAg and 250 sera negative for HIV- and HCV- Ab and HBsAg, according to the results obtained by the reference Architect i2000SR (Abbott Diagnostic, Chicago, IL). Among HCV-seropositive sera, 187 were positive for HCV RNA (chronic infection), whereas 63 were negative (resolved infection), respectively. Serum samples were tested blindly by Exacto® HIV/HCV/HBsAg.

**Results:** Multiplex RDT showed very high sensitivity and specificity, and excellent concordance with Architect results. Lower sensitivity was observed only in individuals who had cleared their HCV infection. Mean lower limit of HBsAg detection was 5.1 IU/ml (PROBIT regression analysis).

**Conclusion:** Advantages of multiplex RDT for HIV, HCV and HBV include the requirement for less overall specimen volume, fewer finger-sticks if capillary whole blood is used, cost savings through lower cost per virus tested, improved patient flow with results for multiple viruses available at the same time, overall service delivery efficiencies with less time required per infected patient; and patient benefits from fewer visits and lower cost associated with each clinic attendance. In high risk population for HIV, HCV and HBV, which may be frequently combined, the screening of chronic HIV, HCV and HBV by multiplex Exacto® HIV/HCV/HBsAg RDT may clearly improve the "cascade of screening" and quite possibly linkage-to-care with reduced cost.

| | Infectious status | Sensitivity (95% CI) | Specificity (95% CI) | Youden's J index | Cohen's κ coefficient |
|---|---|---|---|---|---|
| **Anti-HIV Ab** | Chronic | 100% (99.9-100.0%) | 100% (99.9-100.0%) | 1.0 | 1.0 |
| **Anti-HCV Ab (HCV RNA+)** | Chronic | 100% (99.9-100.0%) | 100% (99.9-100.0%) | 1.0 | 1.0 |
| **Anti-HCV Ab (HCV RNA-)** | Resolved | 96.8% (92.3-100.0%) | 100% (99.9-100.0%) | 0.97 | 0.98 |
| **HBsAg** | Chronic | 100% (99.9-100.0%) | 100% (99.9-100.0%) | 1.0 | 1.0 |

## B-071

### RIG-I Enhanecs IFN-α Response by Promoting Antiviral Proteins Expression in Patients with CHB

Q. Ou. *The 1st affliated hospital of Fujian Medical University, Fuzhou, China*

**Background:** Chronic hepatitis B (CHB) infection is a serious health problem affecting approximately 400 million individuals worldwide. The main purpose in CHB treatment is to suppress viral replication. IFN-α has various biological properties, such as immune modulation, antiviral response, and antiproliferative activity. However, IFN-α has many side-effects, such as bone marrow suppression, influenza-like symptoms, and exacerbation of autoimmune illnesses. Moreover, only approximately 30% of HBV infected patients respond during interferon therapy. Thus, identification of molecular biomarkers to predict IFN-α therapy sensitivity would be useful in the clinic. In this study, we aim to clarify the mechanism of RIG-I in prediction of CHB therapy with IFN-α.**Methods:** A total of 65 CHB patients were recruited from The 1st Affiliated Hospital of Fujian Medical University and Liver Disease Center of Fujian Province between July 2013 and August 2015. All patients with CHB were treated with PegIFN weekly for 48 weeks and followed-up for another 24 weeks. Non-response is defined as less than 1 log10 IU/mL decline in serum HBV DNA level from baseline at three months of therapy or serum HBV DNA level > 2,000 IU/mL at the end of 1 year. Response is defined as serum HBV DNA level < 2,000 IU/mL and ALT normalization. Serum levels of HBV DNA were quantified by qPCR. HBsAg levels and presence of HBeAg and anti-HBe were measured using an immunosorbent assay. Real-time PCR primer sequences for human RIG-I and antiviral proteins were obtained from the online NCBI public resource. Total RNAs from each sample in peripheral blood mononuclear cells (PBMCs) were extracted with use of a TRIzol kit.Group measures were shown as mean ± SEM. A Student's *t* test was used to analyze the differences between the groups. The *p* values were calculated in SPSS 18.0. and the statistical significance level was accepted as $p < 0.05$.**Results:** In this study, we found RIG-I expression was higher in responders than non-responders of CHB patients with IFN-α therapy. Compared with traditional clinical tests such as HBV DNA and HBsAg, RIG-I had more sensitivity and specificity in predicting IFN-α therapeutic response in CHB patients. Mechanistically, RIG-I enhanced IFN-α response by promoting anti-HBV proteins expression such as double-stranded RNA-dependent protein kinase (PKR), Oligoadenylate synthetase (OAS), adenosine deaminase (ADAR1) and Mx protein. Knocking out of RIG-I could downregulate the expression of these proteins above. Moreover, in HBV-transfected HepG2 (PHY106-HBV-HepG2) cell, inhibited RIG-I expression by RIG-I siRNA deceased STAT1 phosphorylation.**Conclusion:** Our results revealed RIG-I enhanced IFN-α response by promoting antiviral proteins expression via STAT1 pathway. RIG-I may be a new predictive factor for prediction of IFN-α efficacy in chronic hepatitis B patients.

## B-072

### Automated and laboratory information system integrated workflow for simultaneous detection of Zika, chikungunya and dengue viruses by RT-qPCR in EDTA-plasma, urine and seminal-plasma: a unique and comprehensive test routine for Brazilian arboviral threats.

T. H. Santa Rita, P. G. Mesquita, R. H. Jácomo, L. F. A. Nery, G. B. Barra. *Laboratório Sabin, Brasília, Brazil*

Background:

The simultaneous detection of Zika (ZIKV), chikungunya (CHIKV) and dengue (DENV) viruses provide a comprehensive diagnostic answer for patients. RT-qPCR is the most reliable method to detect and distinguish these three viruses. The assay is mostly performed on blood subproduts; the limitations are that the viruses RNA last for a few days and the absence of a high-throughput process (majority of RT-qPCR assays are manual/semi-automated). However, prolonged shedding of ZIKV and CHIKV in semen and urine has been reported and platforms for automated molecular tests have been released. Thus, the aim of the present study was to validate an automated and laboratory information system (LIS) integrated RT-qPCR workflow for simultaneous detection of ZIKV, CHIKV and DENV in EDTA-plasma, urine and seminal-plasma.

Methods:

The assay validation enrolled negative EDTA-plasma, urine and seminal-plasma pools/samples spiked with known amounts of each virus. The RT-qPCR workflow was provided by the Flow classic solution (Roche), which accepts different matrixes simultaneously. Nucleic acids were extracted from 500ul of each matrix (seminal-plasma was diluted 1:1 in PBS) by using viral NA universal LV 3.1 protocol. An in-vitro transcribed random RNA sequence was added into samples during the nucleic acids extraction and was co-amplified in all instances to function as a process control. Primers/probes were obtained from literature. ZIKV, CHIKV and DENV were assessed simultaneously, but in independent reaction wells. To determine the limits of detection (LODs), 1:1 dilutions of each virus were tested (from 1000 to 0.98 copies/mL), the LODs were calculated by probit regression analysis. The imprecisions near the clinical decision point (grey zones) were assessed by repeating the LOD experiment in triplicate during 5 days. The viral load ranges that did not allow certainty about the infection status were determined in each matrix. The accuracies were evaluated by a recovery strategy. The following numbers of samples were prepared for all tested specimens: ZIKV n=86 (21 positive), CHIKV n=85 (27 positive) and DENV n=84 (23 positive). Viral loads in positive samples ranged from $1x10^5$ to $5x10^2$ copies/mL in EDTA-plasma and urine and from $2x10^5$ to $1x10^3$ copies/mL in seminal-plasma. The agreements between the obtained and the expected results were evaluated.

Results:

The LODs in EDTA-plasma, urine and seminal-plasma, respectively, were 90.5 (95%CI 58-299), 51.6 (95%CI 40-74) and 136.3 (95%CI 111-183) copies/mL for ZIKV; 172 (95%CI 135-245), 112.6 (95%CI 92-150) and 551.8 (95%CI 451-730) copies/mL for CHIKV and 94.5 (95%CI 65-217), 38.8 (95%CI 31-53) and 1456 (95%CI 1205-2023) copies/mL for DENV. The grey zones in EDTA-plasma, urine and seminal-plasma, respectively, were 250-7.8, 62.5-3.9, 250-7.8 copies/mL for

ZIKV; 250-3.9, 250-7.8, 1000-31.3 copies/mL for CHIKV; and 125-7.8, 62.5-3.9, 2000-250 copies/mL for DENV. In the accuracy assay, the comparison between obtained and expected results revealed total agreement of 100% (95%CI 95-100%), positive agreement of 100% (95%CI 85-100%) and negative agreement of 100% (95%CI 94-100%) for all tested specimens. No cross-reaction was observed.

Conclusion:

The proposed workflow showed acceptable sensitivity, precision and accuracy for ZIKV, CHIKV and DENV detection in multiple body fluids allowing a unique and comprehensive test routine for Brazilian current arboviral threats.

## B-073

### Performance Evaluation of the ADVIA Centaur CMV IgM Assay

S. Shin. *Siemens Healthcare Diagnostics, Tarrytown, NY*

**Objective:** Siemens Healthineers is currently developing a cytomegalovirus IgM (CMV IgM) assay to detect the presence of lgM antibodies to cytomegalovirus (CMV). Anti-CMV IgM antibodies are used as an aid in the diagnosis of recent or current CMV infection in individuals for which a CMV IgM test was ordered. The ADVIA Centaur® CMV IgM assay* is a chemiluminescent magnetic microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate the positive and negative agreement, precision, and cross-reactivity of a prototype automated CMV IgM assay tested on the ADVIA Centaur XP Immunoassay Systems.

**Methods:** The fully automated ADVIA Centaur CMV IgM assay is being developed as an indirect sandwich assay for the detection of CMV IgM antibodies in human serum and plasma for use on the ADVIA Centaur XP Immunoassay Systems. Positive agreement of the assay was evaluated with 104 samples that were positive by bioMerieux VIDAS and Siemens IMMULITE® 2000 CMV IgM assays. Negative agreement was determined by testing 300 pregnancy samples. The results were assessed based on Index values as reactive (index ≥1.0) and nonreactive (index <1.0). Samples from various disease conditions were tested for cross-reactivity. Precision was evaluated per CLSI EP5-A3 by testing six samples with Index values spanning the assay range in two runs per day for 10 days on the ADVIA Centaur XP system for a total of 40 replicates.

**Results:** Positive percent agreement of the ADVIA Centaur CMV IgM assay determined by testing CMV IgM-positive samples was ≥96%. Negative percent agreement determined by testing pregnancy samples was ≥98%. The assay was evaluated for potential cross-reactivity with other viral infections and disease-state specimens and no significant cross-reactivity. The assay demonstrated good precision with repeatability and within-run %CV of <4.0% and <6.0% respectively, for samples yielding Index values between 0.10 and 4.00.

**Conclusions:** The results of these studies demonstrate good performance of the prototype ADVIA Centaur CMV IgM assay on the ADVIA Centaur XP Immunoassay System.

*Under development. Not available for sale. The performance characteristics of this product have not been established.

## B-074

### Serological and molecular diagnosis of arboviruses in Brazil,2016

J. E. Levi, L. Scarpelli, L. Galindo, P. Nishimura, F. Flumian, L. Marani, T. Guerino, L. Pierroti, J. Alves, G. Campana. *DASA Laboratories, Barueri, Brazil*

**BACKGROUND:** Since the introduction of Zika (Zkv) and Chikungunya (Chkv) viruses to Brazil in 2014, several regions are witnessing the co-circulation of these two agents in addition to Dengue (Den) viruses which are endemic. Specific diagnosis of these pathogens is hampered by the cross-reactivity between flaviviruses (Denv and Zkv) and by shared symptoms. Although there is, as yet, no specific antiviral drug, patients shall be managed differently, according to the infectious agent. DASA is the largest Brazilian clinical pathology lab, covering a great extension of the nation, thus, reflecting on-going trends in the epidemiology of these 3 arboviral diseases.

**METHODS:** In year 2016, thousands of samples (table) were submitted to serological (IgG and IgM) or molecular testing upon medical request. Results were compiled and analyzed.

**RESULTS:** 57% (IgG) and 49% (IgM) of the samples submitted to Chkv serology were found reactive, for dengue 40% (IgG) and 23% (IgM) had positive results while these rates were much lower for Zika; 28% (IgG) and 3% (IgM). Among requests for Zkv and Chkv, women were approximately 70% of the patients, while for Denv they were 55%. Chkv, Denv and Zkv RNA were detectable in 18%, 7% and 5% of the samples respectively. Zkv RNA positivity was maximum in January (13%) declining over time.

**CONCLUSIONS:** The clinical hypothesis of Chkv had the highest rate of laboratory confirmation, both serological and molecular, probably reflecting the typical arthralgia associated to this infection. Low positivity for Zika tested samples may be attributed to a large number of symptomless patients being submitted to testing for reproductive planning, since this rate was similar among both genders. The highest rate of viremic samples in January suggests that in 2016 Zika outbreak happened earlier than observed for Dengue, which usually peaks in March/April.

**Table – Number of samples submitted to arbovirus testing at DASA, Brazil, 2016**

| | **MARKER** | | |
|---|---|---|---|
| | IgG | IgM | RNA |
| **AGENT** | | | |
| CHKV | 33,762 | 33,651 | 382 |
| DENV | 18,873 | 21,825 | 150 |
| ZKV | 5,275 | 9,226 | 3,227 |

## B-075

### Improvement to Workflow in Reagent Dispense System Design in the New Beckman Coulter DxM MicroScan WalkAway System

R. Milson, C. Griner, D. Lewis, K. Cullinan, W. Nick, M. Dubost, L. Grenz, A. Silviera, J. Chau, D. E. Roe-Carpenter. *Beckman Coulter, West Sacramento, CA*

The DxM MicroScan WalkAway instrument system is used with MicroScan panels for identification of microorganisms and detection of their susceptibility for relevant antimicrobial agents. In an effort to enhance user workflow, the DxM MicroScan WalkAway instrument reagent dispense system was re-designed to include a more robust liquid level sensing technology paired with a unique indicator for each reagent, oil, and waste container. The indicator lights are visible on the instrument exterior without having to interrupt instrument processing, improving workflow and usability. The bottle cap assembly employs a quick release mechanism that disconnects the reagent bottle and cap from the tubing allowing the operator to more easily replace reagents according to laboratory safety protocols. In conclusion, Beckman Coulter's use of Danaher Business System continuous improvement processes and voice of customer resulted in a new WalkAway system designed to improve productivity and operator safety.

## B-076

### Performance Evaluation of the ADVIA Centaur *H. pylori* IgG Assay

B. Bosch. *Siemens Healthcare Diagnostics, Tarrytown, NY*

**Background**: *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium found in the human stomach that infects nearly 50% of the world's population. Most infected individuals are asymptomatic. However, *H. pylori* infection has been associated with gastritis, peptic ulcers, and gastric cancers. Diagnosis of *H. pylori* infection is mainly performed by a gastric biopsy or the urea breath test. Both methods rely on the direct detection of the bacterium or its activity, are expensive, and can cause discomfort to the patient. By contrast, a serological test with good sensitivity and specificity could be the first choice for diagnosis because of its lower cost and ease of use. The ADVIA Centaur® *H. pylori* IgG assay* is a chemiluminescent two-step magnetic microparticle-based immunoassay that utilizes an *H. pylori* bacterium lysate in the solid phase and an acridinium ester molecule in the lite reagent. The assay is designed for the qualitative detection of *H. pylori* IgG antibodies in human serum and plasma (EDTA and lithium heparin) to rapidly identify *H. pylori* infection in symptomatic populations. The objective of this preliminary study was to evaluate method comparison, precision, and onboard stability of a prototype automated *H. pylori* IgG assay tested on the ADVIA Centaur XP Immunoassay System.

**Methods:** Positive percent agreement (PPA%) and negative percent agreement (NPA%) for *H. pylori* IgG were assessed by testing 313 serum samples against the LIAISON *H. pylori* IgG (DiaSorin) or IMMULITE® 2000 *H. pylori* IgG assays (Siemens Healthineers.). The sample origin was as follows: 233 unselected samples from Banc de Sang i Teixits de Catalunya (Spain) and 80 intended-use samples from Cerba (France). Other relevant characteristics including a 5-day precision study (CLSI EP15-A3) and reagent onboard stability (CLSI 25-A) were also evaluated.

**Results:** Evaluation of the patient samples indicated that the observed PPA% ranged from 95.1 to 95.6% and from 95.9 to 97.1% when compared to the LIAISON *H. pylori* IgG and IMMULITE 2000 *H. pylori* IgG assays, respectively. The observed NPA% ranged from 95.4 to 96.2% (vs. LIAISON assay) and from 94.4 to 95.8% (vs. IMMULITE assay). The assay demonstrated good preliminary precision, with repeatability and within-laboratory %CVs between 2.0 to 5.0% and 4.0 to 5.9%, respectively. In addition, initial reagent onboard stability was verified up to 8 weeks.

**Conclusions:** The results of this study demonstrate good preliminary performance of the prototype ADVIA Centaur *H. pylori* IgG assay in terms of method comparison, precision, and onboard stability.

*Under development. The performance characteristics of this product have not been established. Not available for sale and future availability cannot be guaranteed.

## B-077

### Genotyping of Hepatitis C Virus by a Single-Tube, One-Step RT-PCR, Microarray Based Test

R. Hudspeth[1], R. Fruge-Groulx[2], S. Chang[1]. *[1]Autogenomics Inc., Carlsbad, CA, [2]Personalized Diagnostics, Carlsbad, CA*

**Background:**

Hepatitis C virus (HCV) is a significant medical problem worldwide, as acute infection is typically asymptomatic and 75-85% of patients develop chronic infection, which may lead to cirrhosis and hepatocellular carcinoma decades later if not treated. Hepatitis C virus (HCV) is divided into six major genotypes (1-6) with multiple subtypes. The identification of HCV genotype is an important predictor of the virologic response and is used to select the most appropriate treatment protocol. Commercial tests for HCV genotyping rely on sequencing, real-time PCR or line blot methodologies. The aim of this study is to use pre-characterized HCV specimens to evaluate a single-tube, one-step RT-PCR microarray based test for HCV genotyping.

**Methods:**

A total of 110 plasma specimens were pre-characterized using the Siemens Versant HCV Genotype 2.0 Assay. HCV RNA was extracted from the plasma samples using the MagnaPure 96 Instrument and sample results obtained with the microarray based test were compared. HCV RNA was amplified by a one-step RT-PCR method with thermal activated PCR primers. Fluorescent labelling of PCR products was achieved by primer extension prior to hybridization to a microarray chip.

**Results:**

Of the 110 samples genotyped by the microarray base test, the concordance rate was 93.42% (71/76) for genotype 1, 100% (15/15) for genotype 2, 85.71% (12/14) for genotype 3 and 100% (5/5) for HCV negative samples. While no HCV genotypes 4, 5 or 6 were identified by either test, these genotypes are known to be rare in North America. Overall, the concordance rate was 93.64% (103/110) for all samples tested.

**Conclusions:**

This preliminary study of an HCV single-tube, one-step RT-PCR, microarray based test shows a good correlation with the Siemens Versant HCV Genotype 2.0 Assay. The microarray based test runs on an automated system and provides for reduced hands-on time.

FOR RESEARCH USE ONLY

## B-078

### Exploring Potential Quasispecies Variants of Influenza H1N1pdm09 Virus

K. Tsao[1], Y. Gong[1], S. Yang[2], C. Lin[2]. *[1]Dept. of Laboratory Medicine, Chang Gung Memorial Hospital and Research Center for Emerging Viral Infections, Chang Gung University, Taoyuan, Taiwan, [2]Dept. of Laboratory Medicine, Chang Gung Memorial Hospital and Dept. of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan*

**Background and Objectives:** Swine-origin H1N1 virus, also known as H1N1pdm09, emerged in 2009. Studies in 2011 revealed that a cooperative interaction of two genetic variants, namely viral quasispecies, increased population-level fitness and promoted viral growth, e.g., Asp (as D) and Gly (G) at position 222 in the hemagglutinin gene (HA-222). Incidence of H1N1pdm09 has risen to reach a new high in 2015. An outbreak in Taiwan caused over 1400 severe cases and 110 deaths from July 2015 to March 2016. However, little is known about the association between quasispecies variants and disease severity in this outbreak. Two objectives in this study were to detect genomic variants as potential signatures of viral quasispecies, in particular for 2015-16 outbreak strains, and to further estimate the prevalence of quasispecies variants from 2009 to 2016.

**Methods and Results:** We provided 10 Taiwanese H1N1pdm09 genomes isolated in the 2015-16 season by Sanger sequencing, including five severe (four patients had pneumonia, pleural effusion, and adult respiratory distress syndrome, and one had pneumonia and myocarditis), and five non-severe (four had upper respiratory infections, and one pneumonia case without other complications) cases. Influenza genome in approximately 13.5 Kb contained eight segments which included PB2, PB1, PA, HA,NP,NA, MP, and NS. In the 10 Taiwanese genomes, NA-74, -151, -314, and NS2-22 were detected, exhibiting more diversity than other positions. Only NA-151 showed residue "X" (as codon "RAC") and "D" ("GAC") in all 5 severe and 5 non-severe cases perfectly. This nucleotide ambiguity code "R" presented a dominant variant as "G" and a minor as "A". To estimate the prevalence of this minor variant, 7469 H1N1pdm09 genomes from 2009 to 2016 were downloaded from GISAID database. 94 translated nucleotide sequences exhibited non-D residues at NA-151, including 89"X", 4 "E", and 1 "N". Interestingly, increasing counts of X-residue in 2009, 2013, 2014, 2015, and 2016 were 1, 3, 4, 38, and 43, respectively. According to the prevalence of reported quasispecies signatures (HA-155, HA-222, and NA-275), we additionally identified 3 PB2, 1 PB1, 10 HA, 3 NA, 2 M2, 1 NS1, and 2 NS2 signatures in this study. **Conclusion:** This study began with focusing on exploring potential quasispecies variants in H1N1pdm09 genomes. For the 2015-16 outbreak season in Taiwan, mutation from Asp (D) to Asn (N) at NA-151 was detected in five severe cases, comparing to none in five non-severe cases. We also observed increasing prevalence of this variant from 2009 to 2016. This prevalence was under-estimated because the published sequences in database usually represent the dominant population of sequencing calls. This finding suggested that viral quasispecies at NA-151 was not a sporadic event and it might promote viral growth and further associate with disease severity. Subsequently, a total of 25 potential signatures were identified from 7469 genomes. In summary, these findings could help us to better understand the association of quasispecies variants and clinical outcome. More investigation is needed to demonstrate how those quasispecies signatures contributed to influenza outbreaks.

## B-079

### Analytical Validation of SAA Detection (O-SAA) by Particle-enhanced Immuno-turbidimetric Method

M. GU, K. Wang, H. Chen, Q. Wang, W. Shen, J. GU. *Upper Biotech Pharma Co.,Ltd, SHANGHAI, China*

Background:

Serum amyloid A (SAA) is an acute phase protein with a molecular weight of approximately 12kDa. In the acute phase reaction, SAA is synthesized in the liver and is regulated by interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF). Similar to C-reactive protein (CRP), SAA is a sensitive indicator of inflammation and the concentration of SAA may rise up to 100-1000 times of its base value, which is helpful for inflammation diagnosis, and the initiation of the appropriate therapy. It has been shown that SAA levels increase significantly in viral infections, while the increase in CRP levels are less obvious. Thus, SAA is a sensitive and reliable indicator for evaluating viral infection. In order to demonstrate the usefulness of SAA in early infection, a point-of-care testing method for SAA in human serum, plasma and whole blood, using the O-SAA latex enhanced immunoturbidimetric kit on the Ottoman analyzer (Shanghai Upper Biotech Pharma Co.,Ltd, China) was studied.

Method:

Simultaneous samples of serum, plasma and whole blood were collected from 100 individuals. The serum samples were measured using a SIEMENS BN II specific protein analyzer with the corresponding N Latex SAA kit. The samples of serum, plasma and whole blood (in each case n=100) were analyzed using Upper's O-SAA regent kits on the Ottoman Specific Protein work-station.

Results:

A. Serum samples comparison

Serum samples (n=100) within the reportable range of O-SAA (5-288mg/l) were analyzed on both Ottoman and Siemens system. Linear regression analysis showed excellent correlation ($R^2$=0.9869) between the two systems with a slope of 1.0317 for SAA.

B. Serum and plasma samples comparison on the Ottoman system.

Linear regression analysis showed excellent correlation ($R^2$=0.9874) between the serum and plasma samples with a slope of 0.9891 for SAA.

C. Serum and whole blood samples comparison on the Ottoman system.

Linear regression analysis showed excellent correlation ($R^2$=0.9855) between the serum and whole blood samples with a slope of 1.0037 for SAA.

D. Analytical Concordance

Concordance analysis of SAA showed excellent analytical agreement between O-SAA and BNII with a sensitivity of 100%, specificity of 95.3%, and a concordance of 98% for SAA.

Conclusions:

SAA concentration detected by Upper's O-SAA regent kits correlated well with SIEMENS BN II specific protein analyzer and the corresponding N Latex SAA kit. The plasma and whole blood results obtained with the O-SAA kit compared excellently to the results for the corresponding serum samples. It is concluded that SAA values for serum, plasma and whole blood can be reliably determined using Upper's O-SAA regent kits on the Ottoman system. Whole blood can be used with the O-SAA kit, saving the centrifugation step and allowing faster access to patients' results. The O-SAA kit on Ottoman provides a reliable, rapid and quantitative test which takes less than 5 minutes and is especially useful in evaluating viral infection in emergency situations.

## B-080

### Peformance evaluation of LumipulseG HTLV-I/II

K. Takahashi, M. Murata, H. Karasawa, A. Kaneko, K. Aoyagi. *Fujirebio. Inc, Hachiouji-shi, Tokyo, Japan*

**Background:** HTLV (Human T-cell lymphotropic virus) infects a type of white blood cell called T-cell or T-lymphocyte. There are two types HTLV, HTLV-I and HTLV-II, closely related human C retroviruses. HTLV-I is endemic in the Caribbean, Japan, South America, and parts of Africa. HTLV-I has been recognized as a cause of adult T-cell leukemia (ATL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-II is found among Native Americans and injections drug users in many city of Western Europe and North America. We have developed Lumipulse G HTLV-I/II as screening kit which can detect both anti-HTLV-I and anti-HTLV-II antibodies. This report presents the performance evaluation of Lumipulse G HTLV-I/II.

**Reagent:** Lumipulse G HTLV-I/II is an assay system for the qualitative detection of anti-HTLV in specimens based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE G System. This reagent uses a recombinant protein and peptides derived from three env proteins (HTLV-I gp21, HTLV-I gp46, HTLV-II gp46) and two gag proteins (HTLV-I p19, HTLV-II p19). The two step sandwich assay method is used to detect antibodies against these antigens. The amount of anti-HTLV antibodies in a specimen is automatically calculated from the calibration data. The result of the calculation is reported in cutoff index (C.O.I.) and then it is interpreted if the specimen is "reactive (C.O.I. $\geq$ 1.0)" or "non-reactive (C.O.I. < 1.0)".

**Methods and Results:**Presicion: The four negative specimens, the five positive serum samples and the four positive plasma samples were tested in duplicate per run, two runs per day for 20 test days, total 80 measurements per sample. The proportion of concordant test results was 100.0 % for each sample tested. For the positive samples, the within-run %CV ranged from 1% to 2%, the total %CV ranged from 2% to 3%.Specificity: A total of 5939 anti-HTLV-I/II negative samples were tested. These samples had negative result with Abbot Architect HTLV and included 230 potentially interfering samples. The results were non-reactive for 5933 samples, demonstrating a specificity of 99.9%.Sensitivity: A total of 300 anti-HTLV-I and 100 anti HTLV-II positive samples from different HTLV patients were tested. These samples had positive result with Abbot Architect HTLV. The results of Lumipulse G HTLV-I/II were reactive for 400 samples, demonstrating a diagnostic sensitivity of 100.0%. Dilution sensitivity: The diluted 20 positive samples were tested by Lumipulse G HTLV-I/II and Abbot Architect HTLV. Lumipulse G HTLV-I/II had equivalent or better dilution sensitivity compared with Abbot Architect HTLV.

**Conclusion:** Lumipulse G HTLV-I/II was developed by Fujirebio,Inc which can detect the antibodies against the three env proteins (HTLV-I gp21, HTLV-I gp46 and HTLV-II gp46) and the two gag proteins (HTLV-I p19 and HTLV-II p19) simultaneously. It was indicated that Lumipulse G HTLV-I/II has sufficient performance as HTLV screening kit.

## B-081

### Evaluation of Biochemical and Hematological Markers of Cerebrospinal Fluid in Suspected Patients with Meningitis

A. Hadadi[1], E. Masuadi[1], W. Tamimi[2]. *[1]College of Medicine, King Saud University For Health Sciences, RIYADH, Saudi Arabia, [2]King Abdulaziz Medical City (MNGHA), Riyadh, Saudi Arabia*

**Background**: The cerebrospinal fluid (CSF) is a fluid that frequently received by laboratory to analyze it for different parameters to aid physicians in diagnosis of many diseases. One of particular disease that required CSF fluids is Meningitis. The CSF and blood culture are gold standard test in establishing the diagnosis of Meningitis, however, it is labor intensive and require longer turn-around time before the information is amiable to the physician. There were a lot of effort to explore a better and quick markers in CSF to assist in rapid assessment for Meningitis. The aim of this study was to evaluate the levels of biochemical and hematological markers in the CSF samples received in our laboratory and compare them with the gold standard CSF culture. **Methods**: A total of 248 CSF samples were received from 137 patients, who were admitted to our hospital suspected of having Meningitis. The samples were collected by our physicians and sent to the laboratory to be analyzed immediately. Hematological and biochemical markers were performed in these samples including white cells (WC), glucose (Glu), and total protein (TP). The analyzers used in this study were Advia 2120 from Siemens Company for hematology markers and Architect from Abbott Company for biochemical markers. CSF culture were done for all samples in the microbiology laboratory. Statistics were done using SPSS IBM software version 20. The diagnostic sensitivity, and specificity were calculated. The p value of < 0.05 were chosen to be significant. **Results**: The majority of samples were received from neonate (<1 year) patients 61 (44.5%), children (<18 years) patients 36 (24.3%) and adult (>18 years) patients 40 (29.2%). Female were 47 (34.3%). The culture were performed in 195 CSF samples, 180 (92.3%) were negative and 15 (7.7%) were found positive for bacterial meningitis. The sensitivity for WC, TP and Glu were found to be 73.3%, 86.7%, and 60% respectively, while the specificity were found to be 75.4%, 36.1%, and 30.7% respectively. The positive predictive value (PPV) were calculated 20%, 10.2%, and 6.7% respectively, while the negative predictive value (NPV) were found to be 97.1%, 97%, and 90.2% respectively. The overall accuracy of these tests were found to be 75.3%, 40% and 33% respectively. **Conclusion**: These tests showed a good sensitivity but low specificity with exception of WC. These tests were good enough to rule-out meningitis.

## B-082

### Performance Evaluation of the DxN Zika Virus Assay - EUA on the Beckman Coulter DxN VERIS Molecular Diagnostic System (EUA Version)*

J. D. Tibodeau Jensen[1], B. C. Astry[1], E. M. Cicirelli[1], H. J. Coyne III[1], E. O. Johnson[1], J. M. Prout[2], I. M. Levine[1], K. K. Kapsner[1], J. L. Sackrison[1], S. J. S. Gaston[1]. *[1]Beckman Coulter, Chaska, MN, [2]Beckman Coulter, Danvers, MA*

OBJECTIVE: Zika Virus became a global health crisis in 2016, leading to the FDA granting "Emergency Use Authorization" (EUA) regulatory status to certain Zika assays. In response, Beckman Coulter developed a qualitative molecular Zika assay on the DxN VERIS System. The objective of this study was to evaluate the performance of the assay.

METHODS: The DxN Zika Virus Assay - EUA is a fully automated Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay for the detection of Zika Virus RNA in human serum and EDTA plasma. The assay was evaluated for positive and negative percent agreement (PPA and NPA) via method comparison, sensitivity (Limit of Detection; LoD), sample matrix equivalency, cross-reactivity, and inclusivity. The PPA and NPA of the assay against the Roche LightMix® Zika rRT-PCR Test (EUA) were evaluated using a total of 215 clinical patient samples. Discordant samples were tested on the Hologic Aptima® Zika Virus Assay. To test LoD, a 5-replicate range finding study was first conducted to determine the tentative LoD, followed by a 20-replicate study near the tentative LoD. To test for sample matrix equivalency, 40 paired EDTA plasma/serum samples were evaluated at three target concentrations. Cross-reactivity was evaluated using *in silico* sequence alignments and by testing microorganisms, viruses or purified nucleic acid from the following: Dengue Types 1-4, West Nile Virus, St. Louis Encephalitis, Chikungunya, Mayaro Virus, Parvovirus, Plasmodium falciparum, and Yellow Fever Vaccine Strain. Inclusivity analysis of Zika virus strains was conducted by testing both contemporary and African-origin strains, and by performing a BLAST *in silico* analysis.

RESULTS: Evaluation of patient samples using the DxN Zika Virus Assay - EUA indicated a PPA of 96% (48/50) and the NPA ranging from 45% (25/55) to 100% (50/50 and 60/60) when compared to the Roche assay. The 30 discordant samples from the 45% NPA set were evaluated on the Hologic assay; 24/30 of these samples were positive in disagreement with the Roche assay. The LoD was determined to be 126 copies/mL. This compares well to the reported LoDs of the Roche assay (181 copies/mL) and the Hologic assay (5.9 copies/mL). LoD testing was also performed using FDA Zika reference material and was determined to be 200 RNA NAAT Detectable units/mL for Sample S1 and 500 RNA NAAT Detectable units/mL for Sample S2. Serum and EDTA plasma matrix equivalence was demonstrated across all sample concentrations. No cross-reactivity to other microorganisms was detected in the assay. The assay was determined to be specific to contemporary strains of Zika since African-origin Zika isolates tested were not detected at 1.5xLoD. *In silico* inclusivity analysis showed the contemporary strains had ≥95% homology with the assay primers and probe, while the African-origin strains showed lower (82% to 96%) homology.

CONCLUSIONS: Beckman Coulter's DxN Zika Virus Assay - EUA demonstrates good performance with respect to positive and negative agreement, sensitivity, sample matrix equivalency, cross-reactivity, and inclusivity.

*DxN Zika Virus Assay - EUA and DxN VERIS Molecular Diagnostics System (EUA version) have been submitted to the U.S. FDA for Emergency Use Authorization. Not available for distribution in the U.S.

## B-083

### Clinical Performance of Elecsys® HBsAg II in Subjects with Increased Risk of Hepatitis

S. Jortani[1], S. Jortani[2], P. Jones[3], M. Lessig[4], R. Ostlund Jr.[5], E. Schiff[6], J. Layton[7], R. Dworschack[7]. *[1]Kentucky Clinical Trials Laboratory, Louisville, KY, [2]University of Louisville, Louisville, KY, [3]Formerly South Bend Medical Foundation, South Bend, IN, [4]Formerly Nationwide Laboratory Services, Fort Lauderdale, FL, [5]Core Laboratory for Clinical Studies, Washington University, St. Louis, MO, [6]University of Miami Hepatology Diagnostic Laboratory, Miami, FL, [7]Roche Diagnostics, Indianapolis, IN*

A multicenter clinical performance study of Elecsys® HBsAg II immunoassay and Elecsys HBsAg confirmatory test on **cobas e** 601 analyzer was recently completed at three US sites. Study population consisted of adult, pregnant and pediatric subjects at risk for hepatitis (sexual practice, behavior, medical status or occupation). Elecsys assay is an automated sandwich immunoassay based on the chemiluminescence principle where complexes of sample HBs antigen, biotinylated-/ ruthenylated-anti-HBsAg antibodies, and streptavidin-magnetic microparticles are captured on an electrode. The primary objective was to evaluate percent agreement between Elecsys HBsAg II and reference assay. The secondary objectives included evaluation of specificity, seroconversion sensitivity, and imprecision.

Abbott ARCHITECT HBsAg and HBsAg confirmatory reference testing was performed at two US sites. Final HBsAg interpretation was based on the confirmatory testing of repeatedly reactive samples.

Positive /negative percent agreements with 95% confidence limits for various cohorts are listed below.

| Cohort | Positive | | Negative | |
|---|---|---|---|---|
| | n | Percent Agreement, CL | n | Percent Agreement, CL |
| **Adult** | 39 | 100.00% 90.97-100.00% | 2020 | 99.75% 99.42-99.92% |
| **Supplemental[1]** | 391 | 99.23% 99.77 -99.84% | 6 | 83.33% 35.88-99.58% |
| **Pediatric** | 0 | NA NA | 128 | 100.00% 95.61-99.68% |
| **Pregnant** | 13 | 100.00% 75.29-100.00% | 205 | 100.00% 98.22-100.00% |
| [1]Population to enhance prevalence of acute and chronic hepatitis B subjects | | | | |

Two hundred and sixty-nine specificity specimens representing 21 diseases demonstrated agreement of 99.33% to 100.00%. Additionally samples spiked with bacterial and viral extracts demonstrated concordance with un-spiked control.

Seroconversion sensitivities in test and reference assays were equivalent in thirteen of fourteen commercial panels tested; Elecsys HBsAg II converted one draw later in one panel. Imprecision (CLSI EP5-A3) was evaluated using three reagent lots in three US sites. Three replicates of imprecision pools were tested in two runs per day for five days. Repeatability %CV values for pools (mean COI ≥0.70) ranged from 2.3 to 4.6%. %CVs for Reproducibility ranged from 3.4 to 6.0%. The C5–C95 interval around assay cut-off (CLSI EP12-A2) ranged from 0.93 to 1.06 COI.

Evaluation of Elecsys HBsAg II assay and Elecsys HBsAg confirmatory test on **cobas e** 601 analyzer demonstrated acceptable clinical/analytical comparison against the reference assay.

## B-084

### Update on "Strategies for Improving Rapid Influenza Testing", the New Spanish Version, and "Influenza Preparedness and Response" Courses for Clinicians

L. Williams[1], S. Chitavi[2], R. Thomas[2]. *[1]CDC, Atlanta, GA, [2]The Joint Commission, Oakbrook Terrace, IL*

**Objective**: To enhance the knowledge of clinicians about rapid influenza testing (RIDT) and pandemic preparedness by offering free online courses, "Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS)" and "Influenza Preparedness and Response in Ambulatory Settings" with continuing education credits. Further objectives were to gather feedback from participants about the courses and other educational needs. Based on that feedback, and the need to expand outreach to participants whose primary language is Spanish, a new Spanish version of SIRAS was developed. **Relevance:** RIDTs are the most widely used screening tests for influenza and are often used for making treatment decisions, despite concerns about their variable predictive value. A survey conducted by the CDC and TJC in 2012 identified the need for education on the use of RIDT, interpretation of results, and influenza pandemic preparedness. Feedback from these courses, identified the additional need for a Spanish version of SIRAS. **Methods**: The SIRAS course was developed and launched in Oct. 2012 (http://www.jointcommission.org/siras.aspx). The pandemic preparedness course (http://www.jointcommission.org/topics/influenza_pandemic_preparedness.aspx) was developed and launched in Apr. 2015. Both courses were developed with a technical panel of influenza experts. Specimen collection videos https://www.youtube.com/playlist?list=PLNQfL_CJ36fK08KEPjxu1ZKJn7GuFtn-N, and other e-resources are offered with both courses. Courses are updated annually and relaunched in Oct. of the respective year. In 2016 the SIRAS course was translated into Spanish. TJC uses multiple media channels to market these courses. Course utilization data are compiled annually; e-resource utilization is tracked cumulatively. **Validation**: Since 2012, there were 10,785 unique visitors to the SIRAS webpage, 4,015 unique visitors to the Pandemic Preparedness webpage, and 186,795 views of specimen collection videos. In 2016, 80% (535/668) of participants completed a voluntary SIRAS course evaluation; 33% said the course validated current practices and 29% planned to change practices based upon course content. Of those planning to change practices and describing which practices (119/153), 18% planned to change influenza surveillance practices, 13% infection control practices, 11% influenza diagnostic testing practices, and 18% planned to take the influenza preparedness and response course, offered by CDC and TJC, to improve pandemic planning. Of the participants who evaluated SIRAS in 2016 (n=535), 99% found the course useful, 94% had a better understanding of RIDT, and 89% would recommend the course to others. The Spanish version of SIRAS was launched in Oct. 2016. In 2016, 25% (265/1072) of participants completed a voluntary Pandemic Preparedness course evaluation. Of participants who planned to change practices and described the changes (188/263), 25% planned to develop or change preparedness response plans, 37% to improve communication between their practices and local/state health departments, and 34% to improve specimen collection procedures. Of those completing the course evaluation in 2016 (n=265), 86% were satisfied overall and 82% were likely to recommend the course to others. **Conclusions**: The ongoing opportunity for continued education in influenza testing and preparedness was welcomed by participants. Annual updating of courses before the onset of influenza season attracts increased usage of e-resources and enrollments to the course. Specimen collection videos, and other e-tools continue to be popular.

## B-085

### Evaluation of the Utility of CMS Claim Data for Early Detection of Increasing Influenza Activity

L. Fan[1], R. Astles[1], H. Burkhom[2]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Johns Hopkins University, Applied Physics Laboratory, Baltimore, MD*

Objective: To evaluate the utility of CMS Medicare claim data for early detection of increasing influenza (flu) activity using weekly volume of flu diagnoses (V_dx) or flu rapid tests (V_test) irrespective of the test results, which are not included in CMS data.

Methods: We compared CMS Medicare outpatient claims (OTP) data with positive test volumes using rapid flu tests from the CDC National Respiratory and Enteric Virus Surveillance System (NREVSS) as the "gold standard" for flu activity. We compared both V_dx and V_test to the gold standard for each of 10 selected states (one per HHS region). Spline models were developed and applied to fit the time series of weekly volumes of V_dx, V_test, and NREVSS test positives (dependent variables) for 5 influenza seasons (2007 to 2012), with the week number as the independent

variable. Using the fitted models, we calculated predicted weekly volumes and standard deviations (SD) for V_dx and V_test. Criteria for an initial alert were defined as: 1. an increase of the predicted volume from the previous week by two SD (2 SD criterion), or 2. an increase of the predicted volume from previous week by 0.45 SD for two consecutive weeks (0.45 SD criterion). A flu "episode" was defined as the interval between an initial alert and a drop in volume meeting the same criterion.

Results: We detected 67 flu episodes from the 10 states in NREVSS (2007-12) using spline models. With the 0.45 SD and 2 SD criteria respectively, the OTP predictors using V_dx data identified 60 and 41 out of the 67 episodes, and 64 and 49 episodes out of the 67 episodes using V_test data. With the 0.45 SD criterion, 83% of episodes detected using V_dx and 85% using V_test were also detected in NREVSS. Using the 2 SD criterion, 93% of episodes detected using V_dx and 98% using V_test were also detected in NREVSS.

With the 0.45 SD criterion, V_dx (V_test) detected flu activity an average of 3.1 (5.7) weeks earlier than NREVSS predictions. With the 2 SD criterion, V_dx (V_test) detected flu activity an average of 3.6 (2.6) weeks later than NREVSS predictions.

Conclusions: This study demonstrated the potential use of CMS claims data for early detection of increasing influenza activity in specific regions of the U.S. In this retrospective analysis, spline models performed well for early detection of an increase in flu activity. The 0.45 SD criterion demonstrated greater sensitivity over the 2 SD criterion yielding earlier detection by an average of over three weeks than predictions using the gold standard data. The consecutive week exceedance requirement using 0.45 SD, which required two consecutive changes in the same direction to identify a change in activity, reduced the occurrence of "false peaks" and resulted in reasonably manageable positive predictive values. In our study, V_test was equally effective to V_dx for identifying influenza activity in this convenience sample. In this setting, neither test results nor diagnosis were absolutely necessary for surveillance using CMS data.

## B-086

### Performance Evaluation of the ROCHE E 170 for the Determination of Procalcitonin in blood.

R. Khoury, A. Gandhi, b. P. Salmon, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

**Background:** Sepsis is a systemic inflammation response caused by infection. The rates of hospital admissions for sepsis exceed those of myocardial infection and stroke and it is a major health care expense due to the high incidence and mortality. Early intervention prevents the sepsis to progress to severe sepsis and septic shock. The dilemma begins with proving the presence of infection and the need for a reliable marker to diagnose sepsis; however laboratory tests for sepsis were either non-specific or require longer turn-around time. Procalcitonin could be the promising biomarker; it is precursor for calcitonin and secreted from C-cell in the thyroid but under certain circumstance like systemic infection ptocalcitonin is secreted from other cells making its concentration rises in the blood. Studies have shown that its concentration in the blood increase rapidly in patient with sepsis; in addition, its concentration correlate well with the severity of the infection, the higher concentration of procalcitonin are associated with higher risk to progress to septic shock.

**Methodology:** Roche Elecsys BRAHMS procalcitonin assay is a sandwich assay with a total incubation of 18 minutes. The assay is **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" with 2 incubations; results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

We evaluated: the sensitivity, linearity, within assay coefficient variation for replicates from synthetic materials and from patient sample, between assay coefficient of variation, reportable range, and correlation was done using reference laboratory. Statistical analyses were done using Analyse-it.

**Results:** the sensitivity was 0.02 ng/mL using non-serum matrix sample and 0.06 ng/mL for human sample. Within assay coefficient variations were 9.2%, 2.0%, and 1.0% for a concentration of 0.021 ng/mL, 0.462 ng/mL, and 9.367 ng/mL respectively. Between assay coefficient variations were 4.7% and 6.5% for a concentration 0.47 ng/mL and 9.0 ng/mL respectively. Analytical range was verified from 0.02-100 ng/mL. Regression analysis between the reference laboratory and Roche PCT gave a slope of 1.02 and intercept 0.01 and correlation coefficient of 0.9738.

**Conclusion:** the Roche Elecsys BRAHMS procalcitonin assay gave the benefit of a fully automated, high throughput, high precision and acceptable sensitivity assay. Although the sensitivity of the assay was above the published cut-off for healthy people but it was well below the range for systemic bacterial infection. The accuracy, precision, and sensitivity of the assay make it suitable as a diagnostic marker and a part of the antibiotic stewardship by monitoring the progression of infection and when to begin or stop the antibiotics.

## B-087

### New Molecular Diagnostic Assays for the Detection of *Chlamydia trachomatis*/*Nisseria gonorrhoeae and Trichomonas vaginalis* on the DxN VERIS Molecular Diagnostics System

Z. Wang[1], R. Mathur[1], K. Lykstad[1], J. Fung[1], B. Bastin[1], V. Colinayo[1], G. Stevenson[1], L. Mai[1], C. Sciabica[1], S. Sahar[1], A. Miller[1], S. Chan[1], P. Lee[1], C. Scheele-Bohnstedt[1], J. Buckmeier[1], D. Stone[2], A. Gustafson[2], J. Kumar[2], S. Madden[2], C. Bracesco[2], T. Mallinger[2], J. Slater[3], J. Williams[1], J. Rodriguez[1], N. Tusneem[1]. *[1]Beckman Coulter, Brea, CA, [2]Beckman Coulter, Chaska, MN, [3]Beckman Coulter, Danvers, MA*

**OBJECTIVE:** Beckman Coulter is currently developing assays for the detection of sexually transmitted diseases on its DxN VERIS System including *Trichomonas vaginalis* (TV) and a multiplex assay for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). CT and NG may produce asymptomatic infections, especially in women, and if left untreated, can lead to serious reproductive and other health problems with both short-term and long-term consequences. TV, which colonizes in both men and women, is a protozoan parasite responsible for causing trichomoniasis. The DxN CT/NG and DxN TV Assays are qualitative *in vitro* diagnostic assays for the detection of DNA from theseorganisms in male/female urine, vaginal (patient and clinician collected) and endocervical swabs, and PreservCyt® specimens from symptomatic and asymptomatic individuals. The two assays use magnetic particle extraction and TaqMan® PCR technology on the DxN VERIS platform to detect specific gene sequences of CT/NG and TV. **METHODS:** The DxN CT/NG and DxN TV Assays are designed to detect DNA in a sample collection tube with a pierceable cap. Performance of the two assays was evaluated for analytical sensitivity and specificity. These evaluations included limit of detection, precision and reproducibility, potential interfering substances, and cross reactivity. Study methods were based on Clinical and Laboratory Standards Institute (CLSI) Guidelines. Additionally, the DxN CT/NG and DxN TV Assays were evaluated against two comparator assays with clinical samples. Sensitivity and specificity to the patient infected status (PIS) was calculated. PIS was determined to be positive if two comparator molecular assays produced positive results. PIS was determined to be negative if the two comparator assay produced negative results. If the two comparator assays produced discrepant results, a tie-breaker comparator assay was run to determine the PIS. **RESULTS AND CONCLUSIONS:** Both DxN TV and the DxN CT/NG assays have a turn-around time from sample to result of ≤ 75 minutes. Evaluation of clinical samples across specimen types demonstrated that the DxN CT/NG assay was 96 to 100% sensitivity and 97 to 100% specificity when compared to the PIS for the CT analyte. DxN CT/NG achieved a LoD of 10 to 25 EB/mL for CT Serovars D and H and 5 CFU/mL for NG strains 19424 and 49226. The assay produces reproducible results and demonstrated good precision with a CV of 1-2 % at 3XLoD levels for both organisms. The DxN TV Assay demonstrated a LoD of 1.5 to 4.5 TV/mL with the Metronidazole-susceptible (30001), and Metronidazole-resistant (50143) strains of TV depending on the sample type. Evaluation of clinical samples on the DxN TV assay achieved 97% to 100% sensitivity and 95% to 98% specificity when compared to the PIS. The assay produces reproducible results and demonstrates good precision with a CV of 1.2 to 1.7% at 1X and 3X LoD. *DxN CT/NG and DxN TV Assays are in development. DxN Veris product line has not been submitted to U.S. FDA and is not available in the U.S. market. DxN VERIS Molecular Diagnostics System is also known as VERIS MDx Molecular Diagnostics System and VERIS MDx System.

## B-088

### *Burkholderia Cepacia* Outbreak in Long-Term Care Facilities.

R. Khoury, A. Gandhi, P. Patel, H. Massey, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

**Background:** *Burkholderia Cepacia, a gram negative bacilli,* is a group or complex of bacteria that can be found in soil and water. It is more colonizing bacteria than infecting bacteria; however it may cause problem if found in people with weak immune system, cystic fibrosis, or if found in sterile body fluid. A recent multistate outbreak of *Burkholderia Cepacia* in blood was reported due to contaminated prefilled saline flush was reported. A majority of the cases are reported in long-term care facilities or rehabilitation facilities.

**Methodology:** During the months of 2016 in which the contaminated saline was used in Long-Term Care Facilities, 1276 sets of blood cultures collected from the residents. Every set included two vials (aerobic and anaerobic) which were incubated in a Bactec instrument. Positive cultures were subcultured and then identified using Microscan96 Walkaway conventional panels. Data analyses were done for all the facilities and then isolating the facilities with positive isolate for *Burkholderia Cepacia*. Statistical analyses were done using Analyse-it.

**Results:** 15.4% of the total blood cultures were positive. *Burkholderia Cepacia* accounted for 12.8% of these positive cultures, and was found only in 7 facilities, accounting for127 of the 1276 cultures tested. Of these 127 patients, 28% of them had a positive blood culture. Of those positive cultures 71.4% of the bloodstream infections were due to *Burkholderia Cepacia*. No deaths to our knowledge were reported due to *Burkholderia Cepacia* in the identified patients.

| | All Facilities | Affected facilities |
|---|---|---|
| % positive blood culture | **15.4%** | **28.0%** |
| % B. Cepacia/all blood culture | **2.0%** | **19.7%** |
| % B. Cepacia/total positive blood culture | **12.8%** | **71.4%** |

**Conclusion:** *Burkholderia Cepacia* is a threat if found in blood culture, especially in long-term care facilities where most of the residents are elderly, frail, disable, and are on multiple medications; in addition, *Burkholderia Cepacia* is resistant to common antibiotics. Early detection and appropriate treatment would benefit the patient. Also the awareness of the contaminated saline decreased the spread of the bacteria to other patients.

## B-089

### Performance Evaluation of the VERSANT HCV Genotype 2.0 Assay (LiPA)

M. P. Lau, M. Hennig, N. Shimasaki, A. Bustamante, R. Reid, Y. Labiad, I. Mehlhorn, J. Surtihadi, C. Cosenza, E. Rudie, A. Lal, K. Rehman. *Siemens Healthcare Diagnostics Inc., Berkeley, CA*

**Background:** Determination of the HCV genotype is important to prescribe appropriate HCV treatment and predict response to antiviral treatment. HCV genotyping provides clinicians with important, useful tools to optimize HCV treatment type, dose, and duration, allowing the best chance of eradicating HCV from the patient's body.

**Methods:** The VERSANT® HCV Genotype 2.0 assay (LiPA) is a line probe assay that identifies HCV genotypes 1-6 and subtypes a and b of genotype 1 in human serum or plasma specimens. Using reverse hybridization technology, biotinylated DNA PCR product generated by RT-PCR amplification of the 5' UTR and core regions of HCV, RNA is hybridized to immobilized oligonucleotide probes.

Analytical validity and clinical validity studies were performed at three clinical trial sites to validate the performance of the VERSANT HCV Genotype 2.0 assay (LiPA). The genotyping rate (GR) and genotyping accuracy (GA) were evaluated for each HCV genotype/subtype in order to assess the integrity of the assay results compared to the NS5b reference method. Results were interpreted manually and using the semi-automated method.

The clinical utility of the VERSANT HCV Genotype 2.0 assay (LiPA) was assessed by evaluating the association between HCV genotype and the probability of achieving SVR, defined as undetectable HCV RNA levels (<25 IU/mL) 12 weeks after cessation of treatment ($SVR_{12}$) using clinical samples treated with one of the following combinations: a) sofosbuvir (SOF), pegylated interferon (peg-IFN), and ribavirin (RBV); b) SOF and RBV; or c) SOF and ledipasvir (LDV).

**Results:** The results for the analytical validity study are as follows: The GR for each HCV genotype/subtype ranged from 86.7 to 100% for the manual interpretation method and 83.3 to 100% using the semi-automated method. The GA for each HCV genotype/subtype using either method of interpretation was 100% except for subtype 1a (99%) and 1b (98%).

In the clinical validity study, the $SVR_{12}$ rate for all patients tested was 88.5% (192/217) for both methods. For individual genotypes/subtypes, the observed $SVR_{12}$ rates ranged from 72.5 to 100%. Results indicate patients diagnosed with genotype 1 had significantly lower $SVR_{12}$ rate compared to pooled non-1 genotype patients. The relationship between the HCV genotype/subtype and the $SVR_{12}$ rate is essential for achieving successful clinical outcomes in chronically infected HCV patients treated with direct-acting antiviral (DAA) regimens.

**Conclusions:** The VERSANT HCV Genotype 2.0 assay (LiPA) provides interpretable and accurate diagnosis of HCV genotypes 2, 3, 4, 5, and 6 and subtypes 1a and 1b as shown by an overall GR and GA rate of ≥88% and ≥99%, respectively, in both studies. $SVR_{12}$ rate by genotype/subtype is consistent with published results using similar treatments with DAAs.

The clinical evaluation of the assay demonstrates reliable identification of HCV genotype/subtype for optimal patient therapy.

VERSANT® HCV Genotype 2.0 Assay (LiPA) [Reagents: Genotype 2.0 Kit, Amplification 2.0 Kit, and Control 2.0 Kit] is CE-marked in Europe.For Research Use Only in the United States. Product availability varies from country to country and is subject to local regulatory requirements.

## B-090

### Automated Molecular Detection of *Helicobacter pylori* and its Resistance to Clarithromycin in Human Tissue Biopsies

W. Wu[1], A. Al-Thuwaybi[1], A. Naik[1], S. Topor[2], M. Greene[2], S. Chang[1]. *[1]Autogenomics, Inc., Carlsbad, CA, [2]Select Diagnostix, Northbrook, IL*

Introduction:

According to the World Health Organization (WHO), Helicobacter pylori is a class I carcinogen that affects approximately 50% of the world's population. H. pylori in the gastric mucosa can lead to gastritis, duodenal ulcers and gastric cancer. When an endoscopy is performed in patients with dyspepsia, a gastric biopsy should be taken to evaluate for the presence of H. pylori. Infected patients should be offered some form of eradication therapy. The eradication therapy involves the use of a combination of antibiotics, such as amoxicillin, metronidazole, clarithromycin, combined with proton pump inhibitors; however, antibiotic resistance is a major cause for treatment failure. The gold standard diagnostic procedure for H. pylori detection is the use of a gastric fluid culture and antimicrobial susceptibility testing which typically takes a minimum of 5 days to obtain a definitive result. Other more rapid methods have low sensitivity for the detection of H. pylori including the helicobacter-urease assay from gastric biopsies, antigen testing from stool or breath tests.

Objective:

The objective of our study was to evaluate a molecular based diagnostic test for H. pylori called the Hpylori-Q Assay (Autogenomics, Inc., Carlsbad, CA), an automated multiplexed film-based microarray assay that can simultaneously detect H. pylori and its resistance to clarithromycin from human biopsy samples in a clinic setting.

Results:

A total of 100 gastric biopsies were performed to isolate genomic DNA (50 H. Pylori positive and 50 H. Pylori negative), from specimens previously evaluated with the M-PCR assay (Tsang et al, Gastroenterol Res Pract. 2012). The 100 DNA samples were retested with the Autogenomics Hpylori-Q assay. The results showed that, the Hpylori-Q assay detected 48 out of the 50 H. pylori positive DNA specimens (96% sensitivity) and identified all of the 50 negative DNA as negative for H. pylori (100% specificity). The positive predictive value was 100% with a negative predictive value of 96%. In addition, 17 out of the 50 positive specimens were tested as clarithromycin resistant (34%), while 9 had a A2142G point mutation, another 9 DNA carried A2143G point mutation and one carried both A2142G, and A2143G mutations. The limit of detection for H. pylori was 22 copies per test for the Hpylori-Q assay.

Conclusions:

In this study, we demonstrated that the Hpylori-Q Assay, a molecular based DNA microarray assay is not only extremely sensitive for detecting H. pylori but highly specific for H. pylori and can identify specific clarithromycin resistant strains to better manage treatment of H. pylori. The Hpylori-Q Assay can facilitate the appropriate selection of treatment for patients with H. pylori infection that have clarithromycin resistance.

## B-091

### Panel of Zika virus infection diagnosis.

L. C. Pierrotti, L. Scarpelli, L. Galindo, P. Nishimura, F. Flumian, J. Levi, L. Faro, G. Campana, J. Alves. *DASA Diagnósticos da América, Barueri, São Paulo, Brazil*

**Background.** Laboratory diagnosis of Zika virus (ZIKV) infection depends on the period of infection. During viremic period, diagnosis is based on PCR; and during nonviremic period, over one week from the clinical onset, is based on serology - IgM detectable for 3 to 6 months, and IgG probably lifetime. ZIKV-PCR is detectable on blood within the first week of clinical illness and on the urine up to 21 days after onset of symptoms. We present a retrospective analysis of diagnosis panel of ZIKV infection performed at the laboratory during 2016.

**Methods.** During 2016 we performed 30,799 tests for ZIKV diagnosis; 78 samples were submitted to the full panel of diagnosis, defined as RT-PCR (on blood and/or urine) and ELISA serology performed at the same blood collection.

The serology (Euroimmun kit) is defined as negative (index <0.8), indeterminate (index range from 0.8 to 1.09) and positive (index >1.09).

**Results** Majority of patients were women (60; 78%), average age was 35.8. Seventy-three patients were non-reactive by PCR from blood (table 1).

Only one sample presented positive PCR on urine. At the time of panel sample collection, 18 samples (23%) were IgG positive.

Table 1. PCR and ELISA serology results among 78 patients, Brazil, 2016.

| | | PCR urine | | ELISA_IgM | | Elisa_IgG | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Indet/ Negative | Positive | Indet/ Negative |
| **PCR blood** | **Negative (N=73)** | 1 | 72 | 6 | 67 | 18 | 55 |
| | **Not done (N=5)** | 0 | 5 | 0 | 5 | 0 | 5 |
| **TOTAL (%)** | | 1 (1.3%) | 77 (98.7%) | 6 (7.7%) | 72 (92.3%) | 18 (23.1%) | 60 (76.9%) |

**Conclusions.** Our results demonstrated that concomitant PCR and serology add no value to the laboratory diagnosis. It is important to optimize the laboratory investigation of ZIKV infection to have the highest diagnostic yield and save costs; serology testing should be performed for samples not tested by PCR or those are found to be negative. IgG positivity may be partially attributed to cross-reaction with antibodies due previous flavivirus infection.

## B-092

### Frequency of instrument, environment, and laboratory technologist contamination during routine diagnostic testing of infectious specimens

M. L. Yarbrough, J. H. Kwon, M. A. Wallace, T. Hink, A. Shupe, V. J. Fraser, C. D. Burnham. *Washington University School of Medicine, Saint Louis, MO*

**Background:** Laboratory testing to support the care of patients with highly infectious diseases such as Ebola virus may pose a risk for laboratory workers. However, data on the risk associated with various laboratory procedures and virus transmission during routine laboratory testing are sparse. Our objective was to evaluate contamination of laboratory equipment, the laboratory environment, and the laboratory worker during routine analysis of patient specimens using two approaches—fluorescent markers and a molecular surrogate of a high-titer viral infection. **Methods:** To mimic Ebola virus, which can be present in very high concentrations in blood and body fluids, de-identified remnant specimens were "spiked" with the MS2 bacteriophage, a single-stranded RNA virus that is nonpathogenic to humans, at a concentration of virus of 1.0 x $10^7$ PFU/mL. The exterior of specimen containers was treated with a fluorescent Glo Germ powder (not visible to the eye) to visualize the contamination of laboratory surfaces during routine testing on point-of-care (POC) instruments. Laboratory testing performed and matrix tested included FilmArray Biothreat panel (BioFire, blood), FilmArray Gastrointestinal panel (BioFire, stool), FilmArray Respiratory Panel (BioFire, nasopharyngeal swab in transport medium), FilmArray Blood Culture Identification panel (BioFire, positive blood culture broth), Xpert Flu/ RSV (Cepheid, nasopharyngeal swab in transport medium), urine dipstick (urine), BinaxNOW Malaria antigen (Alere, blood), and a Piccolo comprehensive metabolic panel (Abaxis, plasma). Laboratory testing was performed by two experienced laboratory technologists using standard testing and cleaning procedures and with standard laboratory personal protective equipment (PPE) and procedures. Testing was performed on multiple days. After each test was setup, gloves were removed and samples were run on the corresponding POC instrument or test device. To assess for contamination, laboratory surfaces and the PPE and skin of laboratory technologists was monitored for transfer of fluorescent markers with UV light and the MS2 molecular marker using RT-PCR (Cepheid Smart Cycler). **Results:** Transfer of fluorescence to gloves was observed during all rounds of routine testing. Fluorescence transfer to bare hands and contamination of the biosafety cabinet surface was observed in 6/16 (38%) and 7/16 (44%) tests performed, respectively. Fluorescence was observed on test cartridges/devices and auxiliary equipment/reagents in 4/14 (29%) and 8/12 (67%) tests performed. Importantly, no fluorescence transfer to downstream laboratory instrumentation, hardware, or exposed surfaces was observed.

**Conclusion:** Simulation of grossly contaminated specimens using a fluorescent marker illustrated that proper adherence to PPE and procedures during testing setup prevented further contamination of the laboratory environment. These studies may increase our understanding of the risk for transfer of highly infectious samples to laboratory surfaces to better inform recommendations for laboratory safety practices and reduce the risk of pathogen transmission to laboratory workers.

## B-093

### Zika virus RT-PCR performed in urine and plasma.

L. C. Pierrotti, L. Scarpelli, L. Galindo, P. Nishimura, F. Flumian, L. Faro, G. Campana, J. Levi, J. Alves. *DASA Diagnósticos da América, Barueri, São Paulo, Brazil*

**Background.** Zika virus (ZIKV) infection causes a transient viremia and the diagnosis by RT-PCR has been most successful within one week after the onset of clinical illness. The practical guidelines have recommended search for ZIKV-nucleic acid (RNA) on blood and urine. ZIKV RNA can be detected longer in urine than in serum, extending the period that a definitive diagnosis of ZIKV infection can be established by RT-PCR. The objective is analysis pairs of RT-PCR on urine and blood to assess the positivity of the test on these two different clinical specimens.

**Methods.** During 2016 we evaluated 257 samples of urine and plasma underwent to PCR ZIKA virus (ZIKV) determination on the same day.

**Results.** A total of 12 samples of blood (4.7%) and 24 samples of urine (9.3%) were positive for ZIKV RT-PCR. The majority of samples (84.8%) were negative for ZIKV RT-PCR on both blood and urine. Seven samples were negative RT-PCR on urine but positive RT-PCR on blood; and 19 samples were negative RT-PCR on blood but positive on urine.

| | | Urine | | |
|---|---|---|---|---|
| | | Positive (N=24) | Negative (N=226) | Inconclusive (N=7) |
| Blood | Positive (N = 12) | 4 | 7 | 1 |
| | Negative (N= 243) | 19 | 218 | 6 |
| | Inconclusive (N=2) | 1 | 1 | 0 |

**Conclusions.** Although the ZIKV-PCR is detectable on the blood within the first week of clinical illness and is detectable on the urine up to 21 days after onset of symptoms, we failed to demonstrate a clear superiority of RT-PCR on urine compared to the blood. However, the RT-PCR on blood allowed the diagnosis of 3% of the patients that the ZIKV infection would not be recognized if PCR was performed only in urine, and the RT-PCR on urine allowed the diagnosis of 7% of the patients that ZIKV infection would not be recognized if PCR was performed only in the blood.

## B-094

### Identification of *Non-Tuberculosis Mycobacterial* species by DNA sequencing: an experience from an endemic tuberculosis country.

L. C. Pierrotti, L. Scarpelli, L. Galindo, P. Nishimura, G. Campana, J. Alves, L. Faro, J. Levi. *DASA Diagnósticos da América, Barueri, São Paulo, Brazil*

**Background.** *M. tuberculosis* disease remains a major international health threat causing million of cases and deaths yearly in the world. Reported incidence of nontuberculosis mycobacterial (MNT) disease, however, appears to be increasing worldwide, particularly among higher risk group, such as immunocompromised hosts. Rapid and accurate differentiation of mycobacterial species is important to guide clinical management and appropriate therapy. The accurate identification of NTM, however, is becoming more problematic, mainly because the increased number of species, and their fastidious and low growth rate. In this context, molecular diagnosis and typing is a valuable tool and commercial platforms for that are available. We reported here the distribution of Mycobacteria species isolated by culture and submitted to genotyping from Jan to Dec 2016.

**Methods**. Positive mycobacteria cultures were boiled at 100oC for 30 minutes to obtain DNA which was submitted to PCR amplification and amplicon sequencing using two targets: hsp65 (450 bp) and RPOb (764pb).

Species assignment was performed by sequence alignment and comparison employing BLAST tool (blast.ncbi.nlm.nih.gov).

**Results.** In 2016 a total of 56 positive mycobacterial cultures from 55 patients were sequenced and the results are summarized in tables 1 and 2. One patient had two different Mycobacteria species isolated from different clinical species (*M. mageritense* from breast secretion and *M. fortiutum* from respiratory sample) isolated in samples collected at one-month interval.

*M. tuberculosis* was the most prevalent species identified (23%), followed by *M. kansasii* and *M. abscessus* (16% each). *M. tuberculosis* and *M. kansasii* were the most prevalent species, with 6 cases each. Among 25 respiratory samples, MNT was identified in 76% of the cases.

Table 1. Mycobacteria species identified among 56 positive samples.

| Mycobacterium species | < 18 y | > 60 y | 18 - 60 y | Total |
|---|---|---|---|---|
| *Mycobacterium abscessus* | | 4 | 5 | 9 |
| *Mycobacterium avium* | | 3 | 1 | 4 |
| *Mycobacterium Kansasii* | | 3 | 6 | 9 |
| *Mycobacterium tuberculosis* | 1 | 5 | 7 | 13 |

**Conclusions.** Among a total 56 clinical specimens submitted to Mycobacterial species identification, the majority (77%) was MNT, even among respiratory samples, demonstrating the relevance of additional identification of samples positive for mycobacteria.

## B-095

### Biological Stability Study on the 1st Korea National Standard for anti-Human Immunodeficiency Virus Type 2

Y. Cha. *Chung-Ang University College of Medicine, Seoul, Korea, Republic of*

*Background:* Infection with HIV-2 was mainly confined to western Africa, but the number of people infected with the HIV-2 has recently increased in Europe, India, and the United States. According to the guidelines for HIV testing revised by the Centers for Disease Control (CDC) in the US in 2014, patients who are positive in HIV screening tests should take a further test that differentiates HIV-1 from HIV-2. Thus, the development of products for differentiations of HIV-1/2 is expected to increase in the future. At the same time, it is expected to increase demands for HIV-2 standard material required to develop and approve new products and to carry out quality control.

*Methods:* The 1st Korea National Standard for anti-HIV-2 which is composed of 2 kinds of mixed titer panels consisting of one negative and five positive samples each, has been established in 2015 using plasma source collected and transferred from Togo in Africa. Mixed titer panels for anti-HIV-2 have been characterized with various assays differentiating variable degree of cross-reactivity between HIV-1 and HIV-2. Biological stability has been evaluated with real-time stability assessments and accelerated degradation test (ADT) at various temperature conditions such as 4°C, 20°C, 37°C, and 45°C for 6 months by analyzing at certain intervals. The results were statistically compared with initial values. *Results:* HIV-2 positivity is confirmed by Geenius HIV 1.2 Confirmatory Assay (Bio-Rad Laboratories, Hercules, CA), New LAV BLOT HIV BLOT II (Bio-Rad Laboratories, Hercules, CA), INNO-LIA HIV I/II SCORE (Fujirebio Europe, Gent, Belgium), MP diagnostics HIV BLOT 2.2 (MP Biomedicals, Solon, OH, USA), and SD BIOLINE HIV 1/2 3.0 and cross-reactivity with HIV-1 is evaluated with New LAV BLOT HIV BLOT I (Bio-Rad Laboratories, Hercules, CA), Cambridge Biotech HIV-1 and Serodia HIV-1 PA (Fujirebio Diagnostics, Inc., Japan). HIV-2 RNA is also identified using in-house real-time PCR. As a result of real-time stability, it has confirmed that the antibody titers of the 1st Korea National Standard Panels for anti-HIV-2 established in 2015 are still valid and maintained well. According to the ADT results, the stability was maintained at 4°C for 6 months, slightly decreased but still maintained at 20°C for 6 months, and maintained at 37°C and 45°C for 1 month, when comparing permanently maintained at -80°C. Ten consecutive freeze-thaw cycles and storage of specimens at -20°C and 4°C for 30 days resulted in no loss of anti-HIV-2 reactivity nor false positive samples. *Conclusions:* The 1st Korea National Standard for anti-HIV-2 is an exclusive reference material and will be very useful to resolve cross-reactivity with HIV-1 and to rule out the possibility of co-infection, especially for the accurate profiling of the antibodies. Acknowledgement:This research was supported by a grant (17172MFDS339) from Ministry of Food and Drug Safety and Human Serum Bank (NRF-2015M3A9B8030780) through of the MSIP, Korea

## B-096

### Does Syphilis Chemiluminescent Microparticle Immunoassay(CMIA) correlate with Treponemal Pallidum Agglutination Assay(TPPA)

A. Tan, L. Ong, B. Saw, S. Saw, S. Sethi. *National University Health System, Singapore, Singapore*

**Background:**

Syphilis testing is complicated and requires special algorithms in testing. We reviewed syphilis screening using chemiluminescent microparticle immunoassay (CMIA) against confirmatory treponemal pallidum passive particle agglutination assay (TPPA) and analysed discordant results.

**Methods:**

Syphilis screening is performed in our hospital using Architect CMIA, results with signal >1.0 are considered positive by the manufacturer. All results >1.0 are confirmed using TPPA. 2014 and 2015 results were collated and positive CMIA results were analysed in terms of sensitivity. CMIA and TPPA discordant results were analysed together with other laboratory and clinical results. For patients with repeat testing, the first result was analysed. Rapid Pasma reagin(RPR) was performed for TPPA positive cases.

**Results:**

470 (2.49%) results were>1.0. The median age for CMIA signals>1.0 and <1.0 were 44 and 35 years old, while their female:male ratio were 1:3 and 3:1 respectively.

Out of 470 results with CMIA signal >1.0, 74 were non-reactive, 10 were indeterminate and 386 were reactive on TPPA, with 84% sensitivity. Their median CMIA was 1.62, 1.62 and 17.88, while their median age were 36, 64 and 47 years old respectively. Of 386 TPPA reactive results, 167 were RPR negative, and median RPR titre was 4 for RPR positive results.

The higher the signal value, the higher the sensitivity of CMIA results.

**Conclusions:**

In our population, there were a significant number of false positive results using Architect CMIA as first-line screening for syphilis followed by TPPA for confirmation. The sensitivity in this study is significantly lower than manufacturer report of >99.0% in package insert. Higher CMIA signals were associated with higher TPPA positivity and may be useful to predict true positivity.

## B-097

### Dengue diagnosis challenges in areas cocirculating other arboviroses: Brazilian dilemma situation.

L. C. Pierrotti, M. Guerino da Silva, G. Campana, A. do Nascimento. *DASA Diagnósticos da América, Barueri, São Paulo, Brazil*

**Background.** ZIK virus was first identified in Brazil in March 2015 and since than ZIK and DEN virus are cocirculanting in the country. These two diseases cause similar symptoms (fever, headache, myalgia, arthralgia, and rash) requiring confirmatory diagnosis. Over one week from the onset of symptoms (nonviremic period) the diagnosis is based on serology, but the antibodies present cross-reactivity with related viruses of the same group.

**Methods.** DEN and ZIK serology of four Brazilian kidney transplant recipients who had diagnosis of DEN based on positive IgM from May 2014 to April 2015 were studied to checked which virus are in fact related to symptoms.

The serologies were performed at least one year post-DEN diagnosis. DEN ELISA serology is defined as negative (index <0.9), indeterminate (index range from 0.9 to 1.1) and positive (index >1.1). ZIK ELISA serology is defined as negative (index <0.8), indeterminate (index range from 0.8 to 1.1) and positive (index >1.1).

**Results.** Characteristics of the cases are demonstrated in table 1. All but one presented negative IgG ZIK serology, confirming the previous diagnosis of DEN. However, in one case it was not possible confirm the previous DEN diagnosis since both DEN and ZIK checked serology were positive.

Table 1. Description of DEN infection among four kidney transplant recipients.

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Age | 46 | 41 | 63 | 54 |
| Sex | Female | Male | Male | Female |
| Time from KT (years) | 1.7 | 6.6 | 4.4 | 3.0 |
| DEN diagnosis based on | IgM + | IgM + | IgM + | IgM + |
| Checked IgM-DEN serology | 1.0 | 0.7 | **2.2** | 0.8 |
| Checked IgG-DEN serology | **5.4** | **5.2** | **6.8** | **4.2** |
| Checked IgG-ZIK serology | 0.5 | 0.51 | **2.7** | 0.1 |
| Clinical picture | Asthenia + myalgia | Fever + headache + myalgia + retroocular pain | Fever + myalgia + chills | Fever + asthenia + vomiting + diarrhea |
| Outcome | Recovery | Recovery | Recovery | Recovery |
| KT, kidney transplant. | | | | |

**Conclusions.**

Positive DEN and ZIK serology may represent prior exposure to flaviviruses or prior vaccination for Yellow Fever or Japanese Encephalitis. IgM antibodies is detectable 3 to 5 days and become undetectable in 2 to 3 months after exposure and IgG antibodies appear later and remain detectable for months. In areas of cocirculating these arboviruses it is important perform simultaneously the serology of DEN and ZIK to allow a more accurate flavivirus infection diagnosis. Transplant recipients are peculiar because they have an immunosuppression factor as an additional confounding factor.

## B-098

### Diagnosis of Clostridium difficile hypervirulent strain BI/NAP1/027 using Xpert C. difficile PCR assay in Brazil.

L. C. Pierrotti, L. Scarpelli, P. Nishimura, L. Galindo, J. Levi, G. Campana, J. Alves, L. Faro. *DASA Diagnósticos da América, Barueri, São Paulo, Brazil*

**Background.** *Clostridium difficile*-associated disease (CDAD) is caused bya spore-forming bacterium currently considered one of the most important healthcare-associated infection, being the main cause of hospital acquired diarrhea, associated with a high cost of hospitalizations and treatment. A hypervirulent *C. difficile* strain, denominated BI/NAP1/027, has caused outbreaks in North America and Europe but there are only a few reports of cases in Latin America.

**Methods.** We analyzed the *Clostridium difficile* toxin test in stool specimens collected from patients with suspicion of CDAD from Jan to Dec 2016, tested by Xpert *C. difficile* assay. The Xpert *C. difficile* PCR assay is a real-time pCR that detects the toxin B gene (tcdB), the binary toxin gene (cdt), and the tcdC gene deletion at nt 117. Assay includes reagents for the detection of Toxin producing *C. difficile* and toxin producing C. difficile 027/NAP1/B1.

**Results.** Throughout 2016 a total of 1,544 of CD toxin test were performed from 1,255 patients; 210 patients performed more than one test (average 1.2, range 2 - 6). The general positivity rate was 16% (253/1542) and 2% of samples had indeterminate results. A total of 10 patients presented B1/NAP1/027 positive strain.

**Conclusions.** CDAD caused by BI/NAP1/027 strain was detectable in few samples during 2016; however, laboratory methods that allow the identification of this hypervirulent strain are important both from the point of view of patient care, recognizing the possibility of infections with greater potential for severity, and epidemiologically, with active surveillance.

## B-099

### Performance evaluation of the new rapid AFIAS system to detect hepatitis C virus infection

A. Choi[1], M. Hwang[2], J. Lee[2], M. Kwon[2], J. Moon[2], J. Jeong[3], B. Jung[3], J. Ryu[1], K. Han[1], H. Lee[4], E. Oh[1]. *[1]Seoul St. Mary's Hospital, the Catholic University of Korea, Seoul, Korea, Republic of, [2]R&D Center BODITECH MED, Seoul, Korea, Republic of, [3]KoreaMedical Device Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea, Republic of, [4]SamKwang Medical Laboratories, Seoul, Korea, Republic of*

**Background:** The availability of accurate, rapid and cost-effective screening test for Hepatitis C virus (HCV) infection may be useful in smaller laboratories that cannot afford automated analyzer. This study explored the performance evaluation and diagnostic accuracy of newly developed AFIAS Anti-HCV assay (Boditech Med Inc. ChunCheon, Korea) which is an immune lateral flow cartridge test using small bench-top fluorescence reader for the detection of HCV antibody in serum and whole blood specimens in 20 minutes.

**Methods:** A total of 1,500 samples were used to compare AFIAS Anti-HCV assay with Elecsys anti-HCV II (Roche Diagnostis, Germany). The discrepant results were confirmed by recombinant immunoblot assay, Deciscan HCV Plus (Biorad). The HCV RNA-positive EDTA plasma specimens (SeraCare Life Sciences, Milford, MA), Virotrol I controls (Bio-Rad Laboratories, France), seroconversion panels and samples for crossreactivity or interference test were also used for evaluation.

**Results:** The sensitivity and specificity of AFIAS anti-HCV assay were 99.8% (95% CI: 97.1-99.6%) and 99.1% (95% CI: 98.3-99.6%), respectively. The kappa value for the agreement between two assays' results was 99.0% (k=0.975, 95% CI: 0.962-0.987). AFIAS anti-HCV assay detected all samples with genotype 1, 1a, 1b, 2a, 2b, 4 and 6. Evaluation with 10 seroconversion panels demonstrated the adequate sensitivity. There was no interference or cross-reactivity with other infection, anticoagulant, pregnant-associated, icteric and hemolysis samples. Inter-assay CVs ranged from 4.4 to 5.4% and lot-to lot variation ranged from 3.5 to 6.6%.

**Conclusion:** The AFIAS anti-HCV assay demonstrated acceptable analytical performance and diagnostic accuracy that was equivalent to current automated chemiluminescent immunoassay. It can be very useful in emergency or small clinical laboratory for the detection of anti-HCV due to its simplicity and flexibility.

## B-100

### Comparison of real-time PCR tests with the routine diagnosis technique to detect enteropathogenic bacterias

I. Valledor[1], S. Valledor[1], C. Seral[2], C. Mendoza[3], Ó. Landeta[4], F. Castillo[2]. *[1]Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain, [2]Hospital Clínico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, LIS Aragón, Departamento de Microbiología, Zaragoza, Spain, [3]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain, [4]CerTest Biotec S.L., Zaragoza, Spain*

**Background:** Infectious gastroenteritis is the most common childhood illnesses worldwide and it is caused by different species of bacteria, viruses and parasites, being *Campylobacter*, *Salmonella, Shigella/*EIEC and *Yersinia* four of the main enteropathogens.

**Objective:** Compare real-time PCR tests with the routine diagnosis technique to detect enteropathogenic bacterias.

**Material/methods:** We performed a retrospective study where we tested 400 stool samples from 356 subjects (50% children under the age of 14 years and 50% adults) with gastrointestinal symptoms, from October to December 2015. Total genomic DNA was isolated from stool samples with the "VIASURE RNA-DNA Extraction Kit" (CerTest BioTec S.L). Nucleic acids were amplified on thermocycler AriaMx (Agilent Technologies) using the multiplex assay "VIASURE *Salmonella, Campylobacter, Shigella/EIEC* or *Yersinia* Real Time PCR Detection Kit"" (Certest Biotec S.L) in comparison to "RIDA®GENE Bacterial Stool Panel" (R-biopharm) and "RIDA®GENE EHEC/EPEC" (R-biopharm). All samples were also amplified using the four monoplex assays: "VIASURE *Salmonella, Campylobacter, Shigella* or *Yersinia* Real Time PCR Detection Kit". Conventional PCR was used to identify the different species of *Salmonella* and *Campylobacter.*

Relative to the routine diagnosis method, all samples were cultivated in the routine culture medium. Also MALDI-TOF mass spectrometry was used to identify enteropathogens, and agglutination were used to identify *Salmonella*, *Shigella/*EIEC and *Yersinia enterocolitica.*

**Results: 65/400** (16.25%) were true positive for *Campylobacter*, 42/65 was detected by routine diagnosis, 53/65 by Real Time PCR R-biopharm assay and 65/65 by multiplex Real Time PCR VIASURE assay. Sequencing showed 54.93% C.*jejuni*, 9.86% C.*concisus* and 8.45% C.*coli*.

**23/400** (5.75%) were true positive for *Salmonella*, all of them were detected by routine diagnosis, 21/23 by Real Time PCR R-biopharm assay and 22/23 by Real Time PCR VIASURE assay. Sequencing shows 47.83%S.Typhimurium, 26.09%S. Mbandaka, 4.35% S.Braenderup and 4.35% S. Paratyphi A.

**2/400** (0.5%) were true positive for *Shigella/*EIEC, all of them were detected by routine diagnosis, by Real Time PCR R-biopharm and Real Time PCR VIASURE assay.

**4/400** (1%) were true positive for *Yersinia enterocolitica O:3*, all of them were detected by routine diagnosis, by Real Time PCR R-biopharm and Real Time PCR VIASURE assay.

There is a total concordance between multiplex and monoplex VIASURE PCR assays.

**Conclusions:** Multiplex assay allows the detection of the three enteropathogens in only one reaction, which reduces the cost and is less consuming .For *Salmonella, Shigella/EIEC* and *Yersinia, "*VIASURE Multiplex Real Time PCR Detection Kits" are highly sensitive and specific, being comparable in sensitivity and specificity to culture method and RIDA®GENE Kits.

In the diagnosis of *Campylobacter*, "VIASURE Multiplex Real Time PCR Detection Kits" are the most sensitive one.

Molecular techniques have the additional advantage that detects unusual varieties of *Campylobacter* species in comparison to culture method.

## B-101

### Molecular diagnosis by Real-Time PCR to detect emerging viruses Dengue, Zika, Chikungunya and West Nile Virus

J. Gil[1], L. Muñoz[1], S. Valledor[2], I. Valledor[2], Ó. Landeta[3], R. Benito[4]. *[1]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain, [2]Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain, [3]CerTest Biotec S.L., Zaragoza, Spain, [4]Hospital Clínico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, LIS Aragón, Departamento de Microbiología, Zaragoza, Spain*

**Background:** The rapidly expansion across the globe of the more frequent illnesses caused by arboviruses such as Dengue, Zika, Chikungunya and West Nile Virus and their similar clinical presentations made necessary a proper diagnostic. Dengue virus (DENV) is the most common tropical virus infection and is caused by four distinct serotypes of the Dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4). Dengue serotyped is required to the treatment of patients, control of DENV outbreaks and transmission-blocking strategies targeting the vector, as well as for the development of vaccines and antivirals. The aim of this study is to compare Real-Time PCR assays with the routine diagnosis technique as well as to serotype Dengue positive samples.

**Material/methods:** We performed a retrospective study where we tested 30 samples (28 serum and 2 urine samples), from August 2013 to August 2016. The patients presented typical symptoms for previous named arboviruses. Total genomic RNA was isolated from serum and urine samples with the "QIAamp Viral RNA Mini Kit"(Qiagen). Nucleic acids were amplified on thermocycler AriaMx (Agilent Technologies) using the monoplex assay "VIASURE *West Nile Virus* Real Time PCR Detection Kit" and the multiplex assay "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit"(Certest Biotec S.L) in comparison to Real-Time PCR assay "RealStar® Dengue RT-PCR kit 2.0" (Altona). Dengue serotyping was performed using the multiplex assay "VIASURE *Dengue Serotyping* Real Time PCR Detection Kit" (Certest Biotec S.L).

Relative to the routine diagnosis method, all samples were tested with inmunochromatography by "SD BIOLINE Dengue IgG/IgM (Standard Diagnostics)" and also with enzyme- inmuno assays using "Dengue ELISA IgM CAPTURE (Vircell)".

**Results:** 8/29 samples were positive for Dengue by multiplex Real-Time PCR assays, these results were confirmed by Altona. 1/29 was positive for Zika by VIASURE multiplex assay, being supported by serology and clinical diagnosis. No positive samples for Chikungunya or West Nile Virus were found by VIASURE Real-Time PCR assays.

Considering the phase of the disease, there is a total concordance between serological and Real-Time PCR results.

The positive samples for Dengue were serotyped. 3/8 belong to serotype Dengue 3, 3/8 samples were Dengue 2, 1/8 samples was Dengue 1 and 1/8 was not positive for DEN-1 to DEN-4.

**Conclusions:** A total concordance between serological results and Real-Time PCR has been found in this study. Molecular diagnosis is more accurate during first infection phase, being serological diagnosis proper during convalescence phase.

The multiplex assay "VIASURE *Dengue Serotyping* Real Time PCR Detection Kit" allows the Dengue serotyping, which, in combination with clinical and epidemiological risk factors provides an aid in the patient management.

## B-102

### Evaluation of the Diagnostic Value of Volume, Conductivity and Scatter Parameters Determined by Unicel DxH800 Coulter Cellular Analyzer in Sepsis in Comparison to Chronic Inflammatory and Nonsystemic Infection Cases

T. Cevlik[1], R. Turkal[1], O. Kaya[2], F. Gül[2], Y. Yalçınkaya[3], U. Oguz[3], M. Arslantaş[2], I. Cinel[2], N. Inanç[3], H. Direskeneli[3], O. Sirikci[4], G. Haklar[4]. *[1]Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey, [2]Dept. of Anestesiology and Reanimation, Istanbul, Turkey, [3]Division of Rheumatology, Dept. of Internal Medicine, Istanbul, Turkey, [4]Dept. of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey*

**Background:** Sepsis is a systemic inflammatory response and clinical criteria for organ dysfunction are defined as an increase of 2 points or more in the sequential organ failure assessment (SOFA) score. Laboratory parameters can provide valuable information for the diagnosis and some investigations showed that volume-scatter-conductivity (VCS) parameters of cell counters might be beneficial. However, these parameters are reflections inflammatory status and chronic inflammatory states may cause interferences. Our aim was to evaluate diagnostic significance of VCS parameters in sepsis in comparison to rheumatoid arthritis (RA) and nonsystemic infection cases (NSI).

**Methods:**The study was conducted in Marmara University Pendik E&R Hospital Biochemistry Laboratory. Sepsis (n=22) (diagnosed according to the 2016 Sepsis Consensus Report), RA (n=68), and sputum culture positive respiratory tract infection patients (n=21) were enrolled. Blood samples for cell count and serum markers were collected on the same day of culture collection. VCS parameters were measured by Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). Hs-CRP levels were determined nepholometrically and procalcitonin concentrations measured by electrochemiluminescence immunoassay. For Bacterial diagnosis; sputum samples were stained by Gram stain and examined immediately to analyze the quality of the samples. Qualified samples were inoculated on 5% sheep blood agar, Chocolate Agar, Haemophilus Agar, and MacConkey Agar (BioMerieux, France) by streaking method. After overnight incubation, the samples were evaluated.**Results:** The mean age was 64±15.5 years in sepsis patients, which was not significantly different than other groups. WBC counts were not significantly different. Procalcitonin and hs-CRP levels were significantly high in sepsis group in comparison to RA and NSI. Mean neutrophil volume (MN-V-NE) was the highest in the sepsis group (165±22.3) compared to that in the NSI (144±13.5) and RA (144±10) groups (P=0.002 and P=0.001, respectively). Mean neutrophil SD (SD-V-NE) was higher in the sepsis group (22.2±4.56), which was significantly different than RA group (17.5±3.6, P=0.001). For neutrophils, mean values for median-angle light scatter (MN-MALS-NE), lower-median-angle light scatter (MN-LMALS-NE) and low-angle light scatter (MN-LALS-NE) were significantly lower in the sepsis group. Mean monocyte volume was the highest in the sepsis group (190±15.4) compared to that in the RA (166±8.4) and NSI (169±8.74) groups (P<0.001 and P<0.001, respectively). Mean monocyte volume SD, mean axial light loss (M-AL2) and SD-axial light loss (SD-AL-2) significantly differentiates sepsis from other groups. ROC curves evidenced excellent sensitivity especially in the neutrophil parameters. MN-V-NE at 151, the sensitivity 81.8% and specificity 84.1 %; SD-V-NE at 18.3, the sensitivity 86.4% and specificity 81.8%; MN-MALS-NE at 129, the sensitivity 87.5% and specificity 72.7%; MN-LMALS-NE at 127, the sensitivity 87.5% and specificity 86.4%; SD-V LE at 72.5, the sensitivity 91% and specificity 81.1 %; MN-V LE at 175.5, the sensitivity 81.8 % and specifity 85.2; SD V MO at 21.7, the sensitivity 72.7% and specificity 81.6 %; MN-C MO at 127.5, the sensitivity 72.7% and specificity 81.5 % are achieved in our study.

**Conclusion:** We concluded that VCS parameters might be promising for differentiating between sepsis and non-sepsis cases. Additionally, they are obtained routinely, without any additional cost and time requirement thus making their prospects very promising.

## B-103

### Performance Evaluation of the ADVIA Centaur anti-HCV Assay* in the Pediatric Population

B. Plouffe[1], R. Levine[1], L. McCusker[2]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Siemens Healthcare Diagnostics, Walpole, MA*

**Background:** The HCV antibody immunoassay is clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of the ADVIA Centaur® anti-HCV assay (Siemens Healthcare Diagnostics Inc.) in a pediatric subpopulation consisting of children and adolescents presenting with signs and symptoms of hepatitis or at risk for hepatitis C virus (HCV) infection.

**Methods:** Concordance of the ADVIA Centaur anti-HCV assay* was assessed to a comparator assay commercially available in the U.S. and approved for use in children aged ≥10 years old. Serum samples from suspected or high-risk pediatric populations were tested in singleton on both assays. Samples with initial equivocal test results were retested in duplicate, and the results were interpreted as recommended by the assay manufacturers.

**Results:** A total of 55 subjects were included, aged 2 to 20 years old with a male/female ratio of 57.4/42.6%, respectively. The subjects' specimens yielded the following final results on the comparator assay: 1 equivocal, 6 reactive, and 48 nonreactive results. When excluding the equivocal specimen from the concordance analysis, the percent positive and negative agreements were 100.00% (95% CI 54.07 to 100.00) and 100.00% (95% CI 92.60 to 100.00), respectively. When including the equivocal specimen in the concordance analysis and counting this specimen as a discordant result for the positive agreement, the percent positive and negative agreements were 85.71% (95% CI 42.13 to 99.64) and 100.00% (95% CI 92.60 to 100.00), respectively. All positive anti-HCV results were confirmed to also be positive under RIBA testing.

The specimen which tested equivocal on the comparator assay tested negative on both the RIBA test and the ADVIA Centaur anti-HCV assay.

**Conclusions:** The study showed acceptable concordance between the ADVIA Centaur anti-HCV assay and the comparator assay.

*The pediatric claims mentioned herein are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

## B-104

### Performance Evaluation of the ADVIA Centaur HBc Total* and HBc IgM* Assays in the Pediatric Population

B. Plouffe[1], D. Desai[1], R. Levine[1], M. Hill[2], O. De Armas[2], Y. De La Fuente[2], M. De Medina[2], E. R. Schiff[2]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Center for Liver Diseases University of Miami Miller School of Medicine, Miami, FL*

**Introduction:** Hepatitis immunoassays are clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of ADVIA Centaur® Hepatitis B core Total (HBcT)* and HBc IgM* assays from Siemens Healthcare Diagnostics Inc., in a pediatric population consisting of children and adolescents.

**Objective:** To assess the performance of the ADVIA Centaur HBcT and HBc IgM assays in a pediatric population presenting with signs and symptoms of hepatitis or at risk for hepatitis B virus infection.

**Methods:** Concordance of the ADVIA Centaur HBcT and HBc IgM assays was assessed to comparator assays commercially available in the U.S. and approved for use in children and adolescents. Serum samples from suspected or high-risk pediatric patients were tested in singleton on all assays. Equivocal results were retested, and all test results were interpreted as recommended by the assay manufacturers.

**Results:** ADVIA Centaur HBcT assay: 62 subjects were included, aged 2 to 21 year-old, with a male/female ratio of 58%/42%, respectively. 12 samples tested positive and 50 negative on the comparator assay. The percent positive and negative agreement were 91.67% (95% CI 61.52-99.79) and 100.00% (95% CI 92.89-100.00), respectively.

ADVIA Centaur HBc IgM assay: 142 samples were tested, including 107 native samples (58% male and 42% female, age range from 2 to 21 years) and 35 contrived samples. 41 tested positive and 101 negative on the comparator assay. The percent positive and negative agreement were 100.00% (95% CI 91.40-100.00) and 98.02% (95% CI 93.03-99.76), respectively.

**Conclusion:** The study showed acceptable concordance between the ADVIA Centaur HBcT and HBc IgM assays and the comparator assays.

*The pediatric claims (mentioned herein) are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

## B-105

### Comparison of two algorithms for the diagnosis of syphilis

H. Hwang, H. Park. *Kosin University College of Medicine, Busan, Korea, Republic of*

**Background:** Although the traditional syphilis screening algorithm where nontreponemal screening is followed by confirmatory treponemal testing is recommended in Korea, many laboratories are gradually adopting a reverse syphilis screening algorithm for syphilis testing. We compared the diagnostic performance of the traditional and reverse syphilis screening algorithms in a Korean population.

**Methods:** A total of 201 patient samples were used. We used HiSens Auto rapid plasma regain (RPR) test (HBi corp., Korea) as a nontreponemal test, and ADVIA Centaur Syphilis (Siemens, Germany) test and Treponema pallidum antibody (TPAb) test (HBi corp., Korea) were used as treponemal tests. ADVIA Centaur Syphilis test was used as a first line test in the reverse syphilis screening algorithm. When the tested samples were positive in TPAb and ADVIA Centaur Syphilis test simultaneously, we confirmed the patient samples as an anlytical syphilis in this study.

**Results: :** In traditional syphilis screening algorithm, thirty eight patient samples were positive in RPR test, among which 32(84.2%) were positive in TPAb and 1 of 32(3.1%) was negative in ADVIA Centaur Syphilis. The rest six (15.8%) were TPAb negative. Eleven RPR(-) samples (6.7%) were positive in TPAb and 5 among them were positive in ADVIA Centaur Syphilis as well. Thirty six patient samples were ADVIA Centaur Syphilis (+) in reverse syphilis screening algorithm. Thirty one of them were RPR(+)/TPAb(+). The rest 5(13.9%) were RPR(-)/TPAb(+). Only one of 7 ADVIA Centaur Syphilis(-)/RPR(+)(14.3%) was TPAb(+). Analytical sensitivity and specificity of the traditional syphilis screening algorithm were 81.6% and 96.9%, respectively and those of the reverse syphilis screening algorithm were both 100%. **Conclusion:** The diagnostic performance of reverse syphilis screening algorithm was superior to the traditional syphilis screening algorithm in this study.

## B-106

### Performance of the VITROS® Prototype HTLV I/II Assay*

S. Danielson, T. Grupp, H. Lipshutz, T. Mangan, L. Sprague. *Ortho Clinical Diagnostics, Rochester, NY*

**Background / Objective:**

HTLV-I and HTLV-II are closely related type C retroviruses. HTLV-I is etiologically associated with adult T-Cell leukemia, demyelinating disease, and degenerative retinal disease. HTLV-II has not yet been conclusively established as an etiologic agent for any specific disease. HTLV-I is endemic in some Caribbean countries, Southern Japan, and some areas of Africa and South America. HTLV II is endemic in several American Indian tribes. Transmission of HTLV I/II occurs via sexual contact, transfusion exposure to infected cellular blood components, intravenous drug abuse, or through breast milk. Routine screening of anti-HTLV-I/II is mandatory in many countries. We have assessed the performance of a prototype HTLV I/II Assay* on VITROS Immunodiagnostic Systems. This assay qualitatively detects antibodies to HTLV I/II in human plasma and serum and is suitable for donor screening or as an aid in the diagnosis of HTLV infection.

**Method:**

Antibody detection in the VITROS HTLV I/II Assay* is achieved using recombinant p21 and p24 HTLV I/II antigens coated on microwells which capture HTLV antibodies present in the sample. After addition of the HRP-labeled p21 and p24 HTLV I/II antigens and the VITROS Signal Reagent, the bound HRP conjugate is measured by a chemiluminescent reaction. The signal is compared to a cutoff signal generated using a positive calibrator. All specificity and sensitivity testing was performed using one lot of reagents on a VITROS 3600 Immunodiagnostic System with the exception of the dilutional and performance panels which were tested on a VITROS ECiQ and a VITROS 3600 Immunodiagnostic System to compare performance across systems. Assay specificity was assessed using 1668 fresh serum and plasma blood donor samples. Assay sensitivity was evaluated by testing 151 HTLV-I and 69 HTLV-II serological presumed positive patient samples and 1 commercial performance panel. Results from 4 serially diluted positive patient samples (2 HTLV-I and 2 HTLV-II) were compared to results from the AVIOQ HTLV-I/II Microelisa System run on the ORTHO VERSEIA Integrated Processor. Total within lab precision was evaluated over 15 days in accordance with CLSI EP05-A3 using 1 VITROS 3600 and 1 VITROS ECiQ Immunodiagnostic System.

**Results:**

The assay specificity was 100 % (1668/1668; 95%CI: 99.78 - 100 %) for donor patient samples. The mean S/C for these samples was 0.055 with 1/1668 samples > 0.4 S/C. The assay sensitivity was 100% (212/212; 95% CI: 99.28 - 100.0%) with the presumed positive patient panel. The commercial performance panel was found to be qualitatively concordant with all other commercially available assays reported on both VITROS systems. The VITROS HTLV I/II Assay* generated similar dilutional performance on both VITROS systems and generated reactive results on samples 4 – 10 X more dilute than were reactive on the AVIOQ HTLV-I/II Microelisa System. Within-lab precision of the assay ranged from 3.7 – 5.7 %CV above the cutoff (>1.0 S/C) and 8.9 – 19.8 %CV below the cutoff on both VITROS systems.

**Conclusion:**

The VITROS HTLV I/II Assay* has demonstrated excellent sensitivity and specificity, and acceptable precision.

* Under Development

## B-107

### Performance Evaluation of the ADVIA Centaur HAV Total* and HAV IgM* Assays in the Pediatric Population

B. Plouffe[1], D. Desai[1], R. Levine[1], M. Hill[2], O. De Armas[2], Y. De La Fuente[2], M. De Medina[2], E. R. Schiff[2]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Center for Liver Diseases University of Miami Miller School of Medicine, Miami, FL*

**Introduction:** Hepatitis immunoassays are clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of ADVIA Centaur® Hepatitis A Virus (HAV) Total* and HAV IgM* assays from Siemens Healthcare Diagnostics Inc., in a pediatric population consisting of children and adolescents.

**Objective:** To assess the performance of the ADVIA Centaur HAV Total and HAV IgM assays in a pediatric population presenting with signs and symptoms of hepatitis or at risk for hepatitis A virus infection.

**Methods:** Concordance of the ADVIA Centaur HAV Total and HAV IgM assays was assessed to comparator assays commercially available in the U.S. and approved for use in children and adolescents. Serum samples from suspected or high-risk pediatric patients were tested in singleton on all assays. Equivocal results were retested, and all test results were interpreted as recommended by the assay manufacturers.

**Results:** ADVIA Centaur HAV Total assay: 55 subjects were included, aged 2 to 21 years old, with a male/female ratio of 49.09%/50.91%, respectively. 11 samples tested positive, 42 tested negative, and 2 tested borderline reactive on the comparator assay. The percent positive and negative agreement were 100.00% (95% CI 71.51-100.00%) and 97.62% (95% CI 87.43-99.94%), respectively. Scoring the two borderline results from the comparative assay as discordant results in the % positive agreement calculation yielded a result of 84.62% (95% CI 54.55-98.08%).

ADVIA Centaur HAV IgM assay: 132 native samples were tested (54.55% male and 45.45% female, age range from 2 to 21 years). 31 samples tested positive, 98 tested negative, and 3 tested borderline reactive on the comparator assay. The percent positive and negative agreement were 96.77% (95% CI 83.30-99.92%) and 98.98% (95% CI 94.45-99.97%), respectively. Scoring the three borderline reactive results from the comparative assay as discordant results in the % positive agreement calculation yielded a result of 90.90% (95% CI 75.67-98.08%).

**Conclusion:** The study showed acceptable concordance between the ADVIA Centaur HAV Total and HAV IgM assays and the comparator assays.

*The pediatric claims (mentioned herein) are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

## B-108

### Performance evaluation of the ADVIA Centaur Zika IgM assay*

K. K. Singh, J. Arroyo, M. Smith, S. A. Patibandla. *Siemens Healthcare Diagnostics, Tarrytown, NY*

**Background:** Siemens Healthineers is developing a fully automated Zika IgM (ZikaM) assay* for the ADVIA Centaur® XP and XPT Immunoassay Systems. The ADVIA Centaur ZikaM assay is an IgM capture two-wash immunoassay using direct chemiluminometric technology. The assay uses recombinant Zika virus NS1 antigen for the qualitative detection of Zika IgM antibodies in serum or plasma.

**Methods:** The performance of the ADVIA Centaur ZikaM assay was evaluated with serial draw samples obtained from Zika virus PCR-positive individuals plus samples from normal donors (U.S.), pregnant women (U.S.), and symptomatic (Dominican Republic) and asymptomatic (Dominican Republic and Honduras) individuals. Several potentially cross-reactive samples were also evaluated.

**Results:** ADVIA Centaur ZikaM assay results were reported as reactive for samples with ≥1.00 index and nonreactive for samples with <1.00 index. In 50 Zika PCR-positive serial draw sets (eight draws per individual), all 50 individuals showed reactivity with the ZikaM assay within 2-27 days after the appearance of symptoms, and the majority of these (43/50) were reactive within 14 days post-symptom onset. When the results of all draws from 50 Zika-positive individuals were combined, approximately 85% of the samples were reactive with the ZikaM assay. Evaluation of normal samples from a U.S. population (blood donors and pregnant women) by the ZikaM assay gave a specificity of 94.7% (1418/1497). Specificity in samples collected from Zika-endemic areas was 70.7% (29/41) in individuals with symptoms of Zika and 81.7% (116/142) in asymptomatic individuals. Additionally, cross-reactivity was evaluated using 159 cross-reactive samples, including common flaviviruses (dengue, yellow fever vaccinees, and West Nile).

**Conclusions:** These results demonstrate good performance of the prototype ADVIA Centaur Zika IgM assay.

*Under development. Not available for sale. The performance characteristics of this device have not been established. Product availability will vary from country to country and will be subject to varying regulatory requirements.

## B-109

### Discrimination Between Viral and Bacterial Etiology of Lower Respiratory Tract Infection Using Cell Populaton Data Generated by Unicel DxH800 Coulter Cellular Analyzer

T. Cevlik[1], R. Turkal[1], S. Satılmış[2], A. Ilki[2], O. Sirikci[3], G. Haklar[3]. *[1]Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey, [2]Dept.s of Microbiology, Istanbul, Turkey, [3]Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey*

**Background:** Discrimination of viral vs. bacterial etiology of lower respiratory tract infection is critical. Although WBC and differentials may provide useful information for this discrimination, the sensitivity and specificity of these parameters are not satisfactory. Likewise, C-reactive protein and procalcitonin are not completely satisfactory. Other tests, such as blood culture for bacteria or molecular/antigen studies for viruses are time-consuming, expensive, and labour intensive. The Unicel DxH 800 Coulter Cellular Analyzer with volume, conductivity, and light scatter (VCS) technology generate cell population data (CPD) encompassing morphologic properties of leukocytes. Our aim was to evaluate the usefulness of CPD for the differential diagnosis of viral and bacterial infection etiology in lower respiratory tract infections.

**Methods:** Complete blood count and CPD data measured in the Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA) were collected retrospectively from 65 patients with confirmed diagnosis of viral infection, acute bacterial infection with (+) sputum culture (n=21), and 106 (viral Ag negative 84 and sputum culture negative 22 cases) controls. Viral diagnosis was made by **combo card test** (CerTest, Biotec, Spain), which is a coloured chromatographic immunoassay for the simultaneous qualitative detection of *RSV* and *Adenovirus* antigens. For bacterial diagnosis; sputum samples were stained by Gram stain, and were inoculated on 5% sheep blood agar, Chocolate Agar, Haemophilus Agar, MacConkey Agar (BioMerieux, France). After overnight incubation, the samples were evaluated. The CPD data include mean (MN) and standard deviation (SD) of volume of lymphocytes and neutrophils, and lymphocyte conductivity. Lymphocyte (LI) index was calculated.

**Results:** WBC of bacterial culture (+) and viral Ag (+) cases were not different significantly different (7.8, range 6.7-12.3 and 11.5, range 7.5-12.2; p=0.074, respectively). Mean percent neutrophils were significantly higher in bacterial culture (+) cases 75.7 vs 43.9, P<0.001), on the other hand mean percent lymphocytes were higher in viral Ag (+) cases (12.9 vs 40.3, P<0.001). The lymphocyte conductivity (LyC) were significantly higher in the viral infection than the bacterial infection (P=0.004). In ROC analysis, lymphocyte volume SD (LySDV) sensitivity 71% specificity 63.1, LyC sensitivity 71% specificity 59.4 in diagnosing viral infection.

**Conclusion:** We concluded that for the discrimination of bacterial and viral infections, CPD parameters merits further exploration in larger prospective studies.

## B-110

### Ready-To-Use Real-Time PCR-Based Assay for the Detection of Human Herpesvirus 6

M. Gramegna, M. Ballarini, L. Turner, M. Incandela, L. Spinelli, D. Rigamonti, A. Moiana. *Sentinel CH, Milano, Italy*

**Background:** Human Herpesvirus 6 (HHV-6) is a set of two closely related herpes viruses known as HHV-6A and HHV-6B. HHV-6B infects nearly 100% of human beings, typically before the age of three. Like other herpesviruses, HHV-6 establishes life-long latency and can become reactivated later in life. Reactivation occurs mostly in transplant patients taking immunosuppressant drugs or individuals with immune deficiencies and it can involve brain, lungs, heart, kidney and gastrointestinal tract. Both HHV-6 viruses are highly cell associated and can be detected in plasma briefly during the initial infection or acute reactivation. Detection of HHV-6 DNA in plasma generally means the patient has an active infection. The aim of this work was to evaluate the performance of a new assay designed to detect HHV-6 DNA in human samples.

**Methods:** The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta-globin gene as internal control. The two sets of primers and probes are combined in a

lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. In the present study, several samples obtained from San Raffaele Hospital, previously tested with an "in-house" Real-Time PCR test, were investigated. PCR reactions were performed on nucleic acids extracted from plasma, whole blood and cerebrospinal fluid (CSF).

**Results:** All tested samples were previously diagnosed as positive. The new freeze-dried ready-to-use assay demonstrated robust and accurate target amplification, according to the data obtained at San Raffaele Hospital. This detection kit proved to be specific for HHV-6. The assay did not cross-react with any of the other Human Herpesviruses tested.

**Conclusion:** The described Real-Time PCR assay proved its effectiveness for the detection HHV-6 DNA in samples. The test showed a sensitivity and a specificity of 100%. The high-sensitivity and specificity of this assay, associated with the ready-to-use and room temperature storage, would have a direct impact on the early and correct management of the affected patients.

## B-111

### Circulating Cytokines and Chemokines Disturbance may Influence HBV DNA Replication in Patients with Chronic HBV Infection

B. CAI, Z. SU, X. WU, Y. AN, J. ZHANG, L. WANG. *Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China*

**Background:** Host immune plays a critical role in anti-virus responses and liver injury in patients with HBV infection. Regulatory T cells (Treg) and Th17 cells imbalance has been demonstrated to participated in chronic HBV infection (CHB) progression. IL-10 and IL-17 are the characteristic and effector cytokines of Treg and Th17 cells, respectively. Studies on circulating cytokines or chemokines profiles in anti-virus effects or liver inflammatory responses in CHB were rare. Here we aimed to explore the specific effects the inflammatory, anti-inflammatory cytokines as well as chemokines exerted in HBV DNA replication, e antigen seroconversion and the impairment of liver function.

**Methods:** 159 patients with CHB (including 71 with HBeAg (+) and 88 with HBeAg (-) ) and 29 healthy controls (HC) were recruited from outpatients and inpatients in West China Hospital of Sichuan University. Circulating levels of inflammatory cytokines including IL-1β, IL-6, IL-17 and anti-inflammatory IL-10, as well as chemokines comprising IL-8 and IP-10 were measured by Bio-Plex system and Bio-Plex Pro™ human cytokine reagent kits (Bio-Rad, USA). The clinical information including HBV DNA load, HBV serology biomarkers and liver function were recorded.

**Results:** ① Circulating IL-1β, IL-6, IL-17, IL-10, IL-8 and IP-10 all increased in CHB compared with HC.($P<0.05$). ② In HBeAg positive CHB circulating IL-1β, IL-6, IL-10 and IP-10 were strikingly higher than those in HBeAg negative CHB. ($P<0.05$) And IL-17 significantly decreased, resulting in IL-10/IL-17 ratio rising in HBeAg positive CHB. ③ Compared with HBV DNA>1000 IU/ml group, cytokines and chemokines except for IL-17 were all elevated in HBV DNA≤1000 IU/ml group ($P<0.05$ for IL-1β, IL-6, IL-10, IL-17 and IP-10), as well as IL-10/IL-17 ratio increased. ④The similar differences of circulating cytokines and chemokines levels were observed in ALT group, which showed circulating IL-1β, IL-6, IL-10, IP-10 and IL-10/IL-17 ratio rose distinctly and IL-17 declined in ALT≥40 IU/L group. ⑤ Correlation analysis indicated IL-1β, IL-6, IL-10 and IP-10 were positively related to serum ALT levels ($r_s$=0.360, 0.381, 0.352 and 0.459, respectively; all P values <0.05), and IL-17 had a negative correlation with ALT level (rs= -0.192,$P<0.05$ ).

**Conclusion:** In chronic HBV infection circulating rising IL-1β, IL-6 and IP-10 took part in liver injury and higher IL-17 with lower IL-10 may have a anti-virus role, which was characterized by inhibiting HBV DNA replication and prompting seroconversion to anti-HBe antibodies.

## B-112

### Rapid screening of urinary tract infection using bacteria mode of UF-5000

G. Kwon, S. Kim, J. Lim, H. Kim, S. Koo. *Chungnam National University Hospital, Daejeon, Korea, Republic of*

**Background:** Rapid screening of urinary tract infection (UTI) is advantageous to determine rapidly antibiotic treatment, to decrease inappropriate use of antibiotics, and reduce the unnecessary urine culture. Using a flow cytometry based automated urine analyzer UF-5000 (Sysmex, Kobe, Japan), rapid screening of gram negative and bacteria was made possible. In this study, we compared the results of bacteria mode of UF-5000 and conventional culture and investigated the potential clinical impacts.

**Methods:** A total of 985 urine samples as collected from 813 consecutive patients who were suspected of having UTI and requested for urine culture were tested. Urine samples were inoculated to culture disk using automated Previ-Isola (bioMérieux, Marcy l'Etoile, France) system and bacterial identification were performed by Vitek 2 (bioMérieux). After inoculation by Previ-Isola, the same tubes of the urine were analyzed by Sysmex UF-5000 within 3 hours after inoculation.

**Results:** Among total samples, 587 samples (59.6%) presented bacterial growth, with 216 samples presented gram negative bacteria and 321 samples presented gram positive bacteria in urine samples by culture. By receiver operating curve (ROC) analysis the best-cut off of UF-5000 for suspected UTI (with ≥ 1,000 gram-negative bacteria and ≥ 10,000 gram-positive bacteria) was >122.1 bacteria/μL, with the sensitivity and specificity of 72.6% (67.7%-77.1%) and 89.7% (87.0%-92.0%), respectively. The cut-off for screening significant UTI (with ≥ 100,000 gram-negative and/or -positive bacteria) was >118.8 bacteria/μL, with the sensitivity and specificity of 87.4% (82.6%-91.2%) and 85.0% (82.2%-87.5%), respectively. When the bacterial count and white blood cell (WBC) counts of UF-5000 were analyzed in combination, the area under the curve (AUC) was 0.920, and sensitivity was 85.0% and specificity was 86.7% at cut-off of >425.2 bacteria/μL or >155.6 WBC/μL. When the performance of bacterial identification of UF-5000 was evaluated in samples with ≥ 100,000 bacteria, the sensitivity was 86.2% and specificity was 90.3% for reporting the Gram-negative flag as compared with the final bacterial identification results. The reporting time of final results was significantly reduced for UF-5000 as compared with conventional culture ($P < 0.001$).

**Conclusion:** The use of screening by UF-5000 can give rapid and useful information for determining UTI and identification of gram-negative and positive bacteria. Therefore it can assist prompt and appropriate use of antimicrobial therapy.

## B-113

### Performance Evaluation of the ADVIA Centaur CMV IgG Assay on the ADVIA Centaur CP System

R. Martin, R. Stadnick, W. Zazulak, C. Mesa, A. Austin, S. Patibandla. *Siemens Healthcare, Tarrytown, NY*

**Background:** Siemens Healthcare (Tarrytown, NY) is currently developing a cytomegalovirus IgG (CMV IgG) assay to detect the presence of lgG antibodies to cytomegalovirus (CMV). Anti-CMV IgG antibodies act as a specific marker to aid in the diagnosis of CMV infection. Changes in the seroconversion status of CMV lgG are an indicator of either a reinfection or reactivation of CMV. The Siemens ADVIA Centaur® CMV IgG assay* is a chemiluminescent magnetic microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate the positive and negative agreement, precision, and sensitivity of a prototype, automated CMV IgG assay tested on the ADVIA Centaur CP system.

**Methods:** The fully automated ADVIA Centaur CMV IgG assay is being developed as an indirect sandwich assay for the detection of CMV IgG antibodies in human serum and plasma for use on the ADVIA Centaur CP system. The assay was evaluated for positive and negative agreement (via method comparison), sensitivity, cross-platform alignment, repeatability, and within-lab precision. The positive and negative agreements of the assay were evaluated using a total of 1112 patient samples across two reagents lots. Sensitivity was evaluated using a mix-titer panel obtained from the Center for Disease Control (CDC) containing characterized CMV IgG positive and negative samples. The results were assesses based on Index values as reactive (≥1.00) and nonreactive (<1.00). The serological status of all samples was initially determined by the bioMerieux VIDAS® CMV IgG assay or provided Certificate of Analysis. Discordant samples were tested on the Siemens IMMULITE® 2000 and/or Roche cobas® e 411 CMV IgG assays, when available. Cross platform alignment was evaluated against the ADVIA Centaur XP system, as per CLSI EP9-A2. Precision was evaluated, as per CLSI EP5-A3, by testing four samples with Index values spanning the assay range in two runs per day for 20 days on the ADVIA Centaur CP system for a total of 80 replicates.

**Results:** Evaluation of the patient samples using the ADVIA Centaur CMV IgG assay indicated that the positive agreement ranged from 99.0% to 99.2% and the negative agreement ranged from 95.3% to 95.8%, when compared to the VIDAS CMV IgG assay. In addition, the ADVIA Centaur CMV IgG assay displayed a total agreement of 99.4% (179/180) to the clinical status of the characterized CMV IgG samples provided by the CDC. Regression analysis comparing sample performance on the ADVIA Centaur XP and CP immunoassay systems, across two lots, yielded a slope ranging from 0.946 to 1.00. The assay demonstrated good precision, with an average

repeatability and within-run %CV of <4.0% and <6.0%, respectively, for samples yielding Index values between 0.50 and 30.00.

**Conclusion:** The results of this study demonstrate good performance of the prototype ADVIA Centaur CMV IgG assay on the ADVIA Centaur CP system.

*Under Development. Not available for sale. The performance characteristics of this product have not been established.

## B-114

***Molecular characteristics of HBV virus in patients with HIV/HBV combined infection***

T. Wang, D. Li, Q. Chen, C. Tao*, S. Feng, C. Rao. *West China Hospital of Sichuan University, Chengdu, China*

**Background:** HBV and HIV share common routes of transmission, so HIV/HBV coinfection is common. We analyzed patients with HIV/HBV coinfection and HBV mono-infection cases, and compared the HBV genotype distribution, RT and BCP/PC mutation rate.

**Materials and Methods:** 54 patients with HIV antibody and HBV DNA>$10^3$IU/ml were defined as the research group, while 56 cases without HIV antibody and with HBV DNA>$10^3$IU/ml as the control group. HBV DNA was extracted from serum or plasma. Then, nested PCR was performed for the RT and BCP/PC region. After electrophoresis, PCR products were sequenced. The sequencing results were analyzed.

**Results:**Genotype B was the dominant genotype in HIV/HBV coinfection patients, accounting for 81.48%, while genotype C accounted for 14.82%. The other two cases were C/D recombinant. The total drug resistance rate was 3.70%. A1762T, G1764A and G1896A mutations were the highest mutations in BCP/PC region. A1762/G1764A mutation rate was 35.19%, and G1896A mutation rate was 59.30%. Compared with HBV mono-infection group, there was no significant difference in HBV genotype distribution, drug resistance rate, HBV gene system evolutionary tree and PC mutation rate. And in HIV/HBV coinfection group A1762T/G1764A mutations rate was lower. A1762T, G1764A and G1896A mutation rate of HBeAg negative patients was higher than the HBeAg positive patients for both HIV/HBV coinfected group or mono-HBV infection group.

**Conclusion:** This study was the first time that explored the molecular characteristics of HIV/HBV coinfected patients and the similarities and differences of the HIV/HBV coinfected and HBV mono-infected persons in molecular biology. Research results showed that the genotypes of both groups were mainly genotype B and C, low resistance rates, similar Phyletic evolution, high BCP/PC mutation rate. Yet, HBV mono-infected persons has higher A1762T and G1764A mutation.

HBV genotype comparison of HIV/HBV coinfection group and HBV mono-infection group

| | **HIV/HBV coinfection** | **HBV mono-infection** |
|---|---|---|
| | (n=54) | (n=56) |
| Genotype B | 44(81.48%) | 45(80.36%) |
| Genotype C | 8(14.82%) | 10(17.86%) |
| Genotype D | 0 | 1(1.78%) |
| Recombinant C/D | 2(3.70%) | 0 |

## B-115

**Laboratory Diagnosis of Chikungunya virus: a comparative study of commercial tests for detection of IgM and IgG anti-Chikungunya**

N. Nunes[1], D. Silva[1], A. C. S. Ferreira[2], E. Mateo[2], D. A. G. Zauli[2]. *[1]Hermes Pardini Institute, Vespasiano, Brazil, [2]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

**Background**: The Chikungunya fever is caused by the Chikungunya virus (CHIKV) with symptoms similar to other arbovirus infection, however polyarthritis/arthralgia are clinical signs that can to last months after fever. This disease has affected millions of people and continues to cause epidemics in many countries. According to World Health Organization it occurs in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of Chikungunya have spread to Europe and the Americas. Expansion and endemicity of CHIKV is likely, and large outbreaks of CHIKV infection may continue for the foreseeable future. Clinical laboratories throughout the Americas will need to build and maintain high-volume diagnostic testing capacity and will need validated and reliable commercial CHIKV diagnostic assays to respond to these increased diagnostic testing responsibilities. Commercial kits are available, sometimes with excellent sensitivity and specificity. **Objective**: To compare the performance of two tests for serologic diagnostic of CHIKV on Brazilian samples obtained in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) care routine, using enzyme immunoassay and indirect immunofluorescence. **Methods**: A total of 63 samples were analyzed by Anti-Chikungunya IIFT IgM and IgG kit (Euroimmun AG), and Chikungunya Virus IgM and IgG ELISA kit (Novalisa) according to the manufacturer's instructions. Both tests were approved by a national regulatory agency (ANVISA, Agência Nacional de Vigilância Sanitária). Positive and negative controls were used, which were approved and validated. **Results:** The IgM antibody results showed a concordance of 24 positive samples and 36 negative samples between the tests. The disagreements of 1 negative sample in IIFT and positive in ELISA, and 2 positive samples in IFF and negative in ELISA were evidenced. The negative concordance percentage for IgM was 97.3% and the positive concordance was 92.3%. For IgG antibody, 33 negative and 19 positive samples were observed in both tests, 11 samples were positives in IIFT and negative in ELISA. The percentage of negative agreement was 100% and positive concordance was 63.3%. The Kappa coefficient for IgM was 0,901, with 95% confidence intervals (CI) of 0,654 to 1.0, indicating nearly perfect agreement and for IgG was 0,644, with 95% CI of 0,413 to 0,875, indicating substantial agreement. **Conclusions**: Anti-CHIKV antibodies can be detected in patients shortly after symptom onset, usually after 5 days for IgM and only a few days later for IgG. The CDC published study comparing CHIKV IgM using commercials kits IIF Euroimmun and other ELISA´s and it demonstrate an agreement of >95% to >90%. According to the manufacturers, both techniques show differences in sensitivity (ELISA-Novalisa: > 90%, IIF-EUROIMMUN: 96.2%), which may explain the occurrence of divergences. Despite this, the two kits demonstrate to have equivalent performance. The findings show the importance of evaluating commercial diagnostic kits before using such tools in laboratory routine especially in CHIKV endemic areas and in clinical settings.

## B-116

***Mcr1* gene: first detected case in Ceará, Northeastern Brazil**

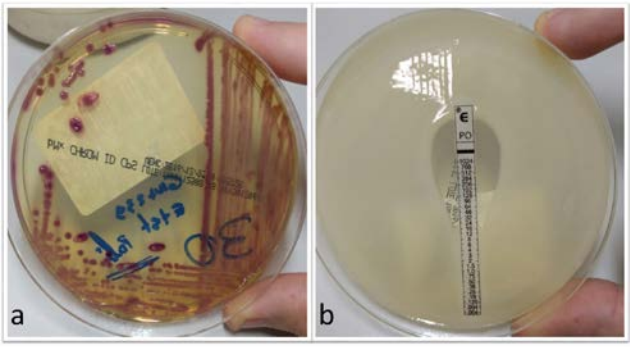
M. C. Gurgel Castelo[1], S. P. Bandeira[2], L. L. A. Cavalcante[3], E. J. U. Kusano[4], A. C. C. Pignatari[4], F. M. P. Ventura[3], R. R. Araújo[3], T. A. C. Palhano[3], G. A. Campana[4], E. S. Girão[5]. *[1]Universidade Federal do Ceará; DASA, Fortaleza, Brazil, [2]Universidade Federal do Ceará;DASA, Fortaleza, Brazil, [3]DASA, Fortaleza, Brazil, [4]DASA, São Paulo, Brazil, [5]Universidade Federal do Ceará;, Fortaleza, Brazil*

**Background:** Resistance to polymyxins is already known in Gram negative bacteria due to mutation in chromosomal sites. In 2015, Liu *et al*. described *mcr1* gene as responsible for plasmid-mediated resistance to polymyxins in *Escherichia coli*strains. Since then, some cases have been described in several countries worldwide. However, few cases have been reported in Brazil and no reports of this gene have been described in strains from Ceará, Brazil.

**Methods:** This work aims to describe the first case of detection of *mcr1* gene in*Escherichia coli* from Ceará, Brazil

**Results:** A 86-year-old female patient was admitted to the hospital with complaints of general decline and drowsiness for 3 days. She had reported sudden respiratory distress. She was transferred to ICU and it was prescribed Piperacillin-Tazobactam EV. Urineculture and hemoculture were requested. Urine sample was seeded in CPS ID3 agar (BioMerieux ™). After 24 hours at 35ºC, a pink colony was detected (figure 1a). Analysis by Vitek 2 (BioMerieux ™) resulted in *Escherichia coli*identification with resistance pattern to ampicillin, ciprofloxacin and colistin. The MIC found for colistin was 8µg/mL. E-test of polymyxin was performed, which confirmed *in vitro* resistance pattern (figure 1b). The isolate was subjected to real-time PCR and the presence of *mcr1* gene was detected.

**Conclusion:** This is the first described case of detection of *mcr1* gene from Ceará, Brazil. Since it is a plasmid-mediated resistance mechanism, early detection of this gene is desirable in order to prevent horizontal dissemination of resistance to other bacterial species, which may limit available therapeutic arsenal for infections by multidrug resistant microorganisms.




## B-117

### Study of the concordance of serological tests for anti-Leishmania antibodies detection

N. C. Nunes[1], J. C. W. Cocovich[1], E. V. C. Benfica[1], E. Mateo[2], D. A. G. Zauli[2], F. A. Brito[1]. *[1]Hermes Pardini Institute, Vespasiano, Brazil, [2]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

**Background**: Visceral Leishmaniases (VL) is a potentially fatal endemic zoonosis, if not promptly diagnosed and treated. In the presence of suggestive clinical and laboratory data, a reactive immune test reinforces the diagnosis of VL. Indirect immunofluorescence (IFI) and ELISA are the serological tests most frequently used as diagnostic of VL. Unfortunately, the knowledge about the agreement of available commercial assays for anti-leishmania antibodies detection is limited. **Objective**: To evaluate the agreement between the commercial kits: Novatec (ELISA), EUROIMMUN (IFI) and R-Biopharm (ELISA) for anti-leishmania antibodies detection. **Methods**: A total of 84 samples obtained in Institute Hermes Pardini routine were selected from results of R-biopharm kit (comparative method), being 40 negative and 44 positive samples. Fifty-five samples were also tested in the IT-LEISH kit (Gold standard). **Results:** The Kappa index was 0.352 (R-Biopharm and EUROIMMUN, 95% CI= 0.206 to 0.498), indicating regular agreement; 0.476 (R-biopharm and Novatec, 95% CI= 0.327 to 0.626), indicating moderate agreement and 0.607 (Novatec and EUROIMMUN, 95% CI= 0.426 to 0.788), indicating moderate agreement. The main causes of disagreement were: positives results by R-Biopharm Kit and negatives by Novatec kit (n=20), positives results by R-Biopharm kit and negatives by EUROIMMUN kit (n=27). The Novatec and EUROIMMUN kits presented the higher degree of agreement. The results of each kit compared to gold standard method (IT-LEISH) are shown in Table 1. The sensitivity and specificity of each kit are described as follows, respectively: R-Biopharm kit = 94% and 32%; Novatec kit = 94% and 81%; Euroimmun kit = 67% and 89%. **Conclusions**: The absence of a good level of agreement between the kits evaluated suggests a lack of standardization among the commercial immunological tests available for the serological diagnosis of VL. Thus, is necessary to develop a standard diagnostic method for VL diagnosis, such as serological tests used by reference centers.

Table 1: Agreement of kits compared to IT-LEISH.

| KIT | IT-LEISH | |
|---|---|---|
| **R-biopharm** | **Negative** | **Positive** |
| Negative | 12 | 1 |
| Positive | 25 | 17 |
| **Novatec** | **Negative** | **Positive** |
| Negative | 30 | 1 |
| Indeterminate | 3 | 2 |
| Positive | 4 | 15 |
| **Euroimmun** | **Negative** | **Positive** |
| Negative | 33 | 5 |
| Indeterminate | 1 | 1 |
| Positive | 3 | 12 |

## B-118

### MALDI-TOF: an application of mass spectrometry for pathogenic bacteria identification in clinical laboratory.

D. A. G. Zauli[1], L. A. Caldeira[2], A. C. S. Ferreira[1], E. Mateo[1], M. Sousa[2]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute, Vespasiano, Brazil*

**Background**: Currently, diagnostic methods for microbiological identification of pathogenic bacteria have been carried out classically by methods involving culture and phenotypic/biochemical tests exploring metabolic differences that exist between different species of microorganisms. These methods of identification are extremely powerful methods of pathogen retrieval, however, they are laborious and the results are obtained after a long period of time depending on the pathogen. In some circumstances, such as bacteremia, identification and appropriate treatment are critical. New methods of identification have been developed. Rapid and accurate molecular genetic tests are commercially available for the identification of species, but they are expensive, which is a limiting factor for their implementation in the laboratory routine. Other methods for rapid diagnosis in the etiology of infections are being used, such as mass spectrometry technology. **Objective**: To analyze the process of bacterial identification by Maldi-Tof in clinical laboratory routine. **Methods**: Bacterial strains were isolated from several clinical samples, including peritoneal fluid, bronchial lavage, urine, blood, sputum and oral secretion that were sent to Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) for culture and identification. The culture was carried out in specific media according to each of clinical samples and medical suspicion. Identification of strains was performed in the Vitek System according to the manufacturer's instructions and/or by conventional Pessoa and Silva method. Bacterial identification by mass spectrometry was performed using Vitek MS (BioMérieux) equipment according to the manufacturer's instructions. For calibration of equipment was used a reference strain of *Escherichia coli* ATCC 8739 according to the manufacturer's specifications. The data obtained from the spectrum were transferred from Vitek MS acquisition station to Myla analysis server (BioMérieux) and compared with SARAMIS 4.12 database. **Results**: A total of 214 bacterial strains were analyzed, including 60 enterobacterial strains, 48 *Staphylococcus* strains, 56 catalase negative cocci bacteria, 50 non-fermenting and fastidious Gram negative rods strains. The concordant results between the two identification techniques were summarize as follows: 90% (54/60) of enterobacterial strains; 92% (44/48) of *Staphylococcus* strains; 98% (55/56) of catalase negative cocci bacteria; 98% (49/50) of non-fermenting and fastidious microorganisms. **Conclusion**: The Maldi-Tof system showed an identification efficiency and workflow robustness compared to routine phenotypic identification in laboratory. This technique has revolutionized microbiological clinical diagnosis because it is a faster workflow, accurate and low cost specimen identification method. The use of mass spectrometry in clinical and research laboratories in European countries is already a reality. In Brazil, the first mass spectrometer with this application was installed in 2010 and this tool is already being used by some research groups and clinical laboratories. Further investigations are need to directly identify bacteria from clinical samples and detect drug resistance among bacteria. These studies are a challenge for clinic microbiologists and might guide patient decision-making regarding bacterial infectious diseases in the near future.

## B-119

### Evaluation of Interference between Biotin and the Streptavidin-Biotin-Based VITROS Hepatitis A-Specific Total and IgM Antibody Immunoassays

J. C. Jara Aguirre, J. D. Yao, N. A. Baumann, B. M. Katzman, T. J. Stier, E. S. Theel. *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*

**Background**: Interference of biotin with certain streptavidin-biotin-based immunoassays has been described for plasma and serum specimens from patients taking oral biotin supplements for certain inherited metabolic diseases, demyelinating diseases, or to enhance hair and nail growth. Such interference may cause false-positive (over-estimation) or false-negative (under-estimation) results in competitive and non-competitive immunoassays, respectively. We conducted a study to investigate the effect of biotin interference in 2 streptavidin-biotin-based, serologic assays, VITROS Anti-HAV Total (HAVT) and VITROS Anti-HAV IgM (HAVM) (Ortho-Clinical Diagnostics, Inc.), used for detection of hepatitis A-specific total antibodies (combined IgG and IgM) and IgM antibodies, respectively.

**Methods**: HAVT and HAVM are competitive and non-competitive immunoassays, respectively, performed with the fully automated VITROS 3600 Immunodiagnostic

System (Vitros 3600; Ortho-Clinical Diagnostics, Inc.). Aliquots of serum were prepared from pooled clinical specimens with 4 different signal/cut-off(S/CO) ratio for HAVT: 1.74 (negative), 1.25 (negative), 0.550 (reactive), and 0.030 (reactive). Aliquots of serum were prepared similarly with 3 different S/CO ratio for HAVM: 0.010 (negative), 1.67 (reactive), and 6.86 (reactive). These aliquots were spiked with a 0.9% NaCl solution containing 10,000 ng/mL biotin (Sigma-Aldrich) to achieve final biotin concentrations of 0, 10, 50, 100, 500 and 1,000 ng/mL. Each concentration of biotin in serum was tested in duplicate for HAVT and HAVM on VITROS 3600 per manufacturer's instructions for use. A difference of >14% between observed and expected S/CO ratios was considered significant change, based on manufacturer's precision data for these two assays.

**Results**: Observed S/CO ratios of HAVT and HAVM at various biotin concentrations are shown below:

| Assay | Expected qualitative result | Mean S/CO ratio observed in serum aliquots with the following biotin concentrations in ng/mL | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 10 | 50 | 100 | 500 | 1,000 |
| HAVT | Negative | 1.74 | 1.75 | 1.60 | 0.980[a] | NT | NT |
| | Negative | 1.25 | 1.17 | 0.990[a,b] | 0.660[a,b] | 0.290[a,b] | 0.120[a,b] |
| | Reactive | 0.550 | 0.540 | 0.470[a] | 0.340[a] | 0.050[a] | 0.035[a] |
| | Reactive | 0.030 | 0.030 | 0.025[a] | 0.020[a] | NT | NT |
| HAVM | Negative | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 | 0.010 |
| | Reactive | 1.67 | 1.67 | 1.63 | 1.60 | 1.64 | 1.62 |
| | Reactive | 6.86 | 6.85 | 6.85 | 6.77 | 6.65 | 6.69 |

[a] >14% difference from corresponding ratio at 0 ng/mL;
[b] Change in qualitative interpretive result;
NT, not tested.

**Conclusion:** HAVT was more susceptible than HAVM to biotin interference, leading to a clinically significant change in qualitative interpretive results of original borderline negative specimens. Clinical laboratories should recognize and evaluate potential interference of biotin in streptavidin-biotin-based immunoassays.

## B-120

### Evaluation of serologic laboratorial test in brazilian patients infected by Zika virus and exposed to Dengue virus and Chikungunya virus.

D. A. G. Zauli[1], N. Nunes[2], W. Pedrosa[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute, Vespasiano, Brazil*

**Background:** Zika virus (ZIKV) is virus whose the newest pandemic is alarming, although, with the paucity of literature the exact details of the disease are not clear. The diagnosis of ZIKA infections can be performed on clinical-epidemiological and laboratorial bases. Overall, your laboratorial diagnosis relies on the same usual strategies used for other arboviruses, with viral genome detection by RT-PCR tests on acute-phase samples and serology (ELISA and immunofluorescence). Usually, the choice of the laboratorial approach used will depend on the goal of the analysis, laboratory infrastructure, technical expertise and sampling availability.The diagnosis of ZIKV infection performed by serological tests can detect specific IgM / IgG antibodies against ZIKV after 5 to 6 days of the onset of symptoms, with increased titers within 2 weeks. **Objective:** To evaluate the performance of commercial Euroimmun ZIKV ELISA test (Euroimmun, Lübeck, Germany) using panels from Brazilian patients exposed to Dengue (DENV) and Chikungunya (CHIKV) infection. **Method:** Samples were obtained in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) routine, with a high potential of causing cross-reactions in serological flavivirus assays. The selection of samples was based on six panels for IgM analysis (n=105) and four panels for IgG analysis (n=65). For IgM assay the panels were as follows: Panel I: 20 DENV IgM-positive samples; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgM-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples; Panel V: 20 Epstein Barr virus IgM-positive samples; Panel VI: 20 Rheumatoid Factor (RF) IgM-positive samples (Concentration: 153.5 to 1350.9 IU/mL). For IgG assay the panels were as follows: Panel I: 20 DENV IgG-positive; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgG-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples. All assays were performed according to the instructions of the manufacturer. **Results**: The ZIKV IgM ELISA was negative in all simultaneously DENV and CHIK negative samples, CHIKV positive, EBV positive and RF positive samples. Of DENV positive samples 10% were ZIKV IgM positive (2/20). For IgG ELISA, 20% of DENV positive samples were ZIKV IgG positive (4/20). All simultaneously DENV IgM and IgG negative samples were ZIKV IgG negative. Of CHIKV IgG positive samples 60% were ZIKV IgG positive (9/15). In addition, 33% of simultaneously CHIKV IgM and IgG negative samples were ZIKV IgG positive (5/15). **Conclusion**: Positive samples for the dengue virus may also be positive for ZIKV. No cross-reactivity of IgM ZIKV with Chikungunya virus has been observed. The IgG positive samples for dengue and Chikungunya viruses may be positive for ZIKV. Despite this it is not established if this situation corresponds to a cross reaction of the test or simply histories of coinfection in different periods in the same individual in endemic areas. Its positivity should be evaluated in the context of the other conditions, such as Epstein Barr virus infections, and in malaria infection. Further studies will be necessary to determine the accuracy of this test and other current assays in a larger set of well-defined samples.

## B-121

### Development and evaluation of a rapid POC multiplex test for the detection of Zika, Dengue and Chikungunya antibodies in patient's blood samples.

D. Gunasekera, K. Paden, K. Addiss, B. Matias-Brand, M. Ashton, L. Sigismondi, A. Gunasekera, P. Lambotte, J. Esfandiari. *Chembio Diagnostic Systems Inc., Medford, NY*

The Chembio Diagnostics proprietary rapid POC DPP® platform is well suited for high sensitivity and multiplex detection of antigens or antibodies in a variety of body fluids. Here, the DPP platform has been expanded to include two test strips in a single cassette, sharing a single sample of fingerstick blood to detect IgM antibodies and IgG antibodies against three possible infectious diseases: Zika, Chikungunya and Dengue. Results are obtained in a few seconds using a small, portable reflectance reader. Differentiating IgM and IgG provides information on the disease phase. This multiplex assay is needed worldwide as the three viruses are carried by the same mosquito vectors and present similar symptoms at the time of infection. Sensitivity of the DPP Zika/Chikungunya/Dengue IgM/IgG System ("DPP Z/C/D") was evaluated against ELISA for IgM and IgG against each of DENV, CHIKV and ZIKV. DENV: of 57 plasma samples, positive for Dengue IgM and/or IgG antibodies, 16 tested positive on DPP Z/C/D, and 15 tested positive by ELISA. Of 46 samples, positive for IgG on DPP Z/C/D, 45 specimens tested positive by ELISA.ZIKV: For IgM, of 24 ZIKV PCR+ serum samples, 7 were positive on DPP Z/C/D and 4 by ELISA. 4/8 plasma samples were positive for Zika IgM on DPP Z/C/D, and only 2 specimens by ELISA. For IgG, 24/24 serum samples tested positive on DPP Z/C/D; 4 were reactive by ELISA. 6/8 plasma samples were reactive for Zika IgG on DPP Z/C/D; 7/8 were reactive by ELISA.CHIKV: Of 54 samples, positive for IgM on the DPP Z/C/D, 51 tested positive for IgM by ELISA. Of 59 samples, positive for IgG by ELISA, DPP Z/C/D found 54 positives.Specificity of the DPP Z/C/D assay was evaluated with 50 whole blood/EDTA specimens and yielded between 94 and 100% specificity for the three analytes.These results suggest that the Chembio DPP Dengue/Zika/Chikungunya IgM/IgG System provides an effective, rapid POC solution with performance equivalent to ELISA, in the field and at the patient's side.

## B-122

### An Evaluation of Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay*

P. Contestable, C. Noeson, T. Mangan, K. Smith, C. Waasdorp, L. Colt, R. Polimeni, I. Tuttle, S. Clark. *Ortho Clinical Diagnostics, Rochester, NY*

**Background:** This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products HIV Combo Assay (VITROS HIV Combo Assay)* on the VITROS® 3600 Immunodiagnostic System. The assay is capable of simultaneous detection of HIV antibodies (Ab) and HIV p24 antigen (Ag) to help enable earlier diagnosis of HIV infection.

**Methods:** Antibody detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane antigens specific to HIV-1 (group M and O) and to HIV-2. The p24 antigen detection is accomplished using monoclonal antibodies (MAb). The antigens and p24 MAb are coated onto the well. Sample is added to the coated wells in the first stage of the reaction, and HIV Ag/Ab from the sample is captured. After washing, HRP conjugated antigens and p24 MAb are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. Specificity was assessed using 6435 samples from low risk populations. Supplemental testing to determine HIV status was conducted on all reactive samples. Sensitivity was evaluated using 1764 antibody positive samples (1535 HIV-1, including 413 from various Group M and O subtypes and 229 HIV-2). In addition, 52 samples with various HIV-1 group M antigen genotypes were tested. Seroconversion sensitivity was assessed by testing 32 commercially available panels on both the VITROS HIV Combo assay and a commercially available Ag/Ab assay. Assay reproducibility was

assessed at three sites using three reagent lots with a 14 member panel. Antigen sensitivity was determined by testing serial dilutions of the NIBSC and the AFSSAPS HIV-1 p24 Ag standards across two reagent lots.

**Results:** The specificity of the VITROS HIV Combo Assay* for the low risk population was 99.59% (6365/6391) [95% exact CI (99.40-99.73%)]. Samples confirmed as HIV positive by supplemental testing were removed from the analysis (n=44). The sensitivity for HIV-1 and HIV-2 antibody positive samples was 99.94% (1763/1764) [exact 95% CI (99.68-100.00%)]. All 413 HIV-1 group M and O antibody positive subtypes and 50 of 52 HIV-1 group M antigen genotypes were reactive with the VITROS HIV Combo assay*. For seroconversion panels the VITROS HIV Combo assay* was reactive at the same panel member as the commercially available Ag/Ab assay for 27 of the 34 panels, was reactive one panel member earlier for 6 panels, and was reactive two panel members later for 1 panel. For the reproducibility study the observed precision for the 12 panel members positioned near the assay cutoff ranged from 9.1 to 17.8 %CV. The overall sensitivity of the VITROS HIV Combo test for the NIBSC HIV-1 p24 Antigen Standard (90/636) was ≤0.48 IU/mL and for the AFSSAPS HIV-1 p24 Antigen Standard was ≤13.1 pg/mL.

**Conclusion:** The VITROS HIV Combo Assay* demonstrates acceptable clinical and analytical performance in the simultaneous detection of antibodies to HIV-1 (group M and O), HIV-2 and HIV p24 antigen.

*Not approved for use in the US.

## B-123

### AccuSpan Zika Linearity Panel Spans the Dynamic Range of Assays and Allows Evaluation of Analytical Sensitivity

H. Lee[1], V. MacKeen[1], J. Wu[2], R. Vemula[2], C. Huang[2], B. Anekella[2]. *[1]SeraCare Life Sciences, Milford, MA, [2]SeraCare Life Sciences, Gaithersburg, MD*

**Background:** An outbreak of the mosquito-borne Zika virus occurred in Brazil in spring of 2015. Since that time, the virus has gained global attention due to its rapid spread throughout countries of the Americas and more recently Pacific island nations, as well as its link to neurological birth defects. In response to this outbreak, several PCR-based Zika assays have been developed and approved under FDA's Emergency Use Authorization. Clinical laboratories as well as test developers need to evaluate assays across the entire reportable range for sensitivity and linearity. SeraCare has developed the Accuspan Zika Linearity Panel to meet this need. The panel uses recombinant Sindbis virus technology; therefore, it is non-infectious but is a whole process reference material that must undergo the entire extraction procedure. This study reports the formulation of the panel as well as initial testing.

**Methods:** The Zika virus is a positive sense RNA virus whose genome is approximately 10.7 Kb. The Zika genome was divided into four (4) segments and each segment was used to generate a recombinant virus using Sindbis vector system. The recombinant viruses were heat inactivated and purified. The strategy of dividing the Zika genome into four different recombinant viruses such that each recombinant virus is not functional assures the safety of the reference material and the heat inactivation serves as an additional safety precaution. Digital PCR assays, specific for each recombinant virus construct (Envelop region, NS2/NS3 region, NS4 region and NS5 region) were designed and used for quantitation of each viral stock. Based on digital PCR quantitation, the viral stocks were mixed together in equimolar ratios such that the combined bulk contained sequences of the entire Zika genome. The combined bulk was then serially diluted in defibrinated human plasma to titers of 1.0E+06, 1.0E+05, 1.0E+04, 1.0E+03, 1.0E+02, and 1.0E+01 copies/mL. Titers of each member were verified by digital PCR testing. A negative member (containing the plasma diluent) is also included in the panel.

**Results:** Panel member testing was performed on various assays, both approved for Emergency use and in development including the Roche LightMix® Zika rRT-PCR Test, Hologic Aptima® Zika Virus Assay, and Beckman DxN Zika Virus Assay - EUA (pending FDA authorization). Testing results indicate these assays all have lower limits of detection of 100 copies/mL or lower. More extensive testing was performed on Roche Zika Assay for the 1.0E+03 and 1.0E+02 cp/mL Panel members. Members were tested in five replicates on three days; all replicates were positively detected. The 1.0E+03 cp/mL member was detected with average cycle threshold value of 30.76 ± 0.099 and the 1.0E+02 cp/mL member was detected with average cycle threshold value of 33.83 ± 0.39.

**Conclusions:** SeraCare has developed a stable, well-characterized linearity panel for Zika virus. This panel will enable laboratories to validate tests and ensure test performance to improve preparedness. The Accuspan Zika Linearity panel demonstrates the utility of recombinant virus technology to produce non-infectious reference materials for dangerous viruses that are difficult to source or propagate.

## B-124

### Biomarkers of inflammation in cerebrospinal fluid and serum to differentiate between bacterial and viral meningitis

J. F. Cuadros-Muñoz, J. D. Santotoribio, C. Cañavate-Solano, M. Mayor-Reyes, S. Perez-Ramos. *Puerto Real University Hospital, Cadiz, Spain*

**Background:** The analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture is used as test for diagnosis of meningitis. There are a great number of works published on biomarkers of inflammation for the diagnosis of meningitis, but there are discrepancies on their utility clinical, not being clear if its determination in CSF presents a greater accuracy that in serum. The aim of this study was to determine the accuracy of the albumin, lactate dehydrogenase (LDH), c-reactive protein (CRP), procalcitonin (PCT) and ferritin in CSF and serum to differentiate between bacterial and viral meningitis.

**Method:** We study patients with clinical suspicion of meningitis. Two types of samples were analyzed: CSF by lumbar puncture and peripheral venous blood obtained by venipuncture. Albumin, LDH and CRP were quantified in the autoanalyzer Dimension EXL (Siemens®), ferritin in the Modular E-170 (Roche Diagnostic®) and the PCT in the Kriptor (Thermo Scientific®). The diagnosis of the patient was obtained from their medical history. Patients diagnosed with bacterial and viral meningitis were selected. The accuracy for diagnosis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

**Results:** We studied 156 patients with age between 1 and 86 years (median = 54 years), 64 women and 92 men. 18 were diagnosed of bacterial meningitis and 12 of viral meningitis. Biomarkers analyzed in CSF and/or serum showed higher concentrations in patients with bacterial meningitis which in the viral. The AUC to differentiate bacterial from viral meningitis with biomarkers analyzed in CSF, serum and with its ratio (CSF/serum) are shown in the following table:

| | CSF | Serum | CSF/Serum |
|---|---|---|---|
| **ALBUMIN** | 0.750 (p=0.0088) | p>0.05 | p>0.05 |
| **LDH** | 0.704 (p=0.0327) | p>0.05 | 0.796 (p=0.0003) |
| **PCR** | 0.935 (p<0.0001) | 0.926 (p<0.0001) | 0.806 (p=0.0001) |
| **PCT** | 0.694 (p=0.0440) | 0.963 (p<0.0001) | 0.870 (p<0.0001) |
| **FERRITIN** | p>0.05 | 0.852 (p<0.0001) | 0.963 (p<0.0001) |

**Conclusions:** Albumina is useful to differentiate viral from bacterial meningitis when is it quantified in CSF; LDH presents greater accuracy when using the CSF/serum ratio; PCR when determined in CSF; the PCT when they are quantified in serum; and ferritin when using ratio CSF/serum.

## B-125

### A Rapid Syphilis Test for Qualitative Measurement of Treponemal Antibody

C. Xu, Q. Shi. *ZBx Corporation, Toronro, ON, Canada*

**Background:** Syphilis is a sexually transmitted disease caused by bacteria called Treponema pallidum (TP). Most people with syphilis tend to be unaware of their infection and they can transmit the infection to their sexual contact or in the case of pregnant woman, to her unborn baby. If left untreated, syphilis can cause serious consequence such as stillbirth. According to WHO, syphilis kills more than one million babies a year worldwide, and 12 million new cases of syphilis occur every year. In addition, as a cause of genital ulcer disease, syphilis has been associated with an increased risk of HIV infection. Early diagnosis is important because timely treatment of syphilis significantly prevents its serious consequence and the spread of the disease. An easy-to-use rapid test for the detection of Treponemal antibody can facilitate an early diagnosis of a syphilis. The objective of this study is to evaluate the performance of a new syphilis rapid test. **Principle:** The ADEXUS-Dx Syphilis Rapid Test ("**Syphilis Test**") is a solid phase immunochromatographic assay. The Syphilis Test uses a sandwich format to detect the presence of Treponemal antibody in blood, plasma, and serum samples. The appearance of a purplish-red band in the test window indicates that the sample contains Treponemal antibody. The Syphilis Test has a unique feature of finger-stick, capillary whole blood sampling needing only 35 µl blood. No additional buffer is needed. The result is ready in 15 minutes. **Performance:** 100 known Treponemal antibody positive samples were tested by the ADEXUS-Dx Syphilis Rapid Test and 99 tested positive. The calculated clinical sensitivity is 99%. Within these 100 positive patient samples, 51 are serum, 26 are EDTA plasma, 11 are heparin plasma, and 12 are citrate plasma. The expected results were obtained for both serum and plasma samples, indicating the test was unaffected

by anti-coagulants. 100 known Treponemal antibody negative serum samples were tested by the ADEXUS-Dx Syphilis Rapid Test and 94 tested negative. The calculated clinical specificity is 94%. Sera containing human anti-mouse antibodies (HAMA) up to 327 ng/mL tested negative suggesting minimal interference by HAMA in a normal population based on the reference range for HAMA (0-188 ng/mL). The syphilis test was compared with a FDA CLIA waived test, Syphilis Health Check, which is also a lateral flow test. Ten known Treponemal antibody positive samples were tested by both methods and 100% agreement (all positive) was achieved although ADEXUS-Dx Syphilis Rapid Test showed relatively stronger signal. Testing of 5 normal fresh capillary blood from finger pricks showed negative results. **Conclusion:** The Syphilis Test is a one-step rapid test with clinical sensitivity and specificity 99% and 94% respectively. No HAMA interference was observed. The fact that the new test is suitable to test capillary blood makes this test a potential point of care and over the counter Syphilis Rapid Test.

## B-126

### Performance Evaluation of the HIV Ag/Ab Combo (CHIV) Assay on the Siemens ADVIA Centaur XP System

A. Macumber, K. W. Simkowski. *Beaumont Health System, Royal Oak, MI*

**BACKGROUND:** A limited number of 4th Generation (4G) HIV tests is currently available to detect both HIV-1 and HIV-2 antibodies and the p24 antigen (HIV-1). Our hospital system currently uses the Abbott ARCHITECT® 4G immunoassay (IA) with reflex to BioRad Multispot HIV-1/HIV-2 Rapid Test kit for routine testing. When the Multispot does not confirm a repeatedly reactive ARCHITECT result, serum is referred for further HIV-1 RNA qualitative testing.

**OBJECTIVE:** The Siemens ADVIA Centaur HIV-1/2 Ag/Ab Combo (CHIV) was recently approved by the FDA. Both the ARCHITECT and ADVIA Centaur report a signal-to-cutoff ratio for combined HIV-1/2 antibodies and p24 antigen (HIV-1). The Abbott ARCHITECT is a manual-loading, stand-alone instrument while the ADVIA Centaur is attached to the Siemens Lab Cell automated line. Therefore, our interest was to evaluate the ADVIA Centaur method to replace the current ARCHITECT method for routine testing.

**STUDY DESIGN:** Remnant sera from 200 patients (100 HIV-1/2 Ag/Ab reactive and 100 nonreactive) originally screened by the ARCHITECT HIV Ag/Ab Combo assay were obtained, re-run on the ARCHITECT and then run on the ADVIA Centaur by the HIV Ag/Ab Combo (CHIV) assay. All repeatedly reactive samples were clarified by performing the BioRad Multispot HIV-1/HIV-2 Rapid Test. All samples initially repeatedly reactive by the ARCHITECT method and nonreactive by the Multispot were sent to a reference lab for HIV-1 Qualitative RNA testing performed by transcription mediated amplification for further clarification of HIV status.

**METHODS:** ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is an *in vitro* diagnostic immunoassay for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1 (including group "O") and type 2 in serum. ARCHITECT HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1 (group M and group O) and/or type 2. **RESULTS:** Inter-assay precision for all positive and negative controls were acceptable. Out of the 100 HIV-1/2 Ag/Ab reactive specimens, 76 were reactive for HIV-1 antibodies, 1 sample was HIV-2 antibody reactive and 1 sample was reactive for HIV-1 antigen by both the ARCHITECT and ADVIA Centaur. There were 22 (11%) discrepant HIV Ag/Ab samples between the two methods (as determined by supplemental testing on the BioRad Multispot and HIV-1 Qualitative RNA testing). All 22 samples were deemed nonreactive by supplemental testing and nonreactive by the ADVIA Centaur XP method but repeatedly reactive by the ARCHITECT method. All 100 HIV-1/2 Ag/Ab nonreactive specimens were nonreactive on the ARCHITECT and ADVIA Centaur for 100% negative agreement. No supplemental testing was performed on these samples.

**CONCLUSIONS:** Based on supplemental testing, it appears the ADVIA Centaur CHIV method demonstrates fewer false positive results as compared to the ARCHITECT 4G HIV testing. Further review of patient histories is warranted to determine if patients were re-tested at 1 to 6 months based on recommendations for repeatedly reactive HIV screening results and nonreactive supplemental testing. The Siemens ADVIA Centaur XP CHIV method would be an acceptable replacement for HIV-1/2 Ag/Ab routine testing.

## B-127

### The FSC PathOne™ Analyzer: A Novel Platform for the Rapid Diagnosis of *Pseudomonas aeruginosa* in Urinary Tract Infection

N. Fowler[1], E. A. Tacheny[1], K. Lohman[1], R. Williams[2], J. L. Lucas[1]. *[1]MRIGlobal, Gaithersburg, MD, [2]Fundamental Solutions Corporation, Easton, PA*

**Objective:** We developed and validated an assay for the rapid diagnosis of *P. aeruginosa* in urine on a novel detection platform, the PathOne™Analyzer from Fundamental Solutions Corporation. **Relevance:** Urinary tract infections are a serious health problem and the second most commonly diagnosed infection of any organ system in the United States. Catheterization is a risk factor for developing UTIs, and catheter-associated UTIs (CAUTI) account for more than 1 million cases in hospitals and nursing homes annually. *P. aeruginosa* is an important pathogen for CAUTI, representing approximately 10% of all cases, of which approximately one third are fluoroquinolone-resistant. *P. aeruginosa* is one of the ESKAPE pathogens and is of particular concern due to its ability to form biofilms on the surface of urinary catheters and its resistance to many antibiotics. Therefore, rapid and accurate diagnosis is needed to provide patients with the correct antibiotics and improve patient outcome. **Methods:** The PathOne analyzer is a novel, rapid, portable diagnostic device and cartridge system that was designed for the true real-time detection of pathogens from sample to result in less than 5 minutes with no enrichment. The technology is a live cell-based biosensor which rapidly detects the presence of an offending target pathogen through a proprietary, light-emitting reaction. In this study, we developed an assay to detect *P. aeruginosa* in contrived urine samples. Assay limit of detection, specificity, range and linearity was determined. Results were obtained using urine pooled from 10 donors and confirmed using contrived samples from individual donors. Contrived samples were generated to mimic bacterial load found in clinical samples. **Validation:** We have in our collection 50 isolates of *P. aeruginosa* with well-characterized resistance profiles against 15 antibiotics representing the major drug classes. These isolates were used to generate contrived urine samples to mimic bacteriuria found in the clinic. Data were analyzed to determine within-run precision, total precision and accuracy. **Results and Conclusions:** We have developed a novel, rapid diagnostic test for *P. aeruginosa* in urine with acceptable sensitivity, specificity, precision and accuracy.

## B-128

### A New One-Step Direct-Sampling Hepatitis B Surface Antigen Rapid Test

M. Tsang, Q. Shi. *ZBx Corporation, Toronro, ON, Canada*

**Background:** Hepatitis B surface antigen (HBsAg) is a group of protein complexes found in serum of patients with hepatitis B virus (HBV) infection and indicates a current hepatitis B infection. HBsAg detection by immunoassay is used in blood screening, to establish a diagnosis of hepatitis B infection in the clinical setting (in combination with other disease markers) and to monitor antiviral treatment. HBsAg usually appears 4 weeks after viral exposure but can be detected any time after the first week. An individual positive for HBsAg is considered to be infected. Persistence of HBsAg is used to differentiate acute from chronic infection. Presence of the antigen longer than 6 months after initial exposure indicates chronic infection. However, the level of antigen does not appear to correlate with disease severity. A rapid one-step test format that accepts a small volume of whole blood will facilitate point-of-care as well as self testing for HBV infection. The objective of this study is to demonstrate the clinical utility of a new one-step HBsAg antigen test. **Principle:** The ADEXUS-Dx HBsAg Test was developed using a direct sampling immunoassay technology for whole blood, plasma or serum. Monoclonal antibodies to HBsAg were employed for the qualitative detection of HBsAg. A small sample volume (35-40µL) is required to run the test and no extra buffer is needed. Capillary blood from a finger tip can be directly applied to the test without any transfer device. When the sample is sufficient to fill the Receiving Channel, the sample flows into a dry porous test strip composed of a membrane array with gold conjugated HBsAg antibodies. The appearance of a visible purplish-red band at the test region indicates the sample contains a detectable level of HBsAg. **Performance:** The new one-step rapid HBsAg test requires less than 40µL of sample and was completed in 15 minutes without additional steps. The test did not have any high dose "hook" effect up to 20 µg/ml of recombinant HBsAg. Samples containing human anti-mouse antibodies up to 327ng/mL measured by Abazyme did not produce false positive results. The test analytical sensitivity is 3.0 IU/ml base on the Third WHO International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2). Clinical sample testing showed that the test recognized HBsAg subtypes adw, ayw, adr, and ayr. A total of 188 clinical samples

(78 positives and 110 negatives measured by Abbott EIA) were tested . The overall relative sensitivity and specificity for the detection of HBsAg were 96% and 94% respectively. Fresh normal capillary blood test showed negative results with good plasma separation. **Conclusion:** The ADEXUS-Dx HBsAg Test is a true one-step rapid test suitable for capillary blood testing. It demonstrated good sensitivity and specificity. It is suitable for use in the detection of HBV infection at the point-of-care settings and for self-testing.

## B-129

### Identifying Significant Association between Host Factors and Improvement of QF TB-Gold Testing with Decreased Incubation Periods

D. Eltoum, M. R. Al-Turkmani, L. V. Rao. *University of Massachussetts Medical School, Department of Pathology, Worcester, MA*

**Background:**

Quantiferon-TB Gold In-Tube test (QF) can be used as an alternative to tuberculin skin testing (TST) for the targeted testing of latent tuberculosis and detection in adults previously exposed to BCG vaccine. Due to many shortcomings with TST, QF usage is increasing. QF has improved specificity, less reader bias and increased cost effectiveness. Shortcomings of the QF include variable sensitivity and high rates of indeterminate results in certain groups. Indeterminate results can be due to manufacturing defects, preanalytical error- in collection, delayed incubation or inadequate processing of the sample, or confounding patient factors, such as immunosuppression. In this study we aimed to evaluate the effect of shorter incubation periods on the rate of indeterminate results as well as to assess the correlation of indeterminate results with multiple clinical variables before and after shortened incubation periods.

**Methods:**

In January of 2016, our institution implemented a one hour incubation period for Quantiferon-TB Gold samples, replacing the previous 16 hour incubation period maximum. In this study, we retrospectively identified 112 patients who received QF testing in the 5 months prior to implementation and 141 patients who received QF testing in the 12 months after implementation of 1hr incubation. Seven clinical risk factors associated with indeterminate results were evaluated.

**Results:**

The rate of indeterminate results at our institution dropped from 11.8% to 5.3% after implementation of 1-hour incubation periods. The distribution and clinical characteristics of patients are summarized in Table 1.

**Conclusion:**

While shortening incubation period from 16 hours to 1 hour resulted in a significant decrease in the rate of indeterminate test results in all risk factor categories, significant associations were found between hypoalbuminemia and cirrhosis and the decrease in indeterminate rates with shortened incubation.

**Table 1:**

| | 16-Hour incubation Period | 1-Hour incubation period | P Value |
|---|---|---|---|
| Hypoalbunemia | 45.7% | 65.2% | 0.040 |
| Cirrhosis | 21.7% | 40.9% | 0.034 |
| Malignancy | 13.0% | 13.6% | 0.928 |
| Autoimmune disease | 50.0% | 47.0% | 0.752 |
| Chronic Inflammatory disease | 50.0% | 43.9% | 0.527 |
| Hepatitis B | 0.0% | 1.5% | 0.402 |
| Hepatitis C | 10.9% | 15.2% | 0.753 |

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Lipids/Lipoproteins

## B-130

### Apo B/ A Ratio: A Predictor of Cardio Vascular Disease in Patients with or without Type II Diabetes Mellitus

P. Rayamajhi[1], S. Malla[1], B. Poudel[2], A. Mittal[1]. *[1]Nepalese Army Institute of Health Sciences, Kathmandu, Nepal, [2]Manipal College of Medical Sciences, Pokhara, Nepal*

**BACKGROUND:** Cardiovascular disease (CVD) is the growing issue of public health problem, and first major cause of mortality and morbidity throughout the globe. DM, Hypertension, Obesity, MetS and Dyslipidemia are the potential risk factors for the development of CVD. Apolipoprotein B (Apo B) transports all potentially atherogenic very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low density lipoprotein (LDL) particles, and Apo A transports and acts as the major antiatherogenic protein in the HDL particles. Although conventional lipid parameters are known to predict CVD, limited data are available regarding Apo B/A ratio. The aim of this study was to find out the association of Apo B/A ratio to predict the risk of CVD with or without DM in patients visiting Shree Birendra Army Hospital, Kathmandu, Nepal.

**METHODS:** A cross-sectional study was conducted at Shree Birendra Army Hospital, Kathmandu from 2014/10/16 to 2016/7/30. We enrolled 180 participants including 45 healthy controls, 45 cases of DM and 90 cases of CVD. CVD was categorized into two groups into CVD with DM and CVD without DM. Demographic and anthropometric data of patient was collected. Fasting blood samples was collected from the patient. Fasting Glucose, Total Cholesterol, Triglyceride (TG), HDL-C, LDL-C, Apoprotein-A and Apoprotein-B were measured. One way ANOVA and Student-t test were applied for the comparison of mean between and among the group respectively. Chi square test was applied to see the categorical association between the group.

**RESULTS:** In this study, we have reported the difference in mean±SD of lipid parameters such as TCHO, HDL-C, LDL-C and TG among controls, DM and CVD were found to be statistically significant. Out of 180 subjects included in this study CVD cases had significantly lower levels of HDL and Apo A and significantly higher levels of TCHO, LDL, TG and Apo B regardless of DM. Apo B/A ratio was significantly elevated in CVD than the DM and control cases. Apo B/A ratio showed a strong positive correlation with CVD. The mean±SD of Apolipoproteins A in control, DM and CVD were found to be 173.9 ± 21.5, 119.5 ± 33.1 and 99.6 ± 28.3 respectively. The mean±SD of Apolipoproteins B in control, DM and CVD were found to be Apo B 104.7 ± 17.9, 126.1 ± 51.8 and 192.1± 79.5) and respectively. Similarly, the ratio of Apo B/A in control, DM and CVD were found to be 0.6 ± 0.1, 1.2 ± 0.8 and 2.2 ± 1.3 respectively. The mean±SD of Apo A and Apo B in CVD with DM and CVD without DM were found to be in significant. Similarly the ratio of Apo A/B were found to be significant.

**CONCLUSION:** This study reveals that Apolipoprotein measurement significantly predict CVD in patients suffering from type II DM and also concludes that the DM can be associated with recognized risk factors for the development of CVD. The predictive ability of Apo B/A ratio was found to be better than any of the routine clinical lipids measurements therefore, their inclusion in further clinical guidelines should not be discarded.

## B-131

### Development of new HDL-Cholesterol Generation 4 assay on Roche Clinical Chemistry Analyzers

H. W. A. Klima[1], C. Schühlmann[1], G. Sobczak[1], B. Löhr[2]. *[1]Roche Diagnostics GmbH, Penzberg, Germany, [2]Roche Diagnostics GmbH, Mannheim, Germany*

**Medical background**

Monitoring of HDLcholesterol (HDL-C) in patients is of clinical relevance as the HDL-C concentration is important in the assessment of atherosclerotic risk. Elevated HDLC concentrations protect against coronary heart disease (CHD), whereas reduced HDL-C concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.

**Test principle**

NonHDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a watersoluble complex. In this complex the enzymatic reaction of cholesterol oxidase (CHER) and cholesterol oxidase (CHOD) towards nonHDL lipoproteins is blocked. The concentration of HDLcholesterol is determined enzymatically by CHER and CHOD.

**Development Goals HDL-Cholesterol Gen.4:**

- Improved specificity for HDL-C in human samples due to reduced sensitivity towards denatured lipoprotein
- Extended measuring range of 0.083.88 mmol/L (3.09150 mg/dL)
- Less sensitivity to denatured lipoproteins and less matrix effects
- Increased reactivity towards apo E enriched HDL(Important for people with reduced CETP (cholesterol ester transport protein) activity)
- Reduced high recovery in samples from patients with liver disease and lipid disorder
- Improved stability over shelf life

**Results**

***Measuring range and lower limits of measurement***

The linear assay range of the HDLC4 assay is from 0.083.88 mmol/L (3.09150 mg/dL). The LoB, LoQ and LoD are 0.08 mmol/L (3.09 mg/dL).

***Traceability***

HDLC4 assay has been standardized against the ultracentrifugation method according to CDC (center of disease control) manufacture's protocol.

***Limitations and interferences***

No interference of bilirubin (conjugated and non-conjugated) up to 1026 µmol/L (60 mg/dL), I-Index of 60, no interference of lipaemia (Intralipid) up to a L-Index of 2000, no significant interference from native triglycerides up to 13.7 mmol/L (1200 mg/dL), no interference of hemoglobin up to 745 µmol/L (1200 mg/dL), H-Index of 1200. Statins (Simvastatin) and fibrates (Bezafibrate) tested at therapeutic concentration ranges do not interfere. Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

***Precision - CLSI EP5 - 21 days***

Repeatability ≤ 1.8% and Intermediate precision ≤ 2.2% (concentration range 0.25 mmol/L (9.67 mg/dL) to 3.66 (141 mg/dL).

***Method comparison study:***

Human serum and plasma samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche **cobas c** 501 analyzer (x) (n = 59). Passing/Bablok regression: y = 1.006x + 0.032 mmol/L. The sample concentrations were between 0.11 and 3.69 mmol/L (4.25 and 143 mg/dL).

**Conclusions**

All development goals for the new HDLC4 assay were met. The use of HDLC4 method in laboratory routine will improve quality of test results for HDL-C.

## B-132

### Development of an HPLC Assay for Measurement of Very Long-Chain Fatty Acids in the Plasma

R. Shrestha[1], S. Hui[1], H. Chiba[1], K. Hirano[2]. *[1]Faculty of Health Sciences, Hokkaido University, Sapporo, Japan, [2]Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan*

**Background**: Measurement of plasma very-chain fatty acids (VLCFA) provides important information to aid the diagnosis of various peroxisomal disorders, including Zellweger syndrome, Rhizomelic chondrodysplasia punctata (RCDP), and X-linked adrenoleukodystrophy (XALD). Furthermore, there are increasing evidence that VLCFA is associated with coronary heart diseases, therefore, can be used to refine the risk stratification of it. In this study, we aimed to develop a simple, rapid and sensitive method for the measurement of plasma VLCFA that can be easily adopted in the clinical laboratories.

**Method**: Fasting blood samples were collected from 200 healthy human volunteers (120 female and 80 male; mean age ±SD – 57.4 ±13.3 years) and 30 subjects with hypertriglyceridemia (10 female and 20 male; mean age ±SD – 59.8 ±10.2 years). 50 µL of the plasma was mixed with 4 nmol of tricosanoic acid (FA23:0) as internal standard (IS). After saponification of esterified fatty acids (FA) in the plasma using 0.3M KOH in ethanol, the liberated free VLCFA are labeled with 2-nitrophenylhydrazine (NPH) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and pyridine. The labeled VLCFA are then extracted in ether, reconstituted in methanol, and subjected to HPLC analysis with a UV/VIS detector. The chromatographic

separation was performed with $C_4$ Mightysil reversed-phase column. Gradient elution was done with the solvent system composed of water (pH adjusted to 4.0 with 1% trifluoroacetic acid) and methanol. A series of experiments were performed to determine optimal condition for the labeling of VLCFA, extraction solvent, type of column and solvents, and its elution profiles for the distinct separation of each VLCFA including IS. Calibration curves of each FA were plotted by analyzing a series of standards with concentration ranging from 0.1 – 20 µmol/L. The assay was validated by evaluating its accuracy, precision, and recovery.

**Results:** Optimal HPLC condition was established with distinct isolated peaks for each VLCFA free from interfering peaks eluting between 18 to 24 min. The lower limit of quantification was 4 pmol for each FA at S/N 5:1 per injection.

The mean concentration of total VLCFA among the healthy controls was found to be 12.5 ±3.8 for male and 13.9 ±4.5 µmol/L for female. Hypertriglyceridemia is associated with significant increase in total VLCFA compared to the healthy controls (33.2 ±18.6 vs 13.3 ±4.3 µmol/L, $p<0.01$). There is a significant difference in mean of arachidic acid (FA20:0), behenic acid (FA22:0), lignoceric acid (FA24:0) and cerotic acid (FA26:0) between healthy and hypertriglyceridemic subjects (7.1 ±2.8 vs 19.9 ±12.4 µmol/L, $p<0.01$; 3.7±1.2 vs 8.7±5.5 µmol/L, $p<0.01$; 2.1 ±0.9 vs 3.8 ±1.6 µmol/L, $p<0.01$; 0.5 ± 0.6 vs 0.8 ±0.7 µmol/L, $p=0.03$, respectively). Significant correlation was observed between concentration of FA20:0, FA22:0, FA24:0, and FA26:0 ($p<0.01$).

**Conclusion**: We established a simple, accurate and reliable method for the quantification of VLCFA in the plasma. Application of this method may be valuable in diagnosis of peroxisomal disorders.

## B-133

### Evaluation of different formulas for estimating LDL-c in Nepalese population

P. Padmavathi[1], G. Brindha[1], V. Sathiyapriya[1], N. Gautam[2]. *[1]A.C.S Medical College & Hospital, Chennai, India, [2]Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Nepal*

Introduction: According to NCEP ATP III guideline the primary target of therapy in cardiovascular disease is based on Low Density Lipoprotein-cholesterol (LDL-c) concentration. Estimating LDL-c by direct homogenous assay is expensive especially in developing countries. Hence in clinical practice most of the clinical laboratory uses calculation method for estimating LDL-c concentration. There are different formulas been used to calculate LDL-c concentration, the most frequently used is Friedewald formula. In recent studies have shown that among them novel 180-c method showed the best agreement with direct LDL-c method when compared with other formulas in Asian population. The aim of the study was to (i) Evaluate different formulas for estimation of LDL-c in Nepalese population. (ii) And to identify which formula shows best agreement with novel 180-c method. (iii) And to determine whether it is really in the need to substitute the Friedewald formula. Materials and methods: This is prospective study conducted at Universal College of Medical Sciences teaching hospital (UCMSTH), Bhairahawa, Nepal. Lipid profile measured (n=1000) out patients samples which were sent to the clinical biochemistry laboratory, from January to December 2013. Total seven formulas used for estimating LDL-c in mg/dl. These are novel 180-cell method and equations suggested by Friedewald et al, Hattori et al, Anandaraja et al, , Cordova et al, , Ahmadi et al, & Rao et al. Two- statistical concepts were utilised for comparison of accuracy of different equations to estimate LDL-c by comparing with novel 180-cell method as reference value. The intra class correlation coefficient ICC was calculated to compare the degrees of agreement between the formulas. Good agreement ICC >0.75, moderate agreement ICC<0.5 or <0.75. Calculated weighted kappa (k) index to distinguish the most accurate equation for estimating LDL-c according to NCEP ATPIII classification guideline. Results: The intraclass correlation coefficient for Friedewald equations (ICC: 0.991), Hattori et al.,(ICC: 0.969), Anandaraja et al.,(ICC:0.979), Cordova et al.,(ICC:0.965), Ahmadi et al.,(ICC:0.623), Rao et al.,(ICC:0.989) ($p<0.001$). Among these Friedewald equation showed the highest agreement in terms of ICC and also highest weighted kappa index (k index: 97.49) which indicating the best agreement with novel 180 cell measurement. Discussion/Conclusion: Studies have highlighted Novel 180 cell method with high accuracy to use in clinical practice which uses adjustable factor for TG: VLDL-c ratio using stratification approach according to TG and non HDL-c levels (TC-HDL-c = non HDL-c) But in routine clinical practice calculating LDL-c by novel 180 cell method for each patient where there is manual entry of reports seems to be burden to the laboratory technologist. We used 180-c method as a reference value for LDL-c estimation with previously reported formulas as the first external validation in Nepalese population. The most commonly used Friedwald equation appeared to be more accurate than the newly derived formulas. This suggests that we still can use Friedwald formula in Nepalese population.

## B-134

### Comparison of Friedewald's Formula with de Cordova's Formula for Calculating Low Density Lipoprotein Cholesterol in a Nepalese Population

S. Pradhan, K. Gautam, D. Pyakurel. *Samyak Diagnostic Pvt. Ltd, Lalitpur, Nepal*

**Background:** As serum Low Density Lipoprotein Cholesterol (LDL-C) concentration is positively related to the increase in incidence of coronary heart disease, its measurement plays vital role in the prevention and management of cardiovascular disease. Ultracentrifugation and beta quantification, the gold standard method, and direct measurement of LDL-C using homogenous assays are quite expensive and inconvenient in laboratory setups for countries like Nepal. To address this problem numbers of formulas have been established which calculate LDL-C level. Among these Friedewald's formula is being widely used in laboratories of Nepal even though it has limitations. Recently Cordova et al developed formula and claimed to outperform Friedewald's formula. This study aims to evaluate the performance of Cordova's formula for calculation of LDL-C in Nepalese population. **Methods:** A total of 270 participants were included in the study. Serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were measured by direct automated method. LDL-C was also calculated using Friedewald's and Cordova's formula. SPSS ver. 22 was used to analyze the data. Pearson's correlation coefficient (r), Passing and Bablok regression, Bland-Altman plots were used to compare performance of different formula. **Results:** The mean values of directly measured LDL-C and LDL-C calculated by Cordova's formula and Friedewald's formula were 120.87±41.55 mg/dl, 106.71±38.60 mg/dl and 104.95±33.88 mg/dl respectively. The correlation coefficient of measured LDL-C for Cordova's formula was 0.857 ($p< 0.01$) and for Friedewald's formula was 0.886 ($p<0.01$). Passing and Bablok regression yielded the equation y= 20.12+0.7x for Cordova's formula and y = 7.17+0.82x for Friedewald's formula. The bias of calculated LDL-C against measured LDL-C were 15.92% for Cordova's formula and 14.15% for Friedewald's formula. **Conclusion:**

This study showed better performance of the Friedewald's formula than Cordova's formula for approximate calculation of LDL-C. However further studies on a large population is required to strengthen this statement.

## B-135

### Serum Bone Morphogenic Protein 4 levels are conversely correlated with high sensitive- C Reactive Protein in Obese Men

T. Yulianti. *Prodia Laboratory, Jakarta, Indonesia*

**Background:** The prevalence of obesity is increasing in many parts of the world, including Indonesia. The reframing of obesity as an inflammatory condition has had a wide impact on the conceptualization of obesity-associated disease. BMP4 is a growth factor of the transforming growth factor-β superfamily. Initially, BMPs were identified as inducer of ectopic bone formation. Recent in vitro study on cultured human adipocytes have highlighted the important roles of BMP4 in the regulation of adipogenesis as an anti-inflammatory. However the correlation between is serum BMP4 and inflammation in adult obese is unknown yet. The objective of this study was to evaluate the correlation between common inflammation marker, serum hs-CRP and BMP4 in obese men.

**Methods:** A total of 80 obese adult male subjects were included in the present study, out of which 33 were non metabolic syndrome (nonMetS) and the remaining 47 were metabolic syndrome, age range from 31 to 60 years old. Serum BMP4 and TNFα concentrations were quantified by ELISA principle. Serum hsCRP were quantified by immulite2000 (DPC cat L2KCRP-2). All assays were performed according to the manufacture instruction. Statistical analysis was performed with SPSS for windows ver 20. Significance value were define as alpha level< 0.05 based on two-tailed tests.

**Results:** The mean levels of serum BMP4 in obese MetS is lower (470 pg/ml) as compared to obese nonMetS group (613 pg/ml). However, inverse results were seen for mean serum hsCRP levels which were higher in obese MetS group (2.69 mg/l) compared to obese nonMetS group (1.89 mg/l). Bivariate analysis (n=80) revealed that serum BMP4 was inversely correlated with hsCRP, TNF-α, triglyceride, blood fasting glucose. A significant inverse correlation were found between BMP4 and hsCRP ($r=-0.277$; $p=0.013$).

**Conclusion:** In conclusion, this study provides evidence that in obese men serum BMP4 inversely correlated with hsCRP. This finding demonstrate the importance of BMP4 as an anti inflammatory protective factor of obesity-related diseases. Further study is needed to validate that BMP4 may serve as a therapeutic target for intervention in the control of metabolic disease.

## B-136

### Evaluation of Free Fatty Acid Assay adapted on the Abbott Architect c8000 analyser

J. W. S. Setoh, C. K. M. Ho, S. P. Peh. *KK Women's and Children's Hospital, Singapore, Singapore*

**Background:**

Fatty acids provide a source of energy alternative to glucose for some organs such as skeletal muscle. In liver, fatty acids can be converted to ketones, which in turn provide energy for the brain during starvation or when blood glucose levels are low. In blood, most fatty acids are esterified with either cholesterol or glycerol, and transported by lipoprotein particles, whereas non-esterified (free) fatty acids are bound to proteins in plasma.

In this study, we aimed to study the performance of the Randox non-esterified fatty acid, also known as free fatty acid (FFA) on the Abbott Architect c8000 analyser.

**Methods:**

We evaluated inter-day imprecision, linearity and limit of quantitation (LOQ) of the FFA assay adapted to the Abbott Architect c8000 analyser and its performance in two external quality assessment (EQA) schemes. Potential interference of the assay by hemolysis and bilirubin was also investigated using serum specimens spiked with known concentrations of hemoglobin or unconjugated bilirubin.

**Results:**

Inter-day imprecision, CV (%), over 10 days was 4.3% at 0.52 mmol/L and 2.5% at 1.59 mmol/L. The FFA assay demonstrated linearity for measurement between 0.10 and 2.97 mmol/L. Limit of quantitation (LOQ) was found to be 0.16 mmol/L with CV = 2.6%.

EQA results were comparable with peers participating in the Randox International Quality Assessment Scheme (RIQAS) and European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM).

Hemoglobin concentration at 300 mg/dL and 600 mg/dL increased FFA results by 11.7% and 23.9%, respectively, at FFA concentration of 0.51 mmol/L ($p<0.001$). Bilirubin concentration at 100 umol/L, 200 umol/L and 400 umol/L decreased FFA results by 12.6%, 21.7% and 29.1%, respectively, at FFA concentration of 0.64 mmol/L ($p<0.001$).

**Conclusion:**

We have evaluated the performance (imprecision, linearity and LOQ) of the Randox free fatty acid assay on the Abbott Architect c8000 analyzer. Assay results were found to be satisfactory when compared to all users' means in both RIQAS and ERNDIM external quality assessment programs. Hemoglobin at 300 mg/dL and above resulted in statistically higher FFA results compared with controls, whereas bilirubin at 100 umol/L and above resulted in statistically lower FFA results.

## B-137

### An Improved Method for LDL-Cholesterol Beta-Quantification for Clinical Drug Trials

H. L. Kincaid[1], S. L. Mankin[1], R. Duncan[1], E. Wagner[1], Y. Olivera[2]. *[1]Covance Central Laboratory Services, Indianapolis, IN, [2]Covance Central Laboratory Services, Geneva, Switzerland*

**Background:** Low density lipoproteins (LDL) are the major cholesterolcarrying lipoproteins in plasma. LDL is primarily cleared from the bloodstream via hepatic LDL receptors, and to a lesser extent, by extra-hepatic tissues. The CDC reference method for the measurement of LDL cholesterol (LDL-C) is beta quantification using ultracentrifugation. The original reference method for LDL-C by beta quantification requires 5 mL of serum; later adaptations apply 1 mL serum. This report describes an adaptation utilizing 0.2 mL of serum with a reduced centrifugation time, which satisfies IRB requirements for pharmaceutical companies investigating PCSK9 inhibitors and other drug development studies designed to improve LDL-C clearance.

**Methods:** Specimens were centrifuged for 3 hours 54 minutes, as opposed to 20 hours 15 minutes (existing method) in a Model Optima XPN-80 ultracentrifuge with titanium rotor Type 42.2 Ti (Beckman Coulter®, Brea, CA). A Beckman CentriTube™ Slicer and Beckman Coulter 7x20 mm cellulose propionate centrifuge tubes were used. Total cholesterol was measured on Modular Analytics or cobas® 8000 instrumentation (Roche Diagnostics, Indianapolis, IN) using Roche cholesterol reagent (Catalog # 05168538190). LDL-C by direct assay was performed using Roche reagents (Catalog #05171369190). An LDL-C determination by Friedewald calculation was calculated from total cholesterol, high density lipoprotein-cholesterol (HDL-C), and triglycerides (glycerol-blanked) measurements. HDL-C was measured by dextran-magnesium sulfate precipitation and subsequent total cholesterol measurement. Triglycerides (glycerol-blanked) were measured using Roche reagents (Catalog #05976006190). Data reduction utilized EP Evaluator® (Data Innovations, South Burlington, VT). The new method was validated in the US and subsequently in Shanghai, PRC and Geneva, Switzerland. Global correlation studies were conducted according to Covance CLS established methods.

**Results:** The new process demonstrated an inter-assay imprecision of 2.6%, which satisfied imprecision requirements using an allowable total error of 10.5%. Accuracy, as determined by method comparisons to the Friedewald calculation and LDL-C by direct assay demonstrated concordance within 20% of the LDL-C ultracentrifugation results. The correlation between the 1 mL and 0.2 mL methods, as well as inter-laboratory global correlations, met acceptance criteria (95% CI for the slope includes 1.00, the 95% CI for the y-intercept includes 0.00, and correlation coefficient, $R \geq 0.95$). Global correlations met acceptance criteria and proved commutability of the method.

**Conclusion:** The method enhancements for LDL-C by ultracentrifugation demonstrated excellent analytical performance, and provided significant value to pharmaceutical clients and laboratory operations both in turnaround time and test cancellation because of sample quantity not sufficient for testing. Test cancellations due to insufficient specimen volume were reduced from 4.3% to 0.2%. The time required for specimen processing was reduced by 48% leading to significant savings in time and labor expense.

## B-138

### Comparison between cholesterol or low-density lipoprotein concentrations obtained by the direct method and those estimated through application of the Martin and Friedewald equations, in a Brazilian population sample: Hypertriglyceridemia limits the accuracy of the Martin equation.

A. C. Dias, J. F. F. Oliveira, L. A. M. B. Gracindo, L. A. Silva, G. Barra, S. F. Fonseca, R. H. Jácomo, A. L. Barbosa, L. F. Abdalla. *Laboratório Sabin, Brasília, Brazil*

Background:

In 2013, Martin et al proposed a new equation for estimating cholesterol or low-density lipoprotein levels (LDL-C), [LDL-C(M)], which, by having an adjustable factor in its formula, would be more accurate than the more traditional Friedewald equation, [LDL-C(F)]. In December of 2016, the Brazilian Consensus for the Normalization of Laboratory Determination of Lipid Profiles was published, and this document recommended using LDL-C(M), regardless of the triglyceride (TG) levels. The objective of the current study was to evaluate the correlation and concordance between LDL-C(M) or LDL-C(F) and the direct dosage determination of LDL-C [LDL-C(D)], and to determine the triglyceride concentrations that may limit the accuracy of the LDL-C(M) values.

Methods:

LDL-C(D), TG, total cholesterol and HDL-C concentrations were determined for 680 serum samples, using the Advia 2400 analyser; LDL-C(M) and LDL-C(F) values were calculated by applying the respective equations of Martin or Friedewald. The clinical concordance was evaluated by using the total minimum error based on the biological variation components (17.84%). Statistical analyses were performed using the Pearson correlation, the Cohen?s Kappa test, the Lin Concordance Correlation Coefficient, ROC curve analysis and the Bland-Altman graph.

Results:

The results are shown in Table. For TG values <400mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 97.4% (p-0.238), and between LDL-C(F) and LDL-C(D) was 84,7% ($p<0.0001$). For TG values ≥400mg/dL, the concordance between LDL-C(M) and LDL-C(D) was 68.3% ($p<0.0001$), and between LDL-C(F) and LDL-C(D) was 43.3% ($p<0.0001$), considering a total maximum error of 17.84%. Analysis of the ROC curve showed that a TG concentration of 432 mg/dL limited the accuracy of the LDL-C(M) results.

Conclusions:

For the LDL-C calculation, the Martin equation is more accurate than that of Friedewald, despite presenting limitations with regard to samples with TG values greater than or equal to 432 mg/dL.

| Concordance and correlation by Friedewald vs Martin Estimates of Low-Density Lipoprotein Cholesterol | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TG < 400 mg/dL (n=620) | | | | TG ≥ 400 mg/dL (n=60) | | | |
| | LDL-C(F) | | LDL-C(M) | | LDL-C(F) | | LDL-C(M) | |
| | Value | 95% CI | Value | 95% CI | Value | 95% CI | Value | 95% CI |
| Percent Agreement based on Total Error | 84.7 | 81.8 to 87.5 | 97.4 | 96.2 to 98.7 | 43.33 | 30.79 - 55.87 | 68.3 | 56.56 - 80.1 |
| Pearson's correlation coefficient | 0.95 | 0.92 to 0.97 | 0.98 | 0.97 to 0.98 | 0.95 | 0.92 - 0.97 | 0.93 | 0.89 - 0.96 |
| Lin's Concordance Correlation Coefficient (Interpretation) | 0.94 (Mod-erate) | 0.92 to 0.94 | 0.92 to 0.94 | 0.97 to 0.979 | 0.78 (Poor) | 0.70 - 0.84 | 0.81 (Poor) | 0.74 - 0.87 |
| Cohen's kappa coefficient (Interpretation) | 0.65 (Good) | 0.61 to 0.70 | 0.85 (Very good) | 0.82 to 0.88 | 0.26 (Fair) | 0,13 - 0,41 | 0.53 (Mod-erate) | 0.38 - 0.69 |
| Mean difference, mg/dL | -5.87 | -6,74 to -5,00 | -0.35 | -0,94 to 0,23 | -19.8 | 25,3 to -14,3 | 16.5 | 11,9 to 21,1 |

## B-139

### Multicenter Evaluation of new HDL-Cholesterol Generation 4 assay (HDLC4) on Roche Clinical Chemistry Analyzers

B. Loehr[1], I. Paege[2], Y. De Rijke[3], D. Plonné[4], H. Klima[5]. *[1]Roche Diagnostics GmbH, Mannheim, Germany, [2]Otto-von-Guericke University, Institut for Clinical Chemistry and Pathological Biochemistry, Magdeburg, Germany, [3]Erasmus Medical Center, Department of Clinical Chemistry, Rotterdam, Netherlands, [4]Medical Center of human genetics Ulm, Department of Laboratory Medicine, Ulm, Germany, [5]Roche Diagnostics GmbH, Penzberg, Germany*

**Background:**

Monitoring of HDL-cholesterol in serum is of clinical importance since an inverse correlation exists between serum HDL-cholesterol concentrations and the risk of atherosclerotic disease. A new formulation of the Roche Diagnostics HDL-Cholesterol Gen.4 reagent (HDLC4) was developed to increase specificity for HDL-C and to widen the measuring range up to 3.88 mmol/L (150 mg/dL). The analytical performance of the assay was tested in three laboratories.

**Assay principle:**

Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a watersoluble complex. In this complex the enzymatic reaction of cholesterol estrase (CHER) and cholesterol oxidase (CHOD) towards nonHDL lipoproteins is blocked. The concentration of HDLcholesterol is determined enzymatically by CHER and CHOD.

**Study Design:**

The analytical performance of the new HDLC4 assay was evaluated in three independent laboratories using **cobas c** 702, **cobas c** 502 and **cobas c** 501 instruments. Recovery of Roche PreciControl ClinChem Multi 1 and 2, recovery of Ring Trial samples from RfB and Instand e.V. and controls from Bio-Rad, within-run precision of human samples pools, precision according to CLSI EP5-A3, method comparisons HDLC Gen.3 vs. HDLC Gen.4, instrument-to-instrument (**cobas c** 701 vs **cobas c** 502), method comparison to HDL-C assays from competitors.

**Methods and Results:**

Repeatability and intermediate precision were measured in the concentration range from 0.66 mmol/L (25.5 mg/dL) to 2.55 mmol/L (98.6 mg/dL) according to the CLSI EP5-A3 protocol using two Roche controls and five human serum pools. For the repeatability the coefficients of variation (CVs) were determined to be less than 1.9 % and for intermediate precision yielded CVs ranging between 1.1 and 2.7 % (two runs/day, 21 days). The recovery of two controls (Roche Diagnostics) was determined in three independent runs measuring 3 aliquots. The recovery of HDL-C target values ranged from 97.4 to 105.6 %. Method comparison experiments were designed in compliance with CLSI EP09-A3, using ≥ 117 serum samples. Passing-Bablok regression analysis of Roche HDL-C methods resulted in slopes in a range of 0.97 to 0.86, intercepts of 0.02 mmol/L (0.77 mg/dL) to 0.13 mmol/L (5.02 mg/dL), and Pearson correlation factors from 0.977 to 0.998. The HDL- C concentration of samples ranged from 0.08 mmol/L (3.09 mg/dL) up to 3.05 mmol/L (117,91 mg/dL). The measured biases at the medical decision points were acceptable (≤ 5 %).

**Conclusions:**

The results of the multicenter evaluation study prove a good analytical performance of the new HDLC4 assay as well as an increased resistance to endogenous interferences and a increased specificity for HDL-C. The assay is well-suitable for routine use.

## B-140

### Specific Sandwich-type Enzyme-Linked Immunosorbent Assay (ELISA) for Oxidized High-Density Lipoprotein and Its Clinical Application

M. Sumida[1], T. Okada[2], Y. Katayama[1], T. Ohama[2], K. Kanno[2], H. Matsuda[2], M. Sairyo[2], Y. Zhu[2], A. Saga[2], T. Kobayashi[2], D. Matsuda[2], M. Koseki[2], M. Nishida[2], N. Kayahara[3], Y. Sakata[2], S. Yamashita[4]. *[1]Research Laboratory, Kyowa Medex Co., Ltd., Shizuoka, Japan, [2]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan, [3]Research and Development Division, Kyowa Medex Co., Ltd., Tokyo, Japan, [4]Department of Cardiology, Rinku General Medical Center, Osaka, Japan*

**Background:** High-density lipoprotein (HDL) has anti-atherogenic functions such as cholesterol efflux. It is also well known that plasma HDL-C levels are negatively correlated with cardiovascular events. Consequently HDL has been considered as ''good'' cholesterol. However, recent studies show that HDL from coronary artery disease patients have poor cholesterol efflux, and such HDL is defined as ''dysfunctional HDL''. Oxidative stress is one of the factors which lead HDL to dysfunctional, so we consider oxidized HDL (Ox-HDL) is useful to assess the quality or functionality of HDL. The aim of this study is to develop a specific sandwich-type enzyme-linked immunosorbent assay (ELISA) for Ox-HDL and to analyze the serum Ox-HDL levels of both normolipidemic healthy subjects and dyslipidemic patients.

**Methods:** We have developed a sandwich ELISA for Ox-HDL with DLH3 antibody that can specifically recognize oxidized phospholipid. We assessed specificity of the assay using native HDL and LDL as well as Cu-oxidized HDL and LDL. Each lipoprotein was separated from human plasma by sodium bromide stepwise density gradient ultracentrifugation. Additionally we assessed the non-specific reaction of unoxidized phosphatidylcholine (PC) by inhibition assay. Next, we defined Ox-HDL obtained by forced-oxidation of 1 mg/L HDL phospholipids (HDL-PL) as 1 U/L Ox-HDL, and analyzed the sera of 118 healthy normolipidemic controls and 177 dyslipidemic outpatients, respectively.

**Results:** *Specificity analysis*-In this ELISA assay, Cu-oxidized HDL was specifically detected and neither Cu-oxidized LDL nor native LDL was detected. Native HDL was also recognized but the signal was low. Inhibition assay with various types of phospholipids revealed that lysophosphatidylcholine (lyso-PC) showed concentration-dependent inhibition. On the other hand, no inhibition by unoxidized PC was observed even in the condition of excess concentration. These results strongly suggested that oxidized phospholipid in HDL is specifically recognized in this ELISA assay. *Clinical evaluation*-The serum Ox-HDL levels in healthy controls were 28.3 ± 4.9 U/L (mean ± SD) and in dyslipidemic patients treated with drugs were 27.7 ± 9.3 U/L. Ox-HDL levels were moderately correlated with HDL-PL levels (r=0.59), therefore we also evaluated Ox-HDL/HDL-PL ratio, which represents oxidation degree of phospholipids in the HDL fraction . Patients treated with probucol, which is a potently anti-oxidative and anti-hyperlipidemic drug, showed significantly lower Ox-HDL level (18.7 ± 6.6 U/L vs 30.0 ± 8.1 U/L, p<0.001) and Ox-HDL/HDL-PL ratio (0.206 ± 0.045 vs 0.224 ± 0.049, p=0.043) than those without probucol, suggesting that probucol may prevent oxidation of HDL. These probucol effects were also seen in Familial Hypercholesterolemia patients (Ox-HDL: 16.1 ± 5.8 U/L vs 30.2 ± 5.4 U/L, p<0.001; Ox-HDL/HDL-PL: 0.205 ± 0.037 vs 0.238 ± 0.043, p=0.015, respectively).

**Conclusion:** Our sandwich ELISA is an assay with a good specificity to Ox-HDL. In addition, clinical evaluation using our assay showed that probucol may prevent the formation of these Ox-HDLs.

## B-141

### Validation of the need for 12-hour fasting and non-fasting for dosages of simple lipid profile parameters in our laboratory

M. C. Feres[1], D. Ramadan[2], R. Bini Jr.[2], J. T. M. Torrecilhas[2], B. B. Perez[2], M. C. De Martino[2], S. Tufik[2]. *[1]Universidade Federal de Sao Paulo- Brazil, Sao Paulo, Brazil, [2]Associacção Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil*

12-hour fasting is a big problem for people who need to do exams, such as children and the elderly. A recent study in the journal, "European Heart Journal," shows

that assessment of lipid profile parameters at 12-hour fasting or after meal does not clinically impact patient outcomes. To improve patient adherence to lipid tests, many laboratories worldwide perform these tests without the need for 12-hour fasting at random times during the day. We know that exams processed immediately after the meal have the effect of postprandial lipemia, which in many laboratory methods undergo turbidity interference. In this context, before adopting non-fasting to perform dosages of the lipid profile in our laboratories, the authors found it prudent to do a study to determine the best time interval after the meal that we could recommend in our laboratory, considering the Inter variability of absorption of Lipids of each individual. The authors of this study aimed to validate within our conditions, equipment and methodologies, so that we can introduce these changes in the pre-analytical phase of our laboratory routine. It was also part of our objectives to determine the mean concentration range of each parameter of the lipid profile studied at 2, 3 and 4 hours after meal. **Casuistic and Methods**: Our sample consisted initially of 62 volunteers from the community and venous blood was collected in a 12-hour fast and after a meal the following day the volunteer returns to the laboratory after having taken his usual breakfast to be collected blood 2, 3 and 4 hours after this meal. The following tests will be carried out: Cholesterol, Triglycerides, C-LDL, C-HDL and VLDL with the Enzymatic/Colorimetric method on Beckman-Coulter® AU5800 equipment and Beckman-Coulter reagent. In addition to dosing the C-LDL was calculated by the Friedewald equation. **Results**: In the comparison of the lipid profile at fasting versus 2, 3 and 4 h after meal, it was observed that there was no significant difference for the parameters of CT and HDL-C and for the calculated LDL-C, with mean indices for TC (p= 0.237), C-HDL (p=0.130) and for C-LDL (p=0.089). However, C-LDL dosed, TG and VLDL presented significant differences with their respective mean concentrations and standard deviation for each hour after 2h C-LDL (112.4 ± 4.8mg/dL, p=0,008), 3h (111.9 ± 5.0mg/dL, p=0.019) and 4h (116.0± 4.9mg/dL; p=0.017) for TG 2h (156.1± 12.5mg/dL, p = 0.000), 3h (150.0 ± 13.3mg/dL, p=0.000) and 4h (144.1 ± 13.5mg/dL, p = 0.000 ), and for VLDL calculated: 2, 3 and 4h after the indices were p =0,000. **Conclusion:** Our data confirmed that the meal did not influence the TC, C-HDL and calculed C-LDL data, but for TG, VLDL and C-LDL dosed, a significant difference was observed in the post-meal concentrations. Although disturbing the difference in C-LDL for methodological reasons, it did not impact on the clinic. Analyzing our data, we observed that the best blood collection time could be between 2 and 3 hours after the meal where the degree of lipemia would already have a lower influence in most individuals.

## B-142

### ApoE-rich HDL in pre-heparin plasma may be HDL remnants which remain in the circulation

S. Usui[1], R. Shinohata[1], K. Miyashita[2], S. Hirohata[1], M. Shibakura[1], I. Fukamachi[2], K. Nakajima[3]. *[1]Okayama University Graduate School of Health Sciences, Okayama, Japan, [2]Immuno-Biological Laboratories, Fujioka, Japan, [3]Gunma University Graduate School of Medicine, Maebashi, Japan*

**Background:** High-density lipoprotein (HDL) containing apolipoprotein E (apoE-rich HDL) represents only a small portion of plasma HDL. Hepatic triglyceride lipase (HTGL) is well known to play a major role in HDL metabolism, and promotes the selective uptake of cholesteryl esters from HDL via the scavenger receptor B1. We previously reported that HTGL may be associated with apoE-rich HDL in post-heparin plasma, but it has not been clear whether this observation is found in pre-heparin plasma. In this study, we propose the possibility of this lipoprotein fraction as HDL remnants based on the interaction with HTGL in pre-heparin plasma. **Methods:** A total HDL fraction was isolated from healthy serum samples (pre-heparin plasma) using 13% polyethylene glycol (PEG), and applied to a cation-exchange column (HiTrap SP HP, 1 mL, GE Healthcare) to obtain apoE-rich and apoE-poor HDL fractions. For the determination of HTGL distribution, the column effluent was collected in 0.4-mL fractions and analyzed by a newly developed HTGL-ELISA. Furthermore, the apoE-rich HDL fraction was applied into a gel-permeation column (Superose 6HR) to investigate the interaction between apoE-rich HDL and HTGL. **Results:** Approximately 90% of serum HTGL mass was recovered in the total HDL fraction (PEG supernatant), indicating apoB-containing lipoproteins were not associated with HTGL in pre-heparin plasma. Only 5% of total HDL-cholesterol was found in apoE-rich HDL fraction but 40% of HTGL in the total HDL was detected in apoE-rich HDL fraction. Therefore, the ratio of HTGL (ng/mL) to cholesterol (mg/dL) was much higher (approximately 7.0-fold) in apoE-rich HDL than apoE-poor HDL. Furthermore, the gel-permeation HPLC analysis revealed that HTGL and apoE-rich HDL were co-eluted at larger HDL position. These findings indicate that HTGL interacts preferably with apoE-rich HDL. **Conclusion:** This is the first report that apoE-rich HDL prevalently carries HTGL in pre-heparin plasma. Although lipoprotein lipase was found in VLDL fraction as remnants, HTGL was found in apoE-rich HDL. Both fractions contain apoE-rich lipoproteins and carry the lipases as bound forms in pre-heparin plasma. Therefore, we propose that apoE-rich HDL is "HDL remnants" which were not rapidly cleared from the circulation.

## B-143

### Comparability of commercial assays & commutability of evaluated materials for apolipoprotein A1 measurement

J. Zeng[1], T. Qi[1], S. Wang[2], T. Zhang[1], W. Zhou[1], H. Zhao[1], R. Ma[1], J. Zhang[1], Y. Yan[1], J. Dong[2], C. Zhang[1], W. Chen[1]. *[1]National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing Engineering Research Center of Laboratory Medicine, Beijing, China, [2]The MOH Key Laboratory of Geriatrics, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:** Harmonization & comparability of apolipoprotein A1 (apo A1) measurement results are crucial to assess cardiovascular risk. Non-commutable materials used as calibrators & external quality assessment (EQA) materials can result in misdiagnosis or provide misleading information leading to limited EQA schemes. Potential reference materials should therefore be selected & evaluated for commutability.

**Methods:** In the present study, we determined apo A1 levels in commercial control materials used in the 2013 EQA Program, human serum pools prepared from leftover samples (LHSPs) & fresh donations (FHSPs), & a set of 50 individual samples using nine commercially available assays. Original or logarithm-transformed results were pairwise-analyzed to estimate the slopes & intercepts by Deming regression. The 95 % prediction interval (PI) & the minimum bias from biological variability were used to analyze the commutability of these materials. Matrix-related biases were also estimated.

**Results:** All commercially available assays in the present study showed acceptable within-run precision, compared with the minimum specification for imprecision derived from biological variability (4.9 %), & almost all combinations correlated well (r>0.975). The slopes varied from 0.65 to 1.35, & the intercepts varied from -0.45 to 0.43. FHSPs were commutable in more combinations than LHSPs or EQA materials, & both 95 % PI & minimum bias (5.6 %) criteria for commutability yielded similar results for FHSPs. The matrix-related biases of EQA materials, LHSPs, & FHSPs varied from -42.28 % to 25.58 %, -7.29 % to 12.53 %, & -8.38 % to 11.22 %, respectively.

**Conclusion:** Human serum pools prepared from fresh donations are thus a more appropriate source of reference materials. The metrological traceability of calibrators is therefore essential for the harmonization & comparability of apo A1 measurement procedures.

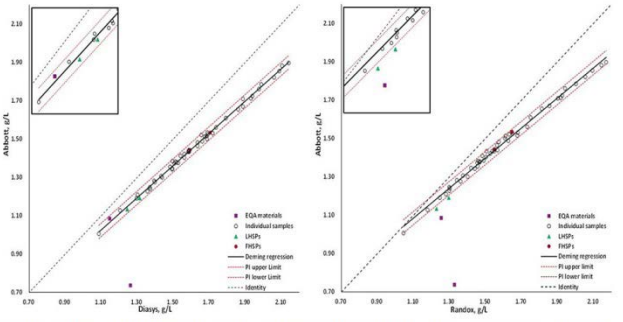



**Fig. Examples for commutability assessment of apo A1 measurements in EQA materials and HSPs.** The Deming fit with 95 % PI (red dotted lines) were calculated from individual sample results. Evaluated materials within 95% PI were considered commutable, otherwise they were non-commutable. The black dotted lines were identity lines (y = x).

## B-144

**The laboratory's contribution in the clinical assessment of cardiovascular risk: evaluation of Imprecision and Reference Change Value of lipid profile.**

F. B. Ronchi, S. Caria, G. Demuro, G. B. Scrocco, G. Serra. *Clinical Pathology Service, Dept. of Services, P.O. N.S. Bonaria, ASSL Sanluri, ATS Sardegna, San Gavino Monreale, Italy*

**Background:** Variability in measurements of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) has important repercussions on clinical decision-making. To test the impact of analytic variability (Va) of lipid measurements on the cardiovascular risk estimation we evaluated our Va close to decision limits suggested by ACC/AHA 2013 and ESC/EAS 2016 guidelines. Since the state of art suggests the use of Reference Change Value (RCV) to evaluate significant changes in serial lipids results we also define our RCV. **Methods:** Analytical imprecision of lipid tests was evaluated throughout intra-(CVr) and inter-assay (CVd) performed in 10 days with Abbott Architect c8000. Samples: Bio-Rad Quality Control third-part ($QC_1$, $QC_2$) and 1 sample with values close to 200, 240, 290 mg/dL TC ($S_1$-$S_2$-$S_3$); 115, 150 mg/dL LDL-C ($S_1$-$S_2$); 150, 180 mg/dL TG ($S_1$-$S_2$); 45 mg/dL HDL-C ($S_1$). We further tested 10 patient samples ($S_{Var}$) close to decision limits (200 mg/dL TC; 115 mg/dL LDL-C; 150 mg/dL TG; 45 mg/dL HDL-C). To define RCV we used biological variation from Ricos's database, we monthly calculated our analytical imprecision ($CV_A$) performing daily measurements of $QC_1$ and $QC_2$ during two years by Unity Real Time software, Bio-Rad Laboratories. Then we compared $CV_A$ with analytical goals (AG) for imprecision derived from biological variation and calculated by Fraser's formulas. **Results:** Table below shows all results expressed as Coefficient of Variation (CV%). RCVs of TC, LDL-C, HDL-C and TG were 17.3, 22.5, 22.1, and 55.5 % respectively ($Z = 1.96$ for bidirectional changes; $p < 0.05$). **Conclusion:** The accordance of CVr and CVd versus $CV_A$ obtained from QC highlight good analytical performances at different concentrations close to decision limits. The definition of CVa and RCV is a useful tool used by our cardiologists to assess cardiovascular risk and response to lipid-lowering therapy of patients.

| | | $QC_1$ CV% | $QC_2$ CV% | $S_1$ CV% | $S_2$ CV% | $S_3$ CV% | $S_{Var}$ CV% | Average $CV_A$% | AG Imprecision CV% |
|---|---|---|---|---|---|---|---|---|---|
| TC | CVr | 0.76 | 0.47 | 0.51 | 0.37 | 0.68 | <0.54 | 1.8 | Desirable 3.0 |
| TC | CVd | 1.51 | 1.43 | 0.86 | 0.88 | 1.32 | - | 1.8 | Desirable 3.0 |
| LDL-C | CVr | 0.79 | 0.64 | 0.50 | 0.68 | - | <0.91 | 2.3 | Desirable 3.9 |
| LDL-C | CVd | 2.24 | 2.34 | 1.52 | 1.55 | - | - | 2.3 | Desirable 3.9 |
| HDL-C | CVr | 1.76 | 0.89 | 0.77 | - | - | <0.71 | 3.2 | Desirable 3.6 |
| HDL-C | CVd | 1.35 | 1.69 | 1.26 | - | - | - | 3.2 | Desirable 3.6 |
| TG | CVr | 0.73 | 0.36 | 0.37 | 0.57 | - | <0.58 | 2.4 | Optimal 5.0 |
| TG | CVd | 1.75 | 1.88 | 1.71 | 1.74 | - | - | 2.4 | Optimal 5.0 |

## B-145

**Fasting Versus Nonfasting serum lipid profile in newly diagnosed Diabetes Mellitus**

R. Shrestha[1], N. P. Bhatt[2], S. Dahal[3], N. Shrestha[4], M. Khanal[3]. *[1]Hokkaido University, Sapporo, Japan, [2]Department of Pharmacology, Kangwon National University, Chuncheon, Korea, Democratic People's Republic of, [3]Nepal Medical College and Teaching Hospital, Kathmandu, Nepal, [4]Chonbuk National University Hospital, Jeonju, Korea, Democratic People's Republic of*

**Background:** Determination of serum lipids in the routine non-fasting state compared to the conventional measurement in the fasting provides several discrete advantages for both patients and the healthcare providers. Importantly, several lines of evidence supported that the non-fasting triglycerides serve as a better risk factor for the future coronary events. However, still, the majority of laboratory and clinician appear to have hesitation and uncertainty in the replacement of fasting lipids testing. This is particularly concerned in the patients with insulin resistance where metabolic handling of dietary lipids is remarkably disordered. Therefore, this study was designed to observe the variation of serum lipids between the fasting and non-fasting state in the newly diagnosed untreated diabetes.

**Methods:** A total of 96 (male:36/female:60) newly diagnosed untreated patients with type 2 diabetes mellitus were recruited (mean age:52.0±10.6) in Nepal Medical College and Teaching Hospital. Equal numbers of age and sex matched healthy control were also included. Blood samples were collected twice from all the volunteers, one after 12-14 hr of fasting and other at 1-5 hr of a recent regular meal. Total cholesterol (TC), LDL-cholesterol, HDL-cholesterol and triglycerides were measured in an automated chemistry analyzer.

**Results:** Diabetic subjects have significantly higher level of TC (176.3±34.6 vs 165.8±27.4; P<0.05), triglycerides (176.2±77.4 vs 126.3±48.8; P<0.05) and LDL-cholesterol (101.9±31.4 vs 89.0±24.1; P<0.05) and significantly lower level HDL-cholesterol (38.7±12.0 vs 51.0±11.5; P<0.05) as compared to non-diabetic controls in the fasting state. There is a minimal increase in TC and LDL-cholesterol in both diabetes and control group in the non-fasting state. Mean TC increased by 5.1 mg/dL and 4.9 mg/dL in diabetes and control groups, respectively. However, we observed no statistical difference in the mean of HDL-cholesterol in both groups. Serum triglyceride shows remarkable difference in both diabetes (mean increased by 46.5 mg/dL) and control (mean increased by 32 mg/dL). Interestingly, when the cutoff limit was set at 150 mg/dL, 52% of diabetic subjects were shifted to hypertriglyceridemia with the non-fasting triglycerides measurement.

**Conclusion:** Despite the non-fasting sampling for lipid measurement appears to be convenient, lipids particularly triglycerides is significantly elevated in diabetes compared to fasting samples. Therefore, it is crucial to determine an optimal cutoff limit of non-fasting triglyceride to be flagged as abnormal.

## B-146

**Establishment of an automated assay for cholesterol uptake capacity, a new concept of high-density lipoprotein functionality**

T. Iino[1], K. Murakami[1], M. Kiriyama[1], T. Kubo[1], K. Yoshikawa[1], K. Miwa[1], R. Toh[2], K. Hirata[2], A. Harada[1]. *[1]Sysmex Corporation, Kobe, Japan, [2]Kobe University Graduate School of Medicine, Kobe, Japan*

**Background:** Recent studies have shown that the capacity of high density lipoprotein (HDL) to stimulate cholesterol efflux from lipid-laden macrophages is a better predictor of cardiovascular disease status than overall HDL cholesterol concentration. Recently, we established a cell-free plate assay system to evaluate cholesterol uptake capacity (CUC) of HDL using a fluorescence-labeled cholesterol and an apolipoprotein A1 (apoA1) specific antibody, and demonstrated feasibility of CUC for potential substitution of cholesterol efflux capacity and coronary risk stratification. To apply this concept in the clinical settings, this study aimed to establish a high-throughput method capable of measuring CUC.

**Methods:** We had developed a fluorescence-based manual plate assay as a pilot method for measuring CUC, which was presented at the AACC 2016 Annual Meeting. In this study, we converted it into automated magnetic bead-based chemi-luminescence detection system. Briefly, HDL was incubated in reaction buffer containing dinitrophenyl (DNP)-labeled cholesterol, followed by its capture to an anti-apoA1 antibody coated onto the beads. Using alkaline phosphatase labeled anti-DNP antibody and specific substrate, chemi-luminescence signals were detected with our automated immunoassay system HISCL®. To investigate the throughput and analytical performance of our method, we quantified CUC of apoB-depleted serum samples and the recombinant apoA-1 as a standard.

**Results:** In this system, measurement of CUC was completed about 40 minutes per sample, notably rapid compared with that of conventional cholesterol efflux capacity assay, which requires 2-3 days. Each assay proceed sequentially every one minutes, so more than 10 samples could be analyzed within an hour. The assay system had high reproducibility (CV < 10%) and was linear from 5 to 200 ng/mL. The LoQ was validated at 5 ng/mL. CUC measured by the present automated method correlated well with that of our previous pilot method, which inversely associated with recurrence of coronary artery disease in patients with optimal control of low-density lipoprotein cholesterol.

**Conclusion:** Our novel automated method can measure CUC quickly and accurately. This method would expand the dysfunctional HDL research from the bench to the clinical arena.

## B-147

### ELISA system for GPIHBP1 levels in human plasma

K. Miyashita[1], K. Nakajima[2], I. Fukamachi[1], A. P. Beigneux[3], S. G. Young[3], T. Machida[2], K. Nakajima[2], M. Murakami[2]. *[1]Immuno-Biological Laboratories, Fujioka, Japan, [2]Gunma University Graduate School of Medicine, Maebashi, Japan, [3]Department of Medicine, University of California, Los Angeles, CA*

**Background:** GPIHBP1, a glycosylphosphatidylinositol (GPI)-anchored protein of capillary endothelial cells, is crucial for the lipolytic processing of triglyceride-rich lipoproteins (TRLs). GPIHBP1 binds lipoprotein lipase (LPL) in the interstitial spaces and shuttles the enzyme to its site of action in the capillary lumen. GPIHBP1 is also required for the margination of TRLs along capillaries—so that lipolytic processing can proceed. A deficiency of GPIHBP1 in humans causes severe hypertriglyceridemia (chylomicronemia). Even though GPI-anchored proteins are tethered to the plasma membrane, it has been possible to detect some GPI-anchored proteins (*e.g.*, uPAR) in the plasma. We hypothesized that it might be possible to detect GPIHBP1 in human plasma. To test this hypothesis, we created a sensitive sandwich immunoassay for human GPIHBP1 with two newly created human GPIHBP1-specific monoclonal antibodies.

**Methods:** We created a solid-phase sandwich ELISA for human GPIHBP1. The wells of 96-well plates were coated with Mab-CH79A4. After first incubating plasma samples at 37°C for 60 min and then washing the plates, the GPIHBP1 captured was detected with horseradish peroxidase-labeled Mab-HE20A6. Recombinant GPIHBP1 was used as a calibration standard.

**Results:** The ELISA detected GPIHBP1 in human plasma. The median concentration of GPIHBP1 in plasma samples from healthy subjects was range, 570-1523 pg/ml; the detection limit was 10 pg/ml. When plasma samples were "spiked" with recombinant GPIHBP1, the recovery of the spiked GPIHBP1 was excellent (ranging from 85 to 115% of the amount added). The assay was linear over a >4-fold dilution of plasma. The intra- and interassay coefficients of variation were <15%. The plasma levels of GPIHBP1 were not different in pre- and post-heparin plasma samples.

**Conclusion:** We developed a solid-phase ELISA for GPIHBP1 in plasma and used the assay to measure levels of GPIHBP1 in human plasma. In future studies, we will determine whether plasma levels of GPIHBP1 correlate with plasma lipid levels and whether plasma GPIHBP1 levels are a useful biomarker for metabolic or cardiovascular disease.

## B-148

### A new enzyme-linked immunosorbent assay system for human serum hepatic triglyceride lipase

K. Miyashita[1], K. Nakajima[2], I. Fukamachi[1], Y. Muraba[3], T. Koga[3], Y. Shimomura[3], T. Machida[2], K. Nakajima[2], M. Murakami[2], J. Kobayashi[4]. *[1]Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan, [2]Gunma University Graduate School of Medicine, Maebashi, Japan, [3]Hidaka Hospital, Takasaki, Japan, [4]Department of General Medicine Kanazawa Medical University, Ishikawa, Japan*

**Background:** There is no previously established method for measuring human hepatic triglyceride lipase (HTGL) concentration in plasma obtained without heparin injection.

**Methods:** We developed new monoclonal antibodies (MoAb mouse 9A1, MoAb rat 141A1) that react with both HTGL in serum and in post-heparin plasma (PHP) and established a novel enzyme-linked immunosorbent assay (ELISA) system that was able to measure both serum HTGL concentration and PHP-HTGL concentration.

**Results:** To confirm the specificity of antibodies, we performed an immunoprecipitation -immunoblotting analysis. Both MoAb mouse 9A1 and MoAb rat 141 A1 were able to immune-precipitate not only recombinant HTGL and PHP-HTGL but also serum HTGL, demonstrating that HTGL exists in plasma obtained without heparin injection. This method yielded a coefficient of variation of less than 6 % in intra- and inter-assays and did not cross-react with lipoprotein lipase (LPL) or endothelial lipase (EL). In clinical analysis on 42 male subjects with coronary artery disease, there were strongly positive correlations of serum HTGL concentration to PHP-HTGL concentration ($r=0.727$, $p<0.01$). Serum HTGL concentrations had positive correlations to serum triglycerides ($r=0.314$, $p<0.05$) and ALT ( $r=0.406$, $p<0.01$) and tendency toward positive correlations to LDL-C , small, dense LDL and γGTP.

**Conclusion:** These results indicate that this new ELISA method for measuring pre-HTGL is applicable in daily clinical practice.

## B-149

### Homogenous LDL Cholesterol assays - How does the Roche Generation 3 LDL-Cholesterol measure up?

S. Y. Wong, C. J. Gea, B. X. S. Quek, N. Iu, C. P. Yeo. *Singapore General Hospital, Department of Clinical Pathology, Clinical Biochemistry Laboratory, Singapore, Singapore*

**Background:**

Low Density Lipoprotein Cholesterol (LDL-C) is a an important risk assessment biomarker for cardiovascular disease and a primary target of cholesterol lowering therapy, hence accurate measurement is pertinent in routine clinical practice. Homogenous assays for direct measurement of LDL-C are gaining recognition due to its capability of being adapted to automated analysers and its' merits in improved assay precision. However not all homogenous assays perform alike, due to differences in the ability of the assays to reliably detect LDL-C particularly in more complex specimen matrix. Our laboratory currently offers, in addition to the more routinely requested traditional Fridewald calculated LDL-C, testing for direct LDL-C measurements using the Wako LDL-C assay on the Beckman Coulter AU 5800 automated analyser.Lack of appropriate peers and sub-optimal performance in external quality assurance (EQA) programs for our current LDL-C assay have propelled our laboratory to proactively explore alternative assays as part of continuous improvement initiatives.Roche Diagnostics has recently launched a new improved formulated LDL-C Generation 3 (Gen 3) assay, which claims to offer improved specificity for LDL-C and better comparison with the beta-quantification reference method.Our study evaluated the analytical performance of the Roche LDL-C Gen 3 assay on the Roche Cobas c502 and compared it against our current existing LDL-C assay on the Beckman Coulter AU5800.

**Methods:**

Performance validation parameters of the Roche LDL-C Gen 3 assay included assay imprecision (within and total), lower limit of detection, linearity and carry-over. Imprecision was assessed using manufacturer's quality control materials measured in triplicates over 5 days. Limit of detection, linearity and carry-over studies were performed using appropriate low and high concentration patient samples.Method correlation studies using fresh patient serum samples (n=120) were performed on the AU5800 and Cobas c502 platforms. EQA performance of the Roche LDL-C Gen 3 assay was also assessed using leftover samples from accuracy-based surveys.

**Results:**

Within run and total imprecision were determined to be ≤1.1% on Roche c502. Roche LDL Gen 3 assay demonstrated linearity across the analytical measurement range of 0.1 - 14.2 mmol/L, with recoveries between 97-100%.The lower limit of detectable concentration was assessed to be in agreement with the manufacturer's claims.Results of carry-over studies were insignificant. Method correlation with Beckman AU revealed Passing Bablok regression slope of 1.16 and intercept of -0.7; mean absolute bias (Altman Bland) of -0.18 mmol/L (95% CI: -0.22 to -0.13) and Spearman's correlation coefficient of 0.98. Indirect assessment of Roche's LDL Gen 3 EQA performance using post-survey samples from accuracy-based surveys showed better agreement with reference method beta-quantification.

**Conclusions:**

Overall, the Roche LDL-C Gen 3 assay performed within manufacturer's specifications and showed good correlation with beta-quantification.A small but insignificant negative bias is detected in the Roche assay when compared with Beckman AU assay (Wako). Our study demonstrated that the Roche Gen 3 LDL-C fulfills the accuracy and imprecision assay goals in the National Cholesterol Education Program (NCEP) requirements and represents a good alternative choice of direct DL-C testing for clinical laboratories.

## B-150

### Small Dense LDL Cholesterol as Strong Predictors for Secondary Cardiovascular Events Compared with LDL Cholesterol in Elder Patients.

Y. Ito[1], S. Koba[2], Y. Yokota[2], K. Sakai[2], Y. Nakamura[2], F. Tsunoda[2], M. Shoji[2], Y. Kobayashi[2]. *[1]Denka Seiken Co.,Ltd., Niigata, Japan, [2]Showa university school of medicine, Tokyo, Japan*

Background: Small dense (sd) LDL particles have been suggested to be highly atherogenic due to their higher penetration into arterial walls, their lower binding affinity for LDL receptors, their prolonged plasma half-life, and their lower resistance to oxidative stress compared to large buoyant (lb) LDL. Though several methods have been developed for the measurement of serum sd LDL levels, the methods are

laborious and time-consuming We have developed a simple fully-automated direct homogeneous measurement method of sd LDL-C . With our assay kit, it has been revealed that the rise of sd LDL-C is a risk factor for coronary heart disease (CHD) independent from LDL-C. However, the significance as a risk factor in the secondary prevention of CHD has been unexplained yet. Method: Subjects were 356 male and 73 female patients aged of 65 years and older with stable coronary artery disease (CAD) who did or did not develop major cardiovascular events (MACEs) during 5-year follow-up period. MACEs were defined as all cause death, onset of acute coronary syndrome, need for coronary and peripheral arterial revascularization, hospitalization for heart failure, surgical procedure for any cardiovascular diseases, and/or requiring hospitalization for stroke. Besides sd LDL-C with our assay kit, the following biomarkers were measured or calculated, and then analyzed: LDL-C, lb LDL-C, non-HDL-C, RLP-C, apoA1, apoB, glucose, HbA1c, BNP, and eGFR.Result: First-time MACEs were observed in 141 patients. Male patients with MACEs had significantly higher levels of LDL-C, lb LDL-C, sd LDL-C, non HDL-C, apoB, RLP-C, fasting plasma glucose, HbA1c, BNP, and high-sensitive CRP, and significantly lower levels of HDL-C, apoA1, and eGFR were observed in the patients. Multivariate Cox regression analysis results showed that all of biomarkers except lb LDL-C and eGFR were significantly associated with MACEs. Among lipid biomarkers, only sd LDL-C had a significant association with MACEs in male patients treated with statins (Hazard ratio (HR) 1.022, 95% confidence interval (CI) 1.003-1.042) and diabetic male patients (HR 1.019, 95% CI 1.001-1.036). Kaplan-Meier MACE-free survival curve analysis was conducted for 2 groups dichotomized by sd LDL-C 25 mg/dL, which is the median of whole subject. Significantly decreased MACE-free Survival ratio was observed in high sd LDL-C group.Conclusion: These results confirm that sd LDL-C is the most important residual risk to predict future MACEs in stable CAD patients including patients treated with statins.

## B-151

### Stability of fatty acids in serum stored at -20°C for up to 5 years

C. D. Powers[1], M. R. Sternberg[1], S. S. Momin[1], E. C. Pendergrast[2], D. C. Scully[3], R. L. Schleicher[1]. *[1]CDC, Atlanta, GA, [2]Battelle, Columbus, OH, [3]ORISE, Oak Ridge, TN*

In a pilot study, we saw up to 45% degradation of polyunsaturated fatty acids (PUFA) in plasma stored for 6 months at -20°C. It is known that PUFA are highly sensitive to oxidative degradation. This study evaluates the stability of a panel of fatty acids in serum stored with and without butylated hydroxytoluene (BHT) at -20°C ≤ 5 years. The panel includes 11 saturated (SFA), 6 monounsaturated (MUFA), and 13 PUFA fatty acids. A modification of Lagerstedt *et al.* (2001) was used to quantitate unfractionated serum fatty acids. Briefly, total fatty acids were hydrolyzed, hexane-extracted, derivatized to pentafluorobenzyl bromide esters, and detected using electron capture negative-ion gas chromatography-mass spectrometry.

Serum pools were prepared (±)BHT (3.33 g/L methanol). The serum pools (±) BHT were stored at -70°C or -20°C for up to 5 years. All vials were kept frozen and analyzed at designated times, (3 months, 1.5 years, and 5 years). To assess assay performance, serum quality control pools (+)BHT were used. Serum was measured in duplicate at each time point except one storage condition, serum (-)BHT stored at -70°C for 5 years was measured in singlicate due to availability.

For each time period and fatty acid in the panel, concentrations were log transformed and a separate two-way analysis of variance was performed using pool and storage condition as the main effects. The reference condition was storage at -70°C (+)BHT for the specified length of time (3 months, 1.5 years, or 5 years). Relative percent changes for storage at -20°C (±)BHT compared to the reference condition for a given time period were calculated from a two-way ANOVA model with no interaction. However, interactions between the storage conditions and pools were noted if the pool-by-storage-condition interaction was both statistically significant and a > 5% difference existed between any of the pools.

The average percent difference for SFA in serum (±)BHT stored at -20°C ranged from -2% to 0.2% at 3 months and 1.5 years ($p \leq 0.05$); MUFA were ≤ 4% different at 3 months ($p \leq 0.05$). PUFA with ≥ 3 double bonds decreased from 0.4% to 9% (+)BHT and from 16% to 36% (-)BHT in serum stored at -20°C for up to 5 years ($p \leq 0.05$). Eicosapentaenoic acid and docosahexaenoic acid were particularly unstable in the absence of BHT, losing 34% and 35%, respectively, after 5 years at -20°C ($p \leq 0.05$). Serum fatty acids stored for 5 years at -70°C (±)BHT were on average < 1% different across all classes compared to the reference condition.

In conclusion, SFA and MUFA in unfractionated serum are relatively stable when stored at -20°C (±)BHT. However, PUFA degrade when stored without BHT at -20°C for longer than 3 months; generally, greater numbers of double bonds were associated with lower stability. To prevent substantial PUFA degradation, BHT should be added to the serum prior to storage at -20°C or preferably serum should be stored at ≤-70°C.

## B-152

### Non-HDL cholesterol secundary therapeutic goals based on LDL cholesterol population percentiles: Contribution of Longitudinal Study of Adult Health (ELSA-Brasil)

F. A. Brito[1], W. Pedrosa[2], C. B. Maluf[1], P. G. Vidigal[1]. *[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Hermes Pardini Laboratory, Belo Horizonte, Brazil*

**Background**: Current international guidelines consider non–high-density lipoprotein cholesterol (non-HDL-C) as a goal for the prevention and treatment of atherosclerotic cardiovascular disease. These guidelines established non-HDL-C targets 30 mg/dL higher than the respective low-density lipoprotein cholesterol (LDL-C) cut-off points, considering the very-low-density lipoprotein (VLDL-C), estimated by the Friedewald equation, when the triglyceride concentration is 150 mg/dL. These definitions do not consider the population distribution of the non-HDL-C values, and recent studies in different populations have shown that they were lower than the guidelines cut-off points. The aim of this study is to establish cut-off points for non-HDL cholesterol (non-HDL-C) at the same population percentiles of LDL-C. The frequency of reclassification of therapeutic category using current guideline-based non-HDL-C and population percentiles-based cut-off points were also assessed.

**Methods**: A total of 14,837 participants from the Longitudinal Study of Adult Health (ELSA-Brasil), aged from 35 to 74 years old and with triglycerides levels < 400 mg/dL were included. Total cholesterol, HDL-C, and triglycerides were measured in the ADVIA Chemistry system after 12 hours of fasting and LDL-C was calculated with the Friedewald equation. Initially, population percentiles were established for the LDL-C values corresponding to the therapeutic goals, and non-HDL-C values equivalent to the respective LDL-C percentiles were assigned. Next, we assessed the reclassification of therapeutic category based on non-HDL-C, defined as the frequency of subjects with LDL-C levels in the recommended treatment category, but non-HDL-C levels higher than the recommended treatment goal.

**Results**: The LDL-C values of 70, 100, 130 and 160 mg/dL corresponded to the population percentiles 3, 18, 52 and 82, respectively. The non-HDL-C cut-off points for the same percentiles were 92, 122, 156 and 191 mg/dL. When using the latter non–HDL-C cut-off points, 2305 (15.5%) participants were reclassified upwards and 2410 (16.2%) downward. Using current guidelines-based cut-off points, 2068 (14.0%) participants were reclassified upwards and 3957 (26.7%) downward. Among participants with triglycerides between 150 and 199 mg/dL and with concurrently LDL-C level < 70 mg/dL, 26.3% were reclassified upward according to the guideline-based non-HDL-C cut-off point (≥ 100 mg/dL), and 65.8% according to the population percentile-based cut-off point (non-HDL-C ≥ 92 mg/dL). Of participants with LDL-C level between 70 and 99 mg/dL, 14.3% were reclassified upward according to the guideline-based cut-off point (non-HDL-C ≥ 130 mg/dL), and 48.9% according to the population percentile-based cut-off point (non-HDL-C ≥ 122 mg/dL).

**Conclusion**: In this large cohort of free living Brazilians, ours results indicates that the recommended target value for non-HDL-C should be reduced to match the same population percentile equivalent to LDL-C goals. This change resulted in a significant increase in the frequency of reclassification of patients to a higher therapeutic category, especially in the presence of low LDL-C level and hypertriglyceridemia.

## B-153

### RNASE L regulates the expression of fatty acid synthase in the mouse liver

R. Wei, A. Zhou. *Cleveland State University, Cleveland, OH*

**Background:**

Interferons (IFNs) are cytokines that participate in the innate immunity against viruses and other microbial pathogens. In addition, they also display anti-tumor, anti-proliferative and immuno-regulatory activities. The function of IFNs is mediated through proteins encoded by IFN-stimulated genes (ISGs). Ribonuclease L (RNase L) is a type of ISGs. Studies have shown that RNase L contributes to anti-viral infection, apoptosis and anti-cell proliferation. RNase L knockout mice are significantly larger than wide type mice in terms of body weight, and have more oil droplets in the organ tissues such as liver and kidney, suggesting that RNase L may be involved in lipid metabolism.

**Method and Results:**

To determine if RNase L regulates the expression of any genes in the liver, liver tissue extracts from RNase L knockout and wild type mice were subjected to SDS-polyacrylamide electrophoresis and Coomassie blue staining. The differentially

expressed protein bands between the two tissue extracts were excised and digested by trypsin, and subsequently analyzed by LC/MS. Interestingly, the results indicated that fatty acid synthase (FAS) was significantly higher expressed in the liver deficient RNase L. The results were further confirmed with Western Blot analysis by using a mouse monoclonal antibody against fatty acid synthase. Further investigation revealed that RNase L regulates its expression at the transcriptional level and is age and gender dependent. This observation is consistent with our previous report that the lipid level is relatively higher in the plasma of RNase L deficient mice.

**Conclusion:**

Our study reveals that FAS is higher expressed in the liver of RNase l deficient mice, as a result, an increased level of lipid in the plasma of the animals was observed. RNase L transcriptionally regulates the expression of FAS, which could be a potential target of hyperlipidemia and a prognostic marker obesity.

## B-154

### The Performance of Calculated and Directly-Measured Low Density Lipoprotein Cholesterol in a Pediatric Population

S. M. Roper[1], J. Cao[1], E. Tam[2], S. Devaraj[1]. *[1]Baylor College of Medicine/ Texas Children's Hospital, Houston, TX, [2]Texas Children's Hospital, Houston, TX*

Introduction: Accurate measurement of low-density lipoprotein cholesterol (LDL-C) is essential for the identification and monitoring of dyslipidemia. Recent studies indicate that the Friedewald equation is biased when LDL-C levels fall below 100 mg/dL and triglycerides are elevated. The objective of this study was to evaluate the use of a novel equation and a direct homogenous assay to correct the bias of the Friedewald equation in a pediatric population. As a secondary goal, the utility of these methods was assessed by determining the proportion of cases which fall into the biased range of the Friedewald equation at Texas Children's Hospital.

Study design: LDL-C concentrations were determined by the Friedewald equation, a direct homogenous assay, the novel equation[1], and the reference method in 127 pediatric samples with triglycerides between 150-399 mg/dL. Bias was measured by regression analysis at selected LDL-C cutoffs and after stratifying samples by triglyceride content. Statistical significance (p=.0001) was determined by the Wilcoxon Signed-Rank test at LDL-C cutoffs of ≤ 70, ≤ 100, and > 100 mg/dL. The concordance of each method, relative to the reference method, was calculated at LDL-C cut-points of <70, 70-99, and 100-129 mg/dL. Retrospective data analysis was carried out to determine the prevalence of low LDL-C (≤100 mg/dL) and elevated TG (150-399 mg/dL) over a 2.5-month period at Texas Children's Hospital in Houston, TX.

Results: The Friedewald equation significantly underestimated pediatric LDL-C concentrations below 100 mg/dL (-21.6%) and the direct-LDL assay was positively biased (10.8%); the performance of both methods worsened with decreasing LDL concentrations and increasing triglyceride content. The novel equation most-effectively reduced the bias (-1.2%) and increased the concordance of sample classification to the reference method. Approximately 19% (157 of 846) of samples submitted for LDL-C and TG measurement between October 2016 and December 2016 fell into the biased range of the Friedewald equation.

Conclusions: The novel equation should be used for accurate measurement of pediatric LDL-C when the concentration is below 100mg/dL and the triglycerides are between 150-399mg/dL. Granted the large percentage of samples having low LDL-C and elevated triglycerides at our institution, values from the novel equation should be included in lipid results.

1. Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310(19):2061-2068.

## B-155

### Monogenic dyslipidemia in a child followed due disnutrition diagnosis: the importance of clinical suspicion and cascading screening

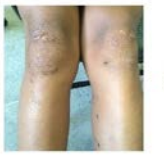
M. C. Gurgel Castelo[1], L. A. A. Batista[2], S. C. Lopes[2], F. L. B. Furtado[2], G. A. Campana[3], R. M. Montenegro Jr[2], R. D. Santos Filho[4], A. C. Pereira[4], C. E. Jannes[5]. *[1]Universidade Federal do Ceará; DASA, Fortaleza, Brazil, [2]Universidade Federal do Ceará, Fortaleza, Brazil, [3]DASA, São Paulo, Brazil, [4]Incor- USP, São Paulo, Brazil, [5]Incor - USP, São Paulo, Brazil*

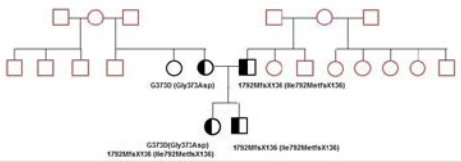
**Background:** The screening to identify children with lipid disorders, and diagnosis of familial hypercholesterolemia (FH) is uncommon in clinical practice.In general, lipid profile measured in early childhood usually do so as a result of a metabolic condition arising from obesity and the family history of cardiovascular disease in the pediatric age group is not always obtained.

**Methods:** Case report

**Results:** We described an 11 year old child attended a pediatric referral center with complains of xanthomas (figure 1) in the knee, elbow and buttocks associated with LDL-c high levels (above 200 mg/dL), despite the rosuvastatin use, since the age 2. Additionally, the child had positive family history with her maternal grandmother surgically revascularized at 47 years old. As the child did not respond to the treatment, the pediatrician decided to refer her for clinical and genetic investigation to a primary dyslipidemia center. At the first visit, the lipid panel showed total cholesterol of 436mg/dL, LDL-c:383mg/dL, HDL-c:38mg/dL and triglyceride:75mg/dL. The calculate Dutch score was 8 with causality classified as "probable" for FH.The genetic sequencing was performed, using the Sanger method and revealed mutation on compound heterozygosis: G373D(Gly373Asp) on exon 8 and 1792MfsX136 (Ile792MetfsX136) on exon 16 of LDL receptor gene. After confirmation of this index-case (IC), a cascade screening was started on her asymptomatic first-degreer elatives and identified the following mutations in heterozygosis at the LDL-c receptor gene: G373D(Gly373Asp) on exon 08 (mother- 27 yrs); 1792MfsX136 (Ile792MetfsX136) on exon 16(father- 45yrs and brother- 5 yrs).

**Conclusion:** Monogenic conditions due to a single gene defect, such as FH is a rare condition in children.Clinical suspicion in non-obese children is uncommon. The most frequent mutation is that of the LDL gene receptor. First-degree relatives should always be screened.





## B-156

### Genetic and clinical-demographic profile of patients with familial hypercholesterolemia (FH) followed on an ambulatory of primary dyslipidemia in a university hospital

M. C. Gurgel Castelo[1], L. A. A. Batista[2], S. C. Lopes[2], F. L. B. Furtado[2], R. M. Montenegro Jr[2], G. A. Campana[3], R. D. Santos Filho[4], C. E. Jannes[4], A. C. Pereira[4]. *[1]Universidade Federal do Ceará; DASA, Fortaleza, Brazil, [2]Universidade Federal do Ceará, Fortaleza, Brazil, [3]DASA, São Paulo, Brazil, [4]Incor - USP, São Paulo, Brazil*

**Background:** FH is a genetic disease caused by mutations in genes related to the metabolism of low-density lipoprotein (LDL-c), resulting in LDL-c high levels and cardiovascular risk (CVR) increase. The course of FH is silent and less than 10% FH patients are diagnosed. **Methods:** This was a cross-sectional study that evaluated patients from a FH screening program, selected through the LDL-c levels ≥210mg/dl (adults) and ≥170mg/dl (children). The patients were classified according to Dutch criteria and genetic data. The DNA sequencing was performed by Sanger's methodology. After the identification of a causal mutation, a cascade screening (CS) in first-degree relatives was pursued.

**Results:** For the 63 index cases (IC), the mean age was 51.44 ± 15.36 years and 72.73% (48) were female. On the 31 related cases, from CS, 22 ± 17.3 years and 27.27% (18) were female. From 63 IC,79.36% (50) completed the genetic sequencing with 20.96% heterozygosis mutation confirmed on LDL-c receptor gene. The positivity for those mutation detection on SC patient was 25.8%. The majority of

mutations were found on exons 8,9,12,14 and 16.From the population (63 IC and 31 CS), 67.02% (63) patients met DUTCH criteria and 52.38% (11) had a positive family history for cardiovascular disease. Overweight or obesity was not identified in the study population. Less than half of the patients received at least one pharmacological agent for dyslipidemia (45.74%).

**Conclusion:** The diagnosis of FH is based on clinical criteriaalthough the genetic diagnosis is very relevant. Most of the patients are heterozygote. The CS is crucial on the FH identification, which allows early intervention and cardiovascular mortality decrease.

## B-157

### Markers of Inflammation in Acne Vulgaris

I. Aslan[1], F. Ozcan[2], T. Karaarslan[1], E. Kıraç[2], M. Aslan[2]. *[1]Antalya Training and Research Hospital, University of Health Sciences, Antalya, Turkey, [2]Akdeniz University Faculty of Medicine, Antalya, Turkey*

**Background:** Acne vulgaris (AV) is a inflammatory skin disease of unknown etiology. Eicosanoids derived from omega-6 (n-6) polyunsaturated fatty acids (PUFAs) have proinflammatory functions whereas eicosanoids derived from n-3 PUFAs have anti-inflammatory properties. Studies have documented that dietary supplementation with n-3 fatty acids ameliorates AV however serum PUFA levels have not been evaluated in patients with AV. Excess levels of secretory phospholipase A2 (sPLA2) also contributes to inflammatory diseases and studies indicate that lipoprotein lipase (LPL) has differential effects on several inflammatory pathways. This study aimed to determine circulating levels of n-6, n-3 PUFAs, assess serum activity of sPLA2, LPL and evaluate changes in circulating protein levels of angiopoietin-like protein 3 (ANGPTL3), ANGPTL4, cyclooxygenase-2(COX-2) and prostaglandin E2 (PGE2) in patients with AV.

**Methods:** Serum from 31 AV patients and 21 age and gender matched control subjects were evaluated for levels of sPLA2, COX, PGE2, LPL, ANGPTL3 and ANGPTL4 via commercial assay kits. Serum PUFA levels were measured by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods using autoanalyzers.

**Results:** No significant differences were found for the lipid profile, routine biochemical and hormone parameters between AV patients and controls. Serum EPA levels were significantly decreased while AA/EPA and DGLA/EPA ratio were significantly increased in AV patients compared to controls. Serum levels of AA, DGLA and DHA showed no significant difference between patients and controls. Serum activity of sPLA2 and LPL were significantly increased in AV compared to controls. No significant differences were found for COX, PGE2, ANGPTL3 and ANGPTL4 levels.

**Conclusion:** The results of this study reveal the presence of a proinflammatory state in AV as shown by significantly decreased serum EPA levels and increased activity of sPLA2, AA/EPA and DGLA/EPA ratio. Increased LPL activity in the serum of AV patients can be protective through its anti-dyslipidemic actions. This is the first study reporting altered EPA levels and increased sPLA2 activity in AV and supports the use of omega-3 fatty acids as adjuvant treatment for acne patients. **Acknowledgement:** This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; #115S940).

## B-158

### Only fresh samples should be allowed for lipid profile evaluation

G. Lima-Oliveira, G. Lippi, G. C. Guidi. *University of Verona, Verona, Italy*

**Background:** This study was aimed to investigate whether the laboratory could assay lipid profile using either fresh or thawed samples.

**Methods:** Serum from 10 volunteers were immediately assayed for total cholesterol (CHOL), high density lipoprotein (HDL), and triglycerides (TG); then each volunteer's sample was divided in 2 identical aliquots. All aliquots were thawed after two-days freezing at -70°C. Immediately afterwards, the sera of the two paired aliquots were treated using two different techniques: (a) no mixing; (b) reference procedure, entailing 6 gentle inversions by overturning; The significance of the differences against the fresh (no frozen samples) was assessed with Wilcoxon signed rank test. The statistical significance was set at $p<0.05$.

**Results:** A significant variability was observed for CHOL, HDL, and TG when compared with thawed samples not mixed; whereas thawed samples mixed by inversion showed significant differences for CHOL, and HDL; but not for TG (Table 1).

**Table 1. Lipid profile assayed on fresh and thawed samples**

| Test | Fresh samples | Thawed samples | |
|---|---|---|---|
| | | Not mixed | Mixed |
| TG | 1.32 [1.11 – 1.87] | 0.82 [0.66 – 1.20] | 1.31 [1.10 – 1.86] |
| | | **P=0.002** | P=0.588 |
| CHOL | 5.43 [5.18 – 5.68] | 3.22 [2.90 – 3.42] | 5.33 [5.10 – 5.65] |
| | | **P=0.002** | **P=0.008** |
| HDL | 1.66 [1.38 – 1.75] | 0.92 [0.80 – 1.02] | 1.65 [1.36 – 1.71] |
| | | **P=0.002** | **P=0.020** |

Data are presented as mean [interquartile range], in mmol/L.

**Conclusion:** Based on our results lipid profile should be assayed on fresh samples in order to guarantee both laboratory outcomes and reliable results, thus patient safety.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Management

## B-159

### Total Laboratory Automation (TLA) - performance and improvements from 2013 to 2016

T. L. Ellison, H. Hassan, M. Alsallom, M. Alfaires, F. Aldayel. *King Faisal Hospital, Riyadh, Saudi Arabia*

**Background and objectives:** The Department of Pathology & Laboratory Medicine (DPLM) at King Faisal Hospital, Riyadh, Saudi Arabia embarked on an ambitious programme to consolidate services with initial discussions starting in 2009. In 2012, DPLM began to implement a TLA, which was the culmination of 5 previous contracts into one, with the following objectives to: be cost efficient (save money), be less labour intensive (use less staff), increase productivity and capacity (be able to do more) and enhance patient safety (reduce turnaround time and enhance predictability / reduce variation). **Method and results:** We went live with a partially implemented TLA in 2013 and have experienced a 7% increase in workload year over year. Routine random glucose was chosen as a sentinel to compare turnaround times (TATs) and whisker boxplots (WBPs) were chosen to visually present TAT by receipt hour of the day for 2012 and 2016. At the peak of workload (11am) we have the greatest variation in TAT; the least predictability in service delivery. The shape of the distribution (timing of workload) has not changed between 2012 and 2016. We present here we present what was delivered in relation to our objectives. There is a 32% reduction in TAT and enhancement in predictability as shown in figure 3 when comparing before (2012) and after (2016) the TLA implementation. **Financial impact:** The cost avoided by the consolidation of services was 5 million SAR per year in direct costs from previous contracts. The staffing costs for the TLA area were 5,411,704 SAR in 2012 (before consolidation); the cost is now 4,275,214 SAR in 2016 which is a saving of 1,136,490 SAR on staffing costs (reduction of 6 full time staff in this area). It is of note that the mix of staff has changed with less senior staff needed; there are now 3 senior staff whereas previously 5 were required (staff attrition posts were not back filled). Staffing and consolidation of contracts will have saved 30 million SAR by March 2018. **Discussion and conclusion:** Total Laboratory Automation, with integrated robotics and IT solutions, is becoming a crucial way to simplify operations - both reducing manual reliance and maximising patient safety. We delivered on our objectives to: be cost efficient (30 million SAR saved by March 2018), be less labour intensive (head count reduced overall by 15%), increased productivity and capacity (70% capacity available for additional work 12 hours per day), enhanced patient safety (reduced turnaround time and enhanced predictability by >30%), designed out opportunity for error (automated practices and adopted IT-logic rules based decisions such as auto-verification) and we have added more services to automation lines (i.e. infectious serology, to improve efficiency). Our experience with the transformation to a TLA has been very positive, resulting in effective and efficient service delivery whilst enabling huge potential for additional work and income generation; with keys to success including strong leadership, robust change management and clear performance metrics (key performance indicators).

## B-160

### Automated Core Facility - enhancing performance, reducing queuing

T. L. Ellison, H. Hassan, M. Alsallom, M. Alfaires, F. Aldayel. *King Faisal Hospital, Riyadh, Saudi Arabia*

**Background:** The Department of Pathology & Laboratory Medicine (DPLM) at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia embarked on an ambitious programme to consolidate services and create an Automated Core Facility (ACF) which went live in 2013. In an on-going effort to further reduce the turnaround time (TAT) and enhance predictability, we reviewed systematically all elements of our ACF using the Roche Cobas 8000 series (Roche Diagnostics Middle East FZCO). The centrifuge spin time had been historically set at 10 minutes (mins). We present here an overview of the impact of reducing the centrifugal spin time down from 10 mins to 5 mins and the unexpected impact on TAT. **Method and Results:** Data was captured on the time from receipt in the ACF clinical laboratory to result availability before and after adjusting the centrifugal spin time down from 10 mins to 5 mins (June 2016). Data was captured for 27 common tests that are performed in clinical biochemistry. Data is presented in Whisker Box Plot (WBP) and histogram formats. 2 unremarkable, representative days were chosen to compare before and after changing the spin time (which had 20,510 and 17,828 tests respectively). The impact of reducing spin time by 5 mins was a significant reduction in some tests (reduction of TAT in mins for AFP 52, amylase 36 and ferritin 81 mins). Before the reduction in spin time, the mean TAT was 80, after this was reduced to 70 mins. There is a 32% reduction in TAT and enhancement in predictability as shown in figure 4 when comparing before (2012) and after (2016) the ACF with reduced spin times. **Conclusion:** The change in spin time of 5 mins delivered a significant change in TAT across many parameters of the ACF which can be explained by the capacity of the system being at a tipping point with the volume of work at the peak time of day. Reducing the spin time by 5 mins resulted in a mean TAT improvement of 10 mins overall. At the busiest time of the day this has resulted in an 18 min TAT improvement. After 3 years' experience with an ACF, there are minor tweaks to be made that may make demonstrable improvement. With a stable automated solution, further enhancements can still be achieved using a Lean approach and focusing on detailed workflow.

## B-161

### 25 OH Vitamin D - Lean production and benefits of immuno-assay

T. L. Ellison, H. Hassan, S. Elimam, M. Alfaires, M. Alsallom, F. Aldayel. *King Faisal Hospital, Riyadh, Saudi Arabia*

**Background and objectives:** The Department of Pathology & Laboratory Medicine has undertaken a systematic review of service provision to ensure that delivery is cost effective and efficient. One of the principles that underpins our service is to embed lean manufacturing principles - so that we are able to meet our customer needs (on time, every time). To that end, we reviewed the provision of 25 OH vitamin D using Xevo TQ-S liquid chromatography mass spectrometry (LC-MS/MS) versus an alternative immuno-assay method on a Roche Cobas 8000 series. It has been estimated that almost one billion people globally have vitamin D deficiency or insufficiency. Vitamin D status in Saudi Arabia is also well documented with >95% of adolescents having deficiency. The workload to screen for 25 OH vitamin D at our hospital has consistently increased by 10% year over year, and currently stands at 40,000 requests per year (September 2016). We present here a 2 instrument comparison study of 25 OH vitamin D using mass spectrometry and immuno-assay and the impact of changing technology on: staffing required, grade of staff and turnaround time (TAT). Our incumbent method required 2 full staff time staff to operate and delivered a mean TAT of 7 days. **Method and results:** EP Evaluator® was used for statistical analysis from the 2 platforms. 25 OH vitamin D was analysed using LC-MS/MS and a Cobas 8000 series system to determine whether the methods are equivalent (total allowable error permitted was 25%). 38 samples were compared which had a range of 8-117 nmol/L. The difference between the methods was within the allowable error for 37 of the 38 samples (97.4). The correlation coefficient (R) was 0.9789. Whisker box plots (WBPs) were chosen to visually show the TAT. Using LC-MS/MS the TAT ranged from 4 to 10 days (25$^{th}$ percentile was 6.89, mean 7.14, 75$^{th}$ percentile 8 days). Scatterplots and index error charts demonstrated good correlation between the methods; correlation coefficient (R) = 0.9789. **Discussion:** Accurate measurement of vitamin D is important as this is an important compound that absorbs calcium and phosphorous. Sufficient vitamin D levels are required for developing and maintaining bones throughout our lives. LC-MS/MS is renowned for its superior analytical performance particularly at the bottom end; our study found that an immuno-assay was comparable in performance. The choice of analytical platform is based on a variety of factors including: quality, hands on time, walk away time, technical expertise needed and cost. Due to the ease of use of the TLA to add another assay, by moving this assay from LC-MS/MS we have:

- Improved TAT (from a mean of 7 days) to a same day service (less than 24 hours),
- Reduced staff time (free-up 2 full time senior staff who were redeployed) and
- Saved money as the cost of immuno-assay less than LC-MS/MS.

**Conclusion:** Moving to an alternative method for vitamin D resulted in lean service improvements, benefits to patients from more timely results benefits to the hospital by cost avoidance and freeing up staff time whilst maintaining quality.

## B-162

### Sigma Metric Quality Framework - Analyte Performance Indicator and Quality Improvement Tool

V. Lo. *Hong Kong Sanatorium & Hospital, Apleichau, Hong Kong*

**Backgrounds:** Although the concept of sigma metrics as advocated by Dr. JO Westgard in 2001 for quality design and control is widely accepted, professional adoption rate is extremely low. Embedded belief of "the more internal quality control (IQC) is done daily the lower is the risk of making mistake and chance of reporting erroneous result" hindered the profession from moving forward. As at 6 November 2016 fifteen PubMed identified publications with keyword search of [sigma metric and internal quality control (IQC)] stated application of sigma metrics as analyte performance indicator, none reported about the POSITIVE outcome of adopting sigma metrics as quality improvement tool. Since 2010 our laboratory established a **Sigma Metric Quality Framework** with which sigma value was applied not only as performance indicator, but also as quality improvement tool.

**Methods:** Starting from 1 January 2010 our laboratory determined sigma value of 54 chemistry and 22 immunoassay analytes in Cobas 6000 (Roche Diagnostic), with formula [Sigma metric = (TEa-bias)/CV]. TEa was obtained from respective defined allowable limit of performance of the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP), which is associated with biological variation and analytical goal. Bias and CV were determined from RCPAQAP end of cycle report and observed imprecision respectively. Sigma 3 or lower analyte was identified and compared with that of the RCPAQAP peer as benchmark, required improvement was executed without any delay. Service of below sigma 3 analyte was temporarily suspended until sigma value was improved. Instead of analysing IQC, every eight hours, frequency became analyte performance specific, once, twice and thrice for analyte of sigma >=5, 4 and 3 respectively.

**Results:** Among 76 analytes, 59(78%), 13(17%) and 4(5%) achieved sigma>=5, 4 and 3 respectively. Four sigma 3 analytes performed equally or better than those of the RCPAQAP peers. Reduction in daily IQC performing frequency of sigma>=5 and 4 analytes contributed to significant reduction in control materials and reagents consumption, which accounted for an annual cost saving of over USD 150,000. Time reduction in IQC preparation, analysis and performance review contributed to an annual man-hour saving of 0.3 full time employee. Without requesting hospital management for additional resource our laboratory increased service scope from 180 analytes to 238, 30% increment.

In 2015 and 2016 our laboratory was categorized by RCPAQAP as GOOD laboratory according to key performance indicator. In October 2016 our laboratory was selected by RCPAQAP as one of the reference laboratories for 2017 target value setting of 5 analytes: Glucose (sigma 8), Lactate (sigma 10), Phosphate (sigma 8), Protein (sigma 5) and Urea (sigma 12) because of past record of good performance and well documented evidence of good analytical performance in the RCPAQAP.

**Conclusion:** With Sigma Metric Quality Framework, incorporating sigma value as performance indicator and quality improvement tool, our laboratory was able to: (1) Identify bad performing analyte(s), (2) Relate analyte required performance clinically, (3) Set clear & achievable target, and (4) Allocate resources wisely.

## B-163

### Using the information system to monitor blood culture contamination rate to improve health care quality

N. Liao, K. Chan, H. Chou, Y. Tsai, L. Wu. *Chi Mei Medical Center, Tainan, Taiwan*

Background

Blood culture (BC) contamination is a common cause lead to misdiagnosis to the interpretation of a positive BC. Treat contaminants as pathogens may leads to unnecessary antibiotic therapy, and induces drug resistant strains. According to Clinical Laboratory Standards Institute (CLSI) standard, BC contamination rate should not exceed 3%. If the nursing staffs fail to follow the correct BC collection standard operating procedures (SOP), they may make the BC contaminated due to insufficient skin disinfection. High contamination rate increase laboratory workload and can cause incorrect changes to patient management. This can prolong patient hospitalization, lead to treatment failure and increase cost to health boards.

Object

In order to effectively monitor the BC contamination rate, our hospital use the information system to regularly feedback BC contamination rate monthly, the number of contaminated cases by each phlebotomist, and the blood volume collected to the emergency department (ED). By data analysis and discussion, we assist the ED to provide educational training and reduce BC contamination rate, then to improve the quality of BC.

Material and Method

To analyze the statistical data from 2015 to 2016, we found 34.0% BC contamination case came from new nursing staffs ; 32.6% contamination occurred at the duplicate staffs in 2015. The BC contamination rates were maintained less than 3% except April to July in 2015 and 2016. This is because the hot climate (>90 degrees) makes patients sweat easily. Routine disinfection methods cannot effectively reduce the number of microbial colonies on the skin surface. The nursing staffs should check patients' skin dirt level and do the appropriate clean before performing blood collection operations.

Result

Through monthly feedback regarding BC contamination rates, and through serious staff education and training in correct BC collection procedures, the number of contaminated BC fell to 13.2% among new nursing staff and 33.3% of duplicate staff were not involved in the collection of any contaminated BC in 2016. Since the ED SOP specified that nursing staff should engage in appropriate skin cleaning before per forming blood collection operations, BC contamination rates decreased from 3.61% to 2.23% from April 2015 to November of 2016. The total decrease was 61.88%.

Conclusion

Laboratory regularly uses the information system to monitor the BC contamination rate, and provide feedback to clinical unit. Through the use of a structured plan and teamwork, it is feasible to assist clinical units in reducing BC contamination rates, patient hospitalization and hospital charges, and to deliver high quality care.

## B-164

### Continuous National survey on Internal Quality Control of Blood Gas in Laboratories in China from 2015 to 2016

Y. Ye, W. Wang, F. He, K. Zhong, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background**: To investigate the imprecision of internal quality control(IQC) practice of blood gas from 2015 to 2016 so as to have an overall knowledge of imprecision level of blood gas in laboratories in China.

**Methods**: Information and IQC data of pH, $pCO_2$ and $pO_2$ were collected via an on-line questionnaire three times a year, respectively in March, June and September. Cumulative coefficient of variations(Cumulative CVs) were analyzed and the percentage of laboratories meeting the quality requirement were calculated in SPSS. The quality specification of pH and $pCO_2$ are derived from biological variation including the optimal(0.9% and 1.2%), desirable(1.8% and 2.4%) and minimal(2.6% and 3.6%) allowable imprecision. Since there is no biological variation data of $pO_2$, quality requirement defined by National Center for Clinical Laboratories in China including 1/3TEa(2.67%) and 1/4TEa(2%) were employed to calculate the acceptable rate.

**Results**: There were 442, 425 and 428 laboratories continuously submitting their results of pH, $pCO_2$ and $pO_2$. The proportion of laboratories meeting different quality specification are shown in table below. For pH, more than 80% participants obtained a satisfied CV. Acceptable rate of $pCO_2$ and $pO_2$ varied largely with different allowable imprecision. And even based on the lowest criteria, the acceptable rate was still not up to 80% and 40%, respectively. There is no significant change of acceptable rate from 2015 to 2016.

**Conclusions**: There exists a fairly good IQC practice of pH currently in China. While only a few laboratories can pass the imprecision requirement for $pCO_2$ and $pO_2$, so much more attention and efforts should be taken on $pCO_2$ and $pO_2$ so as to achieve a better quality and provide more accurate results to patients.

| Analyte | Statistics | | 2015.3 | 2015.6 | 2015.9 | 2016.3 | 2016.6 | 2016.9 | P value |
|---|---|---|---|---|---|---|---|---|---|
| PH | Cumulative CVs | Median(%) | 0.14 | 0.14 | 0.163 | 0.14 | 0.14 | 0.13 | - |
| | | IQR(%) | 0.22 | 0.29 | 0.24 | 0.28 | 0.34 | 0.29 | - |
| | Allowable imprecision specifications based on biological variation | Optimum performance(%) | 83.48 | 83.03 | 85.07 | 85.29 | 84.39 | 84.39 | 0.94 |
| | | Desirable Performance(%) | 88.69 | 89.82 | 89.82 | 90.27 | 89.59 | 89.37 | 0.98 |
| | | Minimum performance(%) | 92.08 | 93.44 | 92.53 | 93.21 | 93.89 | 92.31 | 0.89 |
| $PCO_2$ | Cumulative CVs | Median(%) | 2.63 | 2.50 | 2.56 | 2.44 | 2.46 | 2.45 | - |
| | | IQR(%) | 2.13 | 2.18 | 2.48 | 2.10 | 1.93 | 2.07 | - |
| | Allowable imprecision specifications based on biological variation | Optimum performance(%) | 6.82 | 7.29 | 8.94 | 8.47 | 9.88 | 14.12 | <0.01 |
| | | Desirable Performance(%) | 44.71 | 47.53 | 45.88 | 49.18 | 49.18 | 48.24 | 0.73 |
| | | Minimum performance(%) | 69.18 | 70.35 | 68.00 | 73.88 | 76.00 | 74.35 | 0.05 |
| $PO_2$ | Cumulative CVs | Median(%) | 2.87 | 2.68 | 3.10 | 3.00 | 2.83 | 2.81 | - |
| | | IQR(%) | 2.63 | 2.63 | 2.65 | 2.74 | 2.80 | 2.90 | - |
| | Quality requirement | 1/3TEa(%) | 46.96 | 50.00 | 38.55 | 44.16 | 45.33 | 48.36 | 0.02 |
| | | 1/4TEa(%) | 27.80 | 25.70 | 24.53 | 28.50 | 30.61 | 34.11 | 0.03 |

## B-165

### National Survey on Quality Indicators for the Pre-analytical Phase in China

Y. Ye, W. Wang, F. He, K. Zhong, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background**: Incorrect sample type, incorrect sample container, incorrect fill level and anticoagulant sample clotted are important quality indicators(QIs) for the pre-analytical phase. The survey aims to investigate the current status of the four QIs in laboratories in China.

**Methods**: A survey was performed in 32 clinical laboratory centers in the national wide in 2016. All participants were asked to collect data related to QIs and complete QIs questionnaires. The results were evaluated with sigma scale(σ) and percentages.

**Results**: Totally 7574,7579,7572 and 7149 laboratories involved in the survey. The median of incorrect sample type, incorrect sample container, incorrect fill level and anticoagulant sample clotted is 0.016%, 0.011%, 0.036% and 0.083%, and the median of sigma is 5.096, 5.190, 4.884 and 4.644, respectively. The distribution in detail is shown in table below. The sigma level mainly fall on 4-5, followed by 6(except for incorrect sample container). No laboratories' sigma belong to 0-3.

| Evaluation index | Classification | Incorrect sample type | | Incorrect sample container | | Incorrect fill level | | Anticoagulant sample clotted | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | Proportion (%) | Number | Proportion (%) | Number | Proportion (%) | Number | Proportion (%) |
| Percentage | 0-0.05 | 4992 | 65.91 | 5580 | 73.62 | 4254 | 56.18 | 2850 | 39.87 |
| | 0.05-0.25 | 1753 | 23.14 | 1543 | 20.36 | 2216 | 29.27 | 2576 | 36.03 |
| | 0.25-0.45 | 414 | 5.47 | 245 | 3.23 | 510 | 6.74 | 808 | 11.30 |
| | 0.45-0.65 | 141 | 1.86 | 82 | 1.08 | 206 | 2.72 | 314 | 4.39 |
| | 0.65-0.85 | 83 | 1.10 | 46 | 0.61 | 112 | 1.48 | 193 | 2.70 |
| | 0.85-5 | 191 | 2.52 | 83 | 1.10 | 274 | 3.62 | 408 | 5.71 |
| σ scale | 0-3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3-4 | 286 | 3.78 | 140 | 1.85 | 407 | 5.38 | 638 | 8.92 |
| | 4-5 | 3149 | 41.58 | 2877 | 37.96 | 3960 | 52.30 | 4299 | 60.13 |
| | 5-6 | 1200 | 15.84 | 1568 | 20.69 | 1075 | 14.20 | 350 | 4.90 |
| | 6 | 2939 | 38.80 | 2994 | 39.50 | 2130 | 28.12 | 1862 | 26.05 |

**Conclusions**: The QIs related to sample for pre-analytical phase is an useful tool for laboratories to evaluate the acceptability of sample and determine whether to accept or not, and a premise of guaranteeing the correct result, as well. Laboratories in China have a relatively good performance in the four QIs currently, but more attention should be paid to monitor the QIs and measures should be taken to decrease the number of unacceptable samples.

**Acknowledgment**: We acknowledge the support of centers for clinical laboratory of all the participating provinces in China.

## B-166

### Summary and Analysis of Six Years' Blood Pb Internal Quality Control Practice in China

Y. Huang, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, Beijing, China*

**Background:** The level of blood Pb (BPb) can indicate plumbism especially for children. This survey was initiated to review the six years' status of internal quality control (IQC) practice by statistical analysis of the IQC data of BPb from 2011 to 2016, so as to have an integral knowledge of imprecision level of measurement in laboratories in China.

**Method:** The data of IQC and cumulative coefficient of variation (CVs) of BPb had been collected until September 2016 via the external quality control (EQA) software. Microsoft Excel 2010 and SPSS20.0 had been used to analyze the data. The one third and one fourth of allowable total error (3.33% and 2.5%) was used to evaluate whether laboratories could satisfy the provided quality requirement and to calculate the percentage.

**Results:** There are 209,177,168,167,159,157 laboratories submitted their results from 2011 to 2016, which as shown in table below .The majority of participant laboratories obtain a satisfied CV when 1/3TEa were used. The number of participant laboratories decreased gradually from 209 to 157 while the percentages of laboratories meeting quality requirements were similar when 1/3TEa and 1/4TEa were used in six years from 2011 to 2016. Although median and IQR of Cumulative CVs fluctuated largely among different years, there was no obvious change trend.

**Conclusion:** Although most participant laboratories met the evaluation criteria of 1/3TEa and 1/4TEa, laboratory managers should monitor the current and cumulative CVs of BPb. In order to improve the testing quality of BPb in China, it is recommended that more laboratories participant in IQC program of BPb and participant laboratories take more efforts.

| Item | Year | Number of participant laboratories | Cumulative CVs (%) | | Percentages of laboratories meeting quality requirements | |
|---|---|---|---|---|---|---|
| | | | Median | IQR | 1/3TEa (%) | 1/4TEa (%) |
| BPb | 2011 | 209 | 8.30 | 5.24 | 89.47%(187/209) | 90.43%(189/209) |
| | 2012 | 177 | 8.12 | 5.43 | 90.40%(160/177) | 92.66%(164/177) |
| | 2013 | 168 | 7.52 | 4.00 | 88.10%(148/168) | 93.45%(157/168) |
| | 2014 | 167 | 8.20 | 4.79 | 86.23%(144/167) | 91.62%(153/167) |
| | 2015 | 159 | 7.80 | 4.85 | 88.68%(141/159) | 92.45%(147/159) |
| | 2016 | 157 | 7.63 | 4.98 | 85.35(134/157) | 91.72%(144/157) |

## B-167

### Imprecision investigation and analysis of internal quality control of sex hormones tests in clinical laboratories in China

T. Li, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:** Sex hormones are important indicators for the female endocrine function and diseases associated with endocrine disorders. This study aimed to investigate the imprecision of internal quality control (IQC) for estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, prolactin and testosterone.

**Methods:** IQC information including cumulative coefficient of variation (CV) and measurement method of sex hormones were collected via an on-line questionnaire. To calculate the cumulative CV, laboratories had to collect all the results of controls from the first day on which the same lot of control was used to the last day until August 2016, outliers were removed for analysis. Then the percentages of laboratories meeting quality requirement were calculated according to three kinds of specifications for imprecision derived from within-subject biologic variation including the minimum, desirable and optimal specification. Chi-square ($\chi^2$) test was used to compare the pass rates among different analytes.

**Results:** There were 1046, 1035, 1036, 1036, 1023 and 1027 laboratories submitting their IQC data of estradiol, FSH, LH, progesterone, prolactin and testosterone, respectively. The mainstream testing methods of sex hormones were electrochemiluminescence (32.8% to 33.6%), acridinium ester-chemiluminescence immunoassay (24.3% to 25.0%) and chemiluminescent microparticle immunoassay (24.0% to 24.9%). The percentages of laboratories whose cumulative CVs met 3 imprecision criteria varied significantly among different analytes for both two levels (all $P<0.01$), as shown in table below. The majority of laboratories can obtain a satisfied CV when minimum specification was applied (pass rates > 80%) except testosterone. However, only a few laboratories can meet optimal specification for imprecision (pass rates < 70%).

**Conclusions:** As only a few laboratories can meet the optimal specification, substantial effort is needed for sex hormones tests especially testosterone in China. Laboratories are recommended to adopt suitable imprecision specification so that they can evaluate whether their analysis performance meet quality requirements.

| | Analyte | Number of participating labs | Concentration of control materials | | Cumulative CVs (%) | | Percentages of laboratories meeting quality requirements | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Median | IQR | Median | IQR | Minimum specification | Desirable specification | Optimal specification |
| Level 1 | Estradiol (pmol/L) | 784 | 286.25 | 239.30 | 6.98 | 5.00 | 96.3% (755/784) | 84.1% (659/784) | 35.7% (280/784) |
| | FSH (IU/L) | 946 | 14.60 | 12.92 | 5.35 | 3.47 | 83.6% (791/946) | 52.1% (493/946) | 10.3% (97/946) |
| | LH (IU/L) | 947 | 10.35 | 16.21 | 5.78 | 3.12 | 100.0% (947/947) | 96.8% (917/947) | 50.5% (478/947) |
| | Progestone (nmol/L) | 962 | 8.04 | 22.10 | 6.85 | 4.60 | 95.2% (916/962) | 85.8% (825/962) | 38.6% (371/962) |
| | Prolactin (mIU/L) | 965 | 106.00 | 236.15 | 5.10 | 3.21 | 99.7% (962/965) | 97.1% (937/965) | 60.7% (586/965) |
| | Testosterone (nmol/L) | 997 | 6.56 | 11.77 | 5.72 | 4.14 | 63.9% (637/997) | 32.8% (327/997) | 4.3% (43/997) |
| Level 2 | Estradiol (pmol/L) | 645 | 900.61 | 915.74 | 6.07 | 5.01 | 98.8% (637/645) | 90.7% (585/645) | 45.9% (296/645) |
| | FSH (IU/L) | 480 | 42.61 | 20.71 | 5.00 | 2.97 | 88.1% (423/480) | 60.2% (289/480) | 12.3% (59/480) |
| | LH (IU/L) | 485 | 50.14 | 35.19 | 5.22 | 3.04 | 100.0% (485/485) | 98.1% (476/485) | 61.0% (296/485) |
| | Progestone (nmol/L) | 466 | 36.12 | 47.66 | 5.59 | 3.57 | 99.8% (465/466) | 97.2% (453/466) | 53.0% (247/466) |
| | Prolactin (mIU/L) | 447 | 439.26 | 823.53 | 4.80 | 2.92 | 100.0% (447/447) | 99.1% (443/447) | 67.3% (301/447) |
| | Testosterone (nmol/L) | 416 | 20.62 | 18.23 | 5.20 | 3.78 | 68.5% (285/416) | 42.8% (178/416) | 4.1% (17/416) |

## B-168

### Quality improvement through investigation of unacceptable HbA1c proficiency testing results in China

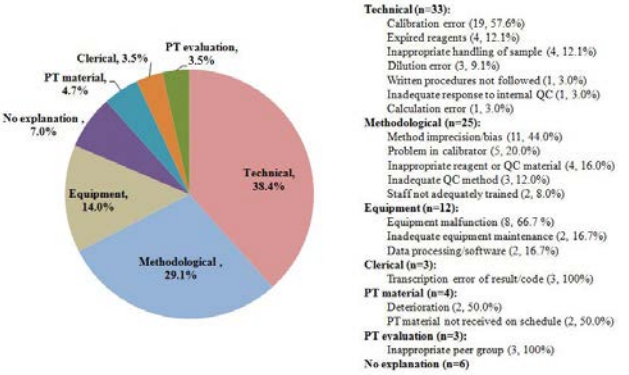
T. Li, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:** National central for clinical laboratories (NCCL) is an official proficiency testing (PT) provider in China. When the laboratory receives a report of unacceptable PT results from NCCL, it should systematically evaluate every aspect of the examination process and correct any problems identified. The investigation aims to provide guidance to laboratories in cause-analysis, identification and monitoring of corrective action.

**Methods:** Participants were given a PT troubleshooting guide for investigating unacceptable PT results. Participants reporting HbA1c PT survey results in March 2016 outside of acceptable limits (±8% of the target value which is the consensus value determined by combining results from participants using a common measurement method) then received the notification to investigate errors and submit their results by Web-based external quality assessment system in November 2016. The responses were categorized as clerical, methodological, technical, equipment, PT material, PT evaluation problems and no explanation after investigation.

**Results:** There were 1394 laboratories participating in the HbA1c PT survey with 124 laboratories (8.9%) not meeting the predetermined acceptable limit. In the end, there were 86 laboratories (69.4%) submitted their causes and corrective action. The causes for unacceptable HbA1c PT results are shown in figure blow. Technical and methodological problems were the main causes for unsatisfactory PT performance and few PT evaluation problems existed except that three participants reported insufficient participant numbers for the specified methodology which leaded to inadequate evidence to evaluate against all methods. Corrective Action: Most corrective actions were appropriate, which included re-writing of procedures and re-education and training of staff.

**Conclusions:** Classifying causes of unacceptable results in PT surveys assists laboratories in identifying opportunities for improvement and developing root cause analysis and corrective action. Meanwhile, participants can share common causes for unacceptable PT results and associated corrective actions with each other so that they can achieve continual improvement.




## B-169

### National survey on critical values notification in 5265 laboratories in China

T. Li, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

**Background:** During post-analytical phase, critical values notification to responsible caregiver in a timely manner has potential to improve patient safety and ineffective notification can lead to diagnostic errors that potentially harm patients. The aim of this study was to investigate the status quo of percentages of critical values notification and timely critical values notification in China.

**Methods:** 5265 clinical laboratories from different provinces in China were enrolled in this quality indicators (QIs) survey in 2016. All participants were asked to submit general information and data related to QIs to National Center for Clinical Laboratories or clinical laboratory centre of corresponding province via network platform. The QI was evaluated in two ways: percentage and sigma(σ). Mann-Whitney Test and Kruskal-Wallis Test were used to perform the group comparison.

**Results:** 92.1% laboratories notified all critical values but only 85.3% laboratories notified all critical values timely. If these two QIs were evaluated with sigma metrics, there were up to 96.7% and 95.6% laboratories whose performance for critical values notification and timely critical values notification were acceptable (σ≥3), respectively. The results of hospitals classified by numbers of beds and information system were shown in table below. Hospitals occupied less than 1000 beds and hospitals where HIS were employed got the higher percentages of critical values notification ($P<0.005$) and timely critical values notification ($P<0.0083$).

**Conclusion:** The defect percentages of critical values notification and timely critical values notification is really low in this survey, but larger hospitals still should pay much attention to critical values notification. Therefore, there is still space for some laboratories to improve the quality of critical values reporting.

| Group | Number of labs % (N/ total) | Percentages of critical values notification | | | | Percentages of timely critical values notification | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean (%) | Median ($P_5$, $P_{95}$) (%) | Mean (σ) | Median ($P_5$, $P_{95}$) (σ) | Mean (%) | Median ($P_5$, $P_{95}$) (%) | Mean(σ) | Median ($P_5$, $P_{95}$) (σ) |
| **Un-grouped** | 100% (5265/5265) | 99.04 | 100.00 (97.41, 100.00) | 5.77 | 6.00 (3.45, 6.00) | 98.84 | 100.00 (94.03, 100.00) | 5.60 | 6.00 (3.06, 6.00) |
| **Beds that hospitals occupy** | | | | | | | | | |
| 0-500 | 67.3% (3542/5265) | 99.10 | 100.0 (98.46, 100.00) | 5.81 | 6.00 (3.66, 6.00) | 98.89 | 100.00 (94.32, 100.00) | 5.69 | 6.00 (3.08, 6.00) |
| 501-1000 | 22.2% (1169/5265) | 99.08 | 100.0 (96.51, 100.00) | 5.70 | 6.00 (3.31, 6.00) | 98.89 | 100.00 (94.14, 100.00) | 5.47 | 6.00 (3.06, 6.00) |
| 1001-1500 | 6.6% (350/5265) | 98.66 | 100.0 (93.33, 100.00) | 5.60 | 6.00 (3.00, 6.00) | 98.79 | 100.00 (94.63, 100.00) | 5.37 | 6.00 (3.11, 6.00) |
| 1501-2000 | 2.3% (120/5265) | 98.39 | 100.0 (92.37, 100.00) | 5.57 | 6.00 (2.93, 6.00) | 97.95 | 100.00 (86.25, 100.00) | 5.11 | 6.00 (2.59, 6.00) |
| More than 2000 | 1.6% (84/5265) | 98.38 | 100.0 (92.81, 100.00) | 5.54 | 6.00 (2.98, 6.00) | 97.73 | 100.00 (90.28, 100.00) | 5.05 | 6.00 (2.80, 6.00) |
| **Information system** | | | | | | | | | |
| Both LIS and HIS were employed | 71.0% (3735/5265) | 99.05 | 100.0 (96.86, 100.00) | 5.74 | 6.00 (3.36, 6.00) | 98.91 | 100.0 (94.28, 100.00) | 5.56 | 6.00 (3.08, 6.00) |
| Only LIS was employed | 7.4% (390/5265) | 99.19 | 100.0 (98.82, 100.00) | 5.81 | 6.00 (3.77, 6.00) | 98.77 | 100.0 (93.74, 100.00) | 5.65 | 6.00 (3.03, 6.00) |
| Only HIS was employed | 9.0% (473/5265) | 99.20 | 100.0 (100.00, 100.00) | 5.85 | 6.00 (6.00, 6.00) | 99.13 | 100.0 (94.32, 100.00) | 5.74 | 6.00 (3.08, 6.00) |
| Neither LIS nor HIS were employed | 12.6% (666/5265) | 98.76 | 100.0 (97.35, 100.00) | 5.80 | 6.00 (3.44, 6.00) | 98.31 | 100.0 (89.62, 100.00) | 5.67 | 6.00 (2.76, 6.00) |

## B-170

### National Survey on Pre-examination and Intra-laboratory Turnaround Time of Biochemical Items of 5975 Laboratories in China

Y. Huang, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, Beijing, China*

**Background:** The pre-examination and intra-laboratory turnaround time are important indicators of timeliness of samples delivery and testing efficiency. The aim of this survey was to investigate the status quo of pre-examination and intra-laboratory turnaround time (TAT) of two biochemical items in China and to improve the quality of TAT.

**Methods:** The national survey of biochemical TAT was performed in 31 clinical laboratory centers in 2016.Results were collected via Clinet-EQA reporting system established by National Center for Clinical Laboratories (NCCL) in China, including two items, serum kalium and cTnI or cTnT. All participants were asked to submit general information, the median and the 90th percentile of pre-examination and intra-laboratory TATs of clinic, hospitalized and stat tests in April 2016 via online questionnaire.

**Results:** Totally 5975 clinical laboratories in China submitted the results of pre-examination and intra-laboratory TATs of biochemical items. The survey included two items, serum kalium and cTnI or cTnT, and three test types, clinic, hospitalized and stat tests. The analysis of medians of pre-examination and intra-laboratory TATs was shown in table below. The distribution of 90th of pre-examination and intra-laboratory TATs among three test types were similar. The 90th TAT of stat tests was lower than clinic and hospitalized tests, no matter which item, both pre-examination and intra-laboratory TATs. The 25th percentile, median, and the 75th percentile of median TATs were calculated as optimum, desirable and minimum quality specifications as shown in table below.

**Conclusions:** Thank every provincial clinical laboratory center for participating in our survey. Remarkable differences were found among different test types of biochemical items in the development and implementation of TATs. Much attention should be paid to improve the TATs of laboratories. To guarantee timely and long-time monitoring, laboratories should submit more biochemical items' data and set goals for different items and test types, respectively.

| | Subject | Items | Test type | Number of laboratories | Average | P5 | P25 | Median | P75 | P95 |
|---|---|---|---|---|---|---|---|---|---|---|
| Pre-examination TATs | Biochemistry | serum kalium | Clinic | 6260 | 33.80 | 2.00 | 10.00 | 22.00 | 36.00 | 90.00 |
| | | | Hospitalized | 6282 | 58.35 | 5.00 | 25.00 | 40.00 | 60.00 | 120.00 |
| | | | Stat | 6267 | 24.13 | 3.00 | 10.00 | 20.00 | 30.00 | 60.00 |
| | | cTnI or cTnT | Clinic | 5417 | 32.40 | 1.00 | 10.00 | 20.00 | 30.00 | 66.00 |
| | | | Hospitalized | 5439 | 52.84 | 1.00 | 20.00 | 30.00 | 60.00 | 120.00 |
| | | | Stat | 5521 | 26.23 | 1.00 | 10.00 | 20.00 | 30.00 | 60.00 |
| Intra-laboratory TATs | | serum kalium | Clinic | 6351 | 67.02 | 5.00 | 30.00 | 45.00 | 80.00 | 180.00 |
| | | | Hospitalized | 6413 | 84.31 | 5.00 | 30.00 | 60.00 | 120.00 | 240.00 |
| | | | Stat | 6381 | 35.50 | 5.00 | 20.00 | 30.00 | 40.00 | 70.00 |
| | | cTnI or cTnT | Clinic | 5492 | 57.37 | 1.00 | 20.00 | 35.00 | 60.00 | 150.00 |
| | | | Hospitalized | 5591 | 73.02 | 1.00 | 30.00 | 50.00 | 90.00 | 210.00 |
| | | | Stat | 5621 | 38.73 | 1.00 | 20.00 | 30.00 | 45.00 | 80.00 |

## B-171

### Summary and Analysis of Unqualified CV Rate of IQC Items and Unqualified Rate of EQA Items in Clinical Laboratories in China

M. Duan, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, P. R., Beijing, China*

**Background:** The aim of this study was to investigate the unqualified CV rate of IQC items and unqualified rate of EQA items in clinical laboratories in China and make a comparison among hospitals with different grades to summarize the quality control condition of these two indicators.

**Methods:** A survey on 15 quality indicators published by National Health and Family Planning Commission was performed in the national wide in 2016. Data of the unqualified CV rate of IQC items and unqualified rate of EQA items and relative basic information were collected via Clinet-EQA reporting system established by National Center for Clinical Laboratories. The results were evaluated with percentages and sigma scales (σ).

**Results:** 6834 laboratories submitted the survey data about the unqualified CV rate of IQC items and 6694 laboratories finished the data about the unqualified rate of EQA items. Unqualified CV rate of IQC items in most laboratories were 0-10% (72.10%) and 10-20%(13.26%). The sigma level mainly focused on 6(38.13%), 2-3 (26.56%) and 3-4 (23.98%). The unqualified rate of EQA items had a similar result with the unqualified CV rate of IQC items. Most of laboratories of which were 0-5% (69.09%) and 5-10%(14.89%). The sigma level mainly focused on 6(41.40%), 3-4(33.36%) and 2-3(19.50%). The median of the unqualified CV rate of IQC items and unqualified rate of EQA items in hospitals of third class A and third class B were 2.35%,2.62%, and 1.43%,1.85%. As for second class A and second class B, it was 4.86%,5% and 2.69%,2.5%.

**Conclusion:** The overall quality control of unqualified CV rate of IQC items and unqualified rate of EQA items in China is satisfactory, but there are differences among hospitals with different grades. Therefore, Laboratories, especially those with lower grades, should reinforce the management and control of the testing items to improve the performance of these two quality indicators.

## B-172

### Investigation and Analysis of the Imprecision of Five Years' Point-of-Care Glucose Meter in Clinical Laboratories in China

M. Duan, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, P. R., Beijing, China*

**Background: :**With the wide application of Point-of-Care(POC) glucose meters all over China, the accurate measurement of blood glucose with POC glucose meters seems more and more essential for correct treatment decisions for glycemic control. The primary objective of this survey is to investigate the imprecision of internal quality control of five years' POC glucose meters from 2012 to 2016, so as to have a general understanding of its imprecision level in laboratories in China.

**Methods:** Internal quality control(IQC) programs for POC glucose meter was organized by NCCL in china from 2012 to 2016, respectively. Data had been collected until 2016 by on-line questionnaire including cumulative coefficient of variation(CV) and other related information. Microsoft Excel 2007 and SPSS had been used to analyze the data. The percentage of laboratories meeting quality requirement were evaluated based on 1/3 TEa(6.67%) and 1/4 TEa(5.00%) defined by National Center for Clinical Laboratories in China.

**Results:** There were 143,86,411,179 and 580 laboratories which submitted the data from 2012 to 2016, respectively. They were divided into five groups according to the year, which as shown in table below. The majority of participant laboratories obtain a satisfied CV when 1/3 TEa were used in all five years. The percentages of laboratories meeting quality requirements increased gradually from 2012 to 2016 no matter 1/3 TEa or 1/4 TEa was used.

**Conclusions:** The imprecision of POC glucose meter in China has been improved gradually from 2012 to 2016. The majority of participant laboratories satisfied the evaluation criteria of 1/3 TEa and 1/4 TEa, but it's still necessary for Laboratories to strengthen internal quality control and improve the detection quality level.

| Item | Years | The number of laboratories | Cumulative CVs | | Percentages of laboratories meeting quality requirements | |
|---|---|---|---|---|---|---|
| | | | Median | IQR | 1/3TEa (%) | 1/4TEa (%) |
| Point-of-Care Glucose Meter | 2012 | 143 | 4 | 3.32 | 79.72 (114/143) | 64.34 (92/143) |
| | 2013 | 86 | 3.98 | 3.59 | 81.40 (70/86) | 66.28 (57/86) |
| | 2014 | 411 | 4.2 | 2.56 | 84.18 (346/411) | 69.10 (284/411) |
| | 2015 | 179 | 4.02 | 2.68 | 84.92 (152/179) | 71.51 (128/179) |
| | 2016 | 580 | 4.02 | 2.38 | 86.21 (509/580) | 73.10 (424/580) |

## B-173

### Comprehensive evaluation of the Internal and External Quality Control Framework annual performance.

M. Fornella, E. Olivera, G. Pacheco, B. Varela, M. Zubillaga. *SIEMBRASUR, Montevideo, Uruguay*

**Background:** Pursuant to the Standard UNIT ISO 15189:2012 "Medical laboratories — Requirements for quality and competence" the direction of the laboratory must annually revise the performance of the External Quality Assurance Services (EQAS) and of the Internal Quality. The aim of this work is to design a tool to make the annual evaluation of the analytical performance of the tests easier for the Direction and to allow a revision of the used Total Allowable Error (ETa), integrating concepts of ETa, bias and sigma metric. **Methods:** The results of twelve surveys of the External Quality Evaluation Program EQAS® BIO-RAD from 2016 were used to evaluate the performance of twenty-four biochemistry tests. The evaluated assays were processed in a homogenous system, analytical platform Architect ci8200 and Abbott manufactured reactive. The performance indicator estimated through EQAS was the bias expressed as the percentage of the ETa [Bias (%ETa)]. The integration of results of the Internal Quality Control System evaluation was represented by the annual sigma metric, which was estimated for each test from the monthly calculation of the performance of the limiting control level, using as reference the results of the inter-laboratory comparison program (BIO-RAD) from 2016. Sigma metric was charted as a function of the Bias $(\%ETa)_{2016}$ and of the relationship Bias $(\%ETa)_{2016}$ / Bias $(\%ETa)_{2015}$. This graph made possible the integration of the performance of the internal and external quality control evaluation programs as well as the evolution of the EQAS performance throughout time. A test performance was considered satisfactory when the Bias $(\%ETa)_{2016}$ was lower than 50% and the sigma higher than 5.15. It was defined that all tests with Bias $(\%ETa)_{2016}$ higher than 25% and a relationship Bias $(\%ETa)_{2016}$ / Bias $(\%ETa)_{2015}$ higher than 1.5 and lower or 0.5 would be evaluated to monitor the evolution of performance. Two areas were identified in the graph for which a revision of the ETa was needed, one defined for an ETa [Bias $(\%ETa)_{2016}$] lower than 25% and a sigma higher than 12 and the other one defined for an ETa [Bias $(\%ETa)_{2016}$] higher than 50% and a sigma lower than 5.15. **Results:** The performance of the 24 evaluated biochemistry tests was satisfactory. No cases showed significant performance modification from one year to the following one. In eight tests a revision of the ETa was needed to evaluate the possibility of reducing it, no tests needed an increase in the ETa. **Conclusion:** The tool allowed for a way to simply and graphically summarize the annual performance of the analytical procedures. The means used for presentation were effective for the communication of the analytical performance to the Laboratory Direction and to carry out a revision of the ETa.

## B-174

### Serum uric acid laboratory test in primary care: a high requested inexpensive test with potentially costly adverse effects

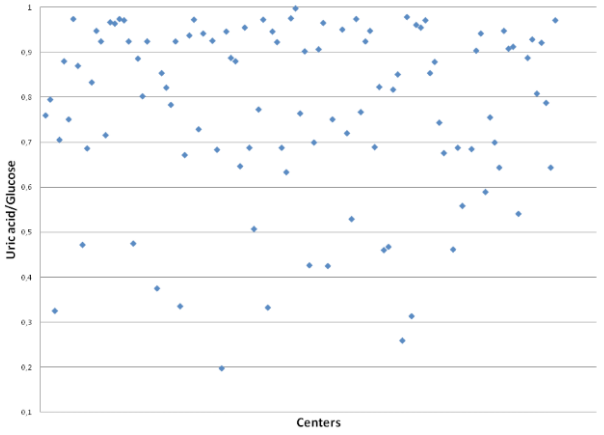
M. Salinas[1], M. López-Garrigós[1], E. Flores[1], Á. Giménez-Marín[2], I. Herrera-Contreras[3], E. Roldán-Fontana[4], L. Rabadán[5], A. Buño[6], M. C. Gallego-Ramírez[7], M. V. Poncela-Garcia[8], M. Ortuño[9], L. F. Colomo[10], V. Villamandos[11], M. C. Plata[12], A. Tapia[13], M. E. Sole-Llop[14], A. Miralles[15], M. A. Rodríguez-Rodríguez[16], L. Máiz-Suárez[17], C. Leiva-Salinas[18]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]Hospital de Antequera, Antequera, Spain, [3]Complejo Hospitalario de Jaen, Jaen, Spain, [4]Hospital La Merced, AGS Osuna, Osuna, Spain, [5]Complejo Asistencial de Soria, Soria, Spain, [6]Hospital Universitario La Paz, Madrid, Spain, [7]Hospital Rafael Mendez, Lorca, Spain, [8]Hospital Universitario de Burgos, Burgos, Spain, [9]Hospital Universitario La Ribera, Alzira, Spain, [10]Hospital de Laredo, Laredo, Spain, [11]Hospital Santos Reyes, Aranda del Duero, Spain, [12]Hospital Campo Arañuelo, Navalmoral de la Mata, Spain, [13]Hospital de Barbastro, Barbastro, Spain, [14]Hospital de Alcañiz, Alcañiz, Spain, [15]Hospital de Sagunto, Sagunto, Spain, [16]Complejo Asistencial Universitario de Palencia (Hospital Rio Carrion), Palencia, Spain, [17]Hospital Lucus Augusti, Lugo, Spain, [18]University of Virginia, Charlottesville, VA*

**Background:** The power of every diagnostic procedure will be closely conditioned to the clinician's use of it. The aim is to study the request of uric acid (UA) from primary care, regional variability, root causes of requesting patterns and potential demand inappropriateness.

**Methods:** A cross-sectional study was designed and conducted at a main core laboratory. Spanish laboratories were invited to report their number of serum glucose and UA tests for year 2014, and their organizational data. A survey was sent to every participant regarding UA inclusion in profiles. The request of UA per 1000 inhabitants and ratio of UA to glucose demand (UA/glucose) were calculated and compared between the different regions, and between laboratories where UA was included or not in "health check profile".

**Results:** 110 laboratories participated (59.8% Spanish population). The overall request of UA per 1000 inhabitants was 296.3 (CI95%: 272.7-312.8). The median UA/glucose ratio was 0.82 (IQR: 0.25); 41 out of the 110 participants had a value above 0.9 (Figure). There was a significant high regional variability for both indicators ($P \leq 0.05$). Laboratories where UA was not included in the "health check profile" had lower results for both indicators ($P \leq 0.05$).

**Conclusion:** There was a high regional variability and overall inappropriate over request for UA in primary care. Inclusion of the test in "health check profile" was probably the main cause behind the observed misuse.



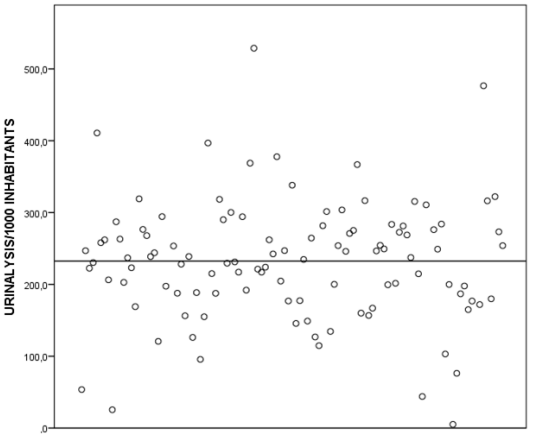

## B-175

### Request pattern, preanalytical and analytical variability and economic costs of urinalysis in primary care

M. Salinas[1], E. Flores[1], M. Lopez-Garrigos[1], C. Mar-Medina[2], M. Herranz-Puebla[3], F. Aguayo-Gredilla[4], E. Prada de Medio[5], F. Gascón[6], P. Garcia-Chico[7], S. Pesudo[8], L. García-Menéndez[9], P. Esteve[10], M. C. Martin-Fernández de Basoa[11], J. C. Garrido[12], J. Domínguez[13], M. D. Calvo-Nieves[14], A. Pérez-Fuentes[15], R. Guillen-Santos[16], F. Cava[16], C. Leiva-Salinas[17]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]Hospital Galdakao, Usansolo, Spain, [3]Hospital General Universitario Gregorio Marañon, Madrid, Spain, [4]Hospital Universitario de Basurto, Bilbao, Spain, [5]Hospital Virgen de la Luz, Cuenca, Spain, [6]Hospital Valle de los Pedroches, Pozoblanco, Spain, [7]Hospital General Universitario de Ciudad Real, Ciudad Real, Spain, [8]Hospital La Plana, Villareal, Spain, [9]Hospital El Bierzo, Ponferrada, Spain, [10]Hospital Ernest Lluch, Calatayud, Spain, [11]Hospital Nuestra Señora de la Candelaria, Tenerife, Spain, [12]Hospital Universitario Marques de Valdecilla, Santander, Spain, [13]Hospital Universitario de Guadalajara, Guadalajara, Spain, [14]Hospital Clínico Universitario de Valladolid, Valladolid, Spain, [15]Hospital Arquitecto Marcide, El Ferrol, Spain, [16]BR Salud, Madrid, Spain, [17]University of Virginia, Charlottesville, VA*

**Background:** The aim was to study the requesting pattern of urinalysis by General Practitioners (GPs), its potential regional variability, and the pre-analytical conditions and economic cost regarding sample collection and analysis. **Methods:** Participating laboratories across Spain reported the number of urinalysis performed in year 2014. We calculated the number of requests per 1000 inhabitants, and the regional differences between autonomous communities (AACCs). A survey was sent to participants regarding pre-analytical sample collection and transport, procedure of analysis, and strip reagent cost.

**Results:** 110 laboratories from 15 AACCs participated in the study. On average 232.5 urinalysis were requested per 1000 inhabitants. Figure displays the number of urinalysis requested per 1000 inhabitants in each laboratory. There were no statistically significant differences between AACCs. The strip cost in our patient cohort for year 2014 was 2526404.4€. There was a 63% in survey participation. It showed that most laboratories used the first morning urine, most frequently collected at home and delivered to a primary care center (PCC), and transported to the laboratory 2 to 4 hours later. Most laboratories combined initial test strip analysis followed by particle analysis based on automatic decision algorithms. The rate of particle analysis varied between laboratories. **Conclusion:** There is a variability in the request of urinalysis by GPs, no differences between regions and when included or not in Health Check Profile. Also we observed an overall lack of compliance with the time between micturition and analysis. Our results suggest the need for efforts to improve pre-analytical conditions and to standardize and systematize the algorithms for particle analysis.




## B-176

### U.S Clinical Laboratories in the Era of the Clinical Laboratory Improvement Amendments (CLIA)

l. fan, y. xia, r. astles, t. taylor, m. earley. *CDC, ATLANTA, GA*

**Background:** To describe the dynamics of the U.S. clinical laboratory system and investigate the characteristics associated with active clinical laboratories.

**Methods:** Using the Centers for Medicare & Medicaid Services CLIA database, we described the dynamics of clinical laboratory history since the inception of CLIA. We compared the characteristics of currently active laboratories vs those no longer active. We calculated lifespans of laboratories, and performed Cox regression to determine the factors associated with active laboratories.

**Results:** Since 1992, there have been 488,829 clinical laboratories ever operating. Among them, 257,621 (52.7%) were still active at the end of 2016. CA, FL, and TX each had more than 20,000 active laboratories; on per capita basis, FL, IA, SD, NE, and WV had more than 10 active laboratories per 10,000 population while CA, NV, NY, and WA had less than 6 active laboratories per 10,000 population. The number of active laboratories has been increasing since 1995. The net annual increase has been between2-6%. Based on self-designated laboratory type, the numbers of physician office laboratories increased the most (84,861 to 122,576). Based on certificate type, numbers of laboratories with certificates of waiver (CoW) increased the most (78,122 to 185,717). Among the 50 States, NE, ND, and SD had the highest proportions of laboratories that remained active (>65%) while CA, DC, DE, LA, ME, NV, NY, OK, and RI had less than 50% laboratories that remained active. When categorized by ownership, government laboratories (federal, state, county, city and other) had a higher proportion of laboratories that remained active (63.4%%) than non-government laboratories (54.8%). When categorized by laboratory types, 63.7% and 48.2% of hospital and physician office laboratories remained active respectively. When categorized by certificate, 64.0% of laboratories with certificates of accreditation (CoA) and 57.0% with CoW remained active. Laboratories that were no longer active had a mean lifespan of 6.6 years versus 12.3 years for laboratories that remained active (December 31, 2016 as cutoff date). For laboratories that were no longer active, the lifespans for government laboratories and non-government laboratories were 7.0 vs 6.6 years; CoA, certificate of compliance (CoC), certificate of PPM (PPM) and CoW laboratory lifespans were 9.4, 8.0, 8.7 and 6.5 years, respectively. Skilled nursing-home laboratories, public health laboratories, and hospital laboratories had the longest lifespans, while home health-agency laboratories, independent laboratories, and pharmacy laboratories had the shortest lifespans. In Cox regression analysis, government laboratories were more likely to remain active compared to non-government laboratories. CoA and PPM laboratories were more likely to remain active compared to CoW. Hospital laboratories, skilled nursing-home laboratories, ambulatory surgery center laboratories, and end stage renal disease dialysis laboratories were more likely to remain active compared to physician office laboratories.

**Conclusion:** The U.S. clinical laboratory system is dynamic. The number of laboratories has increased since 1995particular CoW laboratories. The lifespan was 12.3 years for current active laboratories as of Dec. 31, 2016. While other factors may have been reported as impacting laboratory sustainability, this study reveals new insight in that geographic location, ownership, certificate, and laboratory type are associated with laboratories' active status.

## B-177

### Clinical Significance of Duplicate Results for Components of Chemistry Panels Ordered on Inpatients

E. W. Holmes. *Department of Pathology, Loyola University Medical Center, Maywood, IL*

**Background:** There is a consensus that as many as 30% of the laboratory tests that are ordered on hospitalized patients might be unnecessary. While the sheer frequency of excessive ordering seems to be sufficient to justify limitations on the number of panels that can be ordered per hospital day, a more compelling reason to limit replicate ordering would be to demonstrate that the differences between the test results of frequently ordered panels are not clinically significant. This study was undertaken to evaluate the analytical and clinical significance of changes over time in the individual analytes of duplicate CMP and BMP panels that were ordered on hospital inpatients.

**Methods:** I evaluated the differences between 48052 replicate test results for each of the eight components of the BMP and 13200 replicate results for each of the 6 components that are unique to the CMP in 19995 inpatient encounters (of > 24 hr.duration) that occurred between 10/1/2014 and 1/31/2015. Data were obtained from my institution's HIS, de- identified, imported into a SQL database and analyzed with a query that compared the first result for each component of the first panel that was ordered with the result obtained for each component of the second panel (i.e. the first duplicate) that was ordered. The results for the second panel were, likewise, compared with the results for the third panel ordered (i.e. the second duplicate), etc. The query returned many details about each series of replicated panels including the absolute and percent differences between the analyte values for each pair of duplicate results, whether or not the difference between duplicate results exceeded the analytical error or the critical difference for the particular analyte, or differed from the previous result relative to the reference range for the particular test.

**Results:** In the case of the differences between duplicate results for the BMP components, an average of 50.7% (min: 31.8, max; 63.4) were within the analytical error of the respective test, an average of 19.8 %(min: 12.0, max: 41.2) percent exceeded the respective critical difference for a serially monitored test, and an average of 14.9 % (min: 7.5, max: 22.5) of the duplicates changed relative to the reference limits for the respective test. In the case of the duplicated results for the CMP components, an average of 61.0 % (min: 42.9, max: 88.8) were within the analytical error of the test, 13.5 % (min: 2.9, max: 24.8) exceeded the critical difference; and 9.5 % (min: 6.4, max: 14.7) changed relative to the reference limits for the test.

**Conclusion:** My data indicates that the majority of changes in the results of the BMP and CMP panels, as revealed by replicate testing within a 24-hour period, were not clinically significant. The time intervals between replicate panel orders suggests that most of the testing during the study period was driven by standing orders in the HIS rather than by changes in the clinical status of the patients.

## B-178

### Innovative use of Sunquest Blind Duplicate function for expanded quality control

J. Baker[1], K. Colburn[1], L. Steier[1], P. Dolph[1], C. Vilk[1], K. Congo[1], J. Watson[2]. *[1]MultiCare Health System, Tacoma, WA, [2]Sunquest Information Systems, Tucson, AZ*

Background:

"Blind duplicates" provide a quality control process for using duplicate patient specimens to enhance additional quality control. Sunquest Laboratory™ has a blind duplicates function (BDUP) that allows Multicare Health System (Multicare) to easily check one instrument against another using established criteria to evaluate variations in actual patient results obtained and then graph them on a Levy-Jennings plot. We perform this daily at sites with two or more instruments and monthly between all additional sites to help validate that each instrument is turning out correlated patient results.

Multicare uses this function to both test similar instruments (i.e, Abbott Architects ci8200, ci4100) and check the analyte on different instruments. For example, daily we run BDUP patient samples on the Abbott Architect for five analytes and correlate this with four NOVA Stat Profile CCX instruments. This helps monitor the correlation of analyte results with other instruments and methodologies on a more frequent basis, instead of every six months, and removes the need to perform linear regression correlations. This frequent monitoring uses a software function to plot the values. The Sunquest BDUP results are then monitored using quality control rules and rule failure documentation like standard quality control materials.

Methods:

In all cases, we select an analyte and a primary instrument. We define the BDUP mean as zero and identify a standard deviation that is acceptable or targeted. The specimen is run on the primary instrument and the results are accepted in Sunquest. The same specimen is analyzed on different instruments and Sunquest will plot the variance on a Levy –Jennings plot. For example, the BDUP potassium QC mean is set to zero and the standard deviation is defined (we use 0.15 mmol/L – for one standard deviation). The specimen is analyzed on the primary instrument and a potassium value of 3.5 mmol/L is obtained. The same specimen is analyzed on a different instrument with a result of 3.7 mmol/L. Sunquest BDUP will plot this difference for Potassium BDUP as +0.2 mmol/L (which is within 2 standard deviations). Sunquest quality control flagging occurs so we can view results on a daily or weekly report and supporting graph.

Results:

Multicare has 11 Abbott Architects located at six laboratories and 18 Sysmex hematology instruments in 13 laboratories. Monthly: we run 25 chemistry and 19 hematology assays using three to four specimens on primary instruments in the core lab and compare to all other instruments. Daily: BDUP function is performed on 16 chemistry analytes every eight hours for labs with two or more analyzers.

Conclusion:

The BDUP quality control strategy is a fully automated report from Sunquest. We utilize our couriers to transport the monthly specimens to other laboratories. These comparisons allow us to identify shifts due to reagent lots, instrument and assay errors, and validate on a daily, weekly or monthly basis that all of our instruments are comparable and reporting consistent correlated patient results.

## B-179

### Reducing Unnecessary Bilirubin Measurements

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

**Introduction:** Serum index measurements, including icterus index (IC) measurement, are routinely performed on all clinical specimens in many laboratories. This studies examines whether IC values can predict hyperbilirubinaemia and can potentially be used to avoid unnecessary total bilirubin measurements. **Methods:** In our laboratory, serum index measurements are automatically performed on all samples analysed on the 3 Beckman Coulter DxC-800 clinical chemistry analysers. Anonymised details of all simultaneous IC and total bilirubin (diazo, manufacturer supplied reagent) measurements for 6 months (Jan to June 2016) were extracted from the laboratory database. The locally derived total bilirubin reference interval is 7-31 umol/L. Excel 2003 and Analyse-it were used to prepare a ROC curve to assess the ability of IC to predict TBil > 31 umol/L. **Results:** There were 51111 paired IC and total bilirubin results. In 5562 (10.9%) cases, total bilirubin > 31 umol/L. The sensitivity and specificity, with 95% CIs, for IC to predict total bilirubin > 31 umol/L were: for IC >0 (i.e >=1): sensitivity 1.000 (0.999-1.000), specificity 0.004 (0.003-0.005); IC >1 (i.e. >=2): sensitivity 0.992 (0.989-0.994), specificity 0.753 (0.749-0.757). Using an IC>0, 183 tests are avoided (at a cost of 2 false negatives) and IC>1, 34345 tests are avoided (at a cost of 47 false negatives). **Conclusion:** Withholding total bilirubin measurement on specimens with icterus index (IC) of 0 or 1 would reduce total bilirubin test volumes by 67% with only a 0.08% false negative rate. The false negative rate could potentially be reduced further on analytical systems reporting smaller IC increments. The logistics of performing IC measurement prior to initiating bilirubin measurement would require close collaboration with diagnostic manufacturers and middleware/ laboratory information system vendors.

## B-180

### Spermal quality for fertility studies in a health area

D. González Benito, R. Escobar Conesa, V. García Moreira, A. Llorente Torres, E. Fernández Rodríguez. *Hospital Universitario de Cabueñes, Gijón, Spain*

Background: Spermiogram are useful for the diagnosis of male sterility. Over the years, semen quality has deteriorated mainly due to environmental factors and life styles, which has caused that millions of partners are affected by fertility problems. The aim of this study is to analyze sperm quality of our received in our laboratory over the last three years (April 2013-March 2016) according to the latest recommendations of the World Health Organization (WHO).

Methods: 754 spermiograms were conducted following the recommendations of the WHO in 2010 and they were analyzed following these parameters: volume, sperm

count, motility, vitality and morphology. For the sperm count (million of sperm by milliliter) the Neubauer improved chamber was used, to analyze the motility, we employed the Makler chamber, for the vitality, slide extensions with Eosin-Nigrosin dye, and for the morphology also slide extension with Diff-Quick.

Results: From the examined samples, 38.59% (291) were Normozoospermic, whereas, 14.19% were Hipospermic (<1.5 ml), with a median volume of 2.80 ml and a percentile 5 of 0.9 ml. Concerning the count sperm, the median was 39 million of sperm by ml, the percentile was between 5 of 4.6 million by ml and 20.82% of the samples were Oligozoospermic (<15 million / ml). In regard to the sperm progressive motility, the median percentage was 38% with a 5 percentile in 10%, suffering 36.34% of patients from Asthenozoospermia (<32%). In the vitality study, the median percentage of livie sperm was 83% and 58% were placed in the 5 percentile, ranking the Necrozoospermia (<58%) in just 4.77%. Finally, thenormal morphology sperm median stood at 5% with a 1% placed in the 5 percentile and Teratozoospermia was in 31.70% (<4%).

in the same simple, according to the deficit of several parameters, 13.62% suffered from Oligoastenozoospermia, while 8.09% were affected by Oligoastenoteratozoospermia.

Conclusions: Over all the studied samples, 38.59% met the new WHO normality principles. The remained, failed to meet one or more parameters. The most frequent sperm abnormality was the motility (36.34%), followed by the morphology (31.70%). On the other hand, the less common abnormality was the alteration in the vitality (5.57%).

## B-181

### Project management tools for implementation of instruments across a multi-site academic medical center

M. R. Peddicord, R. Kerlin, J. L. Phillips, B. Fenske. *The Ohio State University Wexner Medical Center, Columbus,, OH*

**Background:** The decision to change hematology platforms with new analyzers presented the issue of how to streamline the process of installing and validating thirteen instruments in five separate labs. A structured process required preliminary meetings with the hematology director. Project co-chairs were determined and a shared network folder was established. Participants in the implementation would include staff at varied levels of depth including directors (both clinical and administrative), managers, lead technologists and key operators. Clinical Laboratory Improvement Amendment (CLIA) and College of American Pathologist (CAP) guidelines would be followed for the instrument method validation.

**Methods:** Microsoft OneNote was utilized for the initial planning which included stakeholders, timelines, renovations, water system installations, expense tracking, and training resources. Managers were able to add individual lab information which could be viewed by all project members. Weekly meetings were established with the vendor project coordinator as well as internal project team meetings. A question and answer spreadsheet was created to address and follow-up with concerns. A shared network drive included specified folders for method validation subsets (accuracy, precision, carryover, linearity, correlations, raw data, differential flagging, mixing studies, reference range and stain validation), meeting minutes, safety, instrument specifications and vendor white papers. A method validation checklist was used to ensure all components were completed as required by CLIA. A task list and associated checkoff spreadsheet were created with targeted due dates. The checkoff included varied tasks from method validation to asset tracking to monitor each lab's progress. Staff training was accomplished using multiple methods: a key or lead tech spent one week with the clinical applications specialist, all staff were required to complete e-learning modules prior to using the instrument, and a virtual classroom was offered as an optional training setting.

**Results:** The implementation process required logical and strict timelines to complete the project. Staff were assigned to e-learning in segments to align education with implementation stages (overview, instrumentation, QC and software). Once instruments were installed and clinical applications completed, the raw data was evaluated by the division director. During this timeframe, the Laboratory Information Systems (LIS) department partnered with the vendor to develop and test the middleware application. Procedure writing, mixing studies, reference range validation, reagent purchasing application, and safety requirements were completed. One global procedure was instituted versus five individual procedures. Final testing with specimens led to the unified go-live date.

**Conclusion:** The implementation process required engagement by project members at weekly web conference meetings. Using OneNote facilitated a global overview of the project with each lab able to see the progress. However, due to most staff being unfamiliar with OneNote, this could be easily manipulated with the potential for data loss. Structured folders were used for storing and evaluating data to secure information. The project required a degree of trust that all components were being addressed, but needed to take place in an organized fashion. A participant survey was conducted after the live date to gauge the overall process. The project was effective and efficient utilizing the management tools with aligned communications.

## B-182

### Correlating Clinical Laboratory Characteristics to Proficiency Testing Misuses and Good Laboratory Practices

M. C. Earley, L. Fan, J. R. Astles. *Centers for Disease Control and Prevention, Atlanta, GA*

**Background:** In 2013, the Centers for Disease Control and Prevention and the Association of Public Health Laboratories implemented a national survey about proficiency testing (PT) use and scored responses as good laboratory practices (GLP) or PT misuse [not allowed by the Clinical Laboratory Improvement Amendments (CLIA)]. Using statistical analysis, we investigated if specific laboratory characteristics correlated with PT GLPs or PT misuses.

**Methods:** The dependent variables were summary scores calculated from 10 PT GLP and 6 PT misuse questions. Higher GLP scores indicated better laboratory PT practices and lower PT misuse scores denoted less PT misuse. Descriptive statistical analysis characterized laboratories using laboratory certification, laboratory type, size by annual test volume, and rural/urban status according to Rural Urban Commuting Area codes (RUCA) as the independent variables. ANOVA analyses compared scores among laboratories with different attributes and multivariate linear regression identified independent factors associated with summary scores. The F test was used to examine if each independent variable was significant as a group because the independent variables were categorical variables.

**Results:** Among the 769 laboratories that responded to the survey, 309 (40.2%) laboratories were CLIA Certificate of Compliance (CoC) laboratories and 460 (59.8%) were under Certificate of Accreditation (CoA). There were 486 (63.2%) laboratories with annual test volumes of less than 300,000; 116 (15.1%) with volumes between 300,000 and 700,000; and 167 (21.7%) with volumes of more than 700,000. There were 468 (60.9%) laboratories in urban areas, 111 (14.4%) in large rural areas, 107 (13.9%) in small rural areas, and 75 (9.8%) in isolated rural areas. The mean score for PT GLPs was 5.6 (range 0-10). ANOVA analyses indicated differences in scores were statistically significant for laboratory certification, laboratory type, size, and RUCA classification. Multivariate linear regression results revealed statistically significant differences between CLIA CoC laboratories and CoA Laboratories. Compared to physician office laboratories (POLs), all other laboratory types had statistically significant higher GLP scores. Medium and large laboratories had statistically significant higher GLP scores compared with small laboratories. Compared to laboratories in urban areas, laboratories in small rural areas had statistically significant higher GLP score; however, rural /urban locations were not statistically significant as a group. The mean score for PT misuse was 0.4 (range 0-6). Most laboratories (552; 71.8%) had no PT misuse. Compared to (POLs), only hospital laboratories had statistically significant lower misuse score. Compared to laboratories in urban areas, laboratories in large rural areas had statistically significant lower misuse score. These differences were statistically significant at the p= 0.1 level. Laboratory certification and size were not statistically significant.

**Conclusion:** The results show that most responding laboratories did not misuse PT and suggest that medium and large laboratories tend to have higher PT GLP scores as do non-POLs. Laboratory size and certification did not correlate with PT misuse scores. The results can be used to identify strategies to target laboratories for efforts to reduce PT misuses and promote PT GLPs.

## B-183

### Impact of targeted test utilization strategy for Vitamin D testing

U. Uddayasankar[1], A. A. H. Sokoro[2]. *[1]University of Manitoba, Winnipeg, MB, Canada, [2]Diagnostic Services Manitoba, Winnipeg, MB, Canada*

**Background and objective:** Optimizing test utilization is an important responsibility of Clinical laboratories. Of particular interest is Vitamin D testing. Manitoba, Canada, has observed a significant increase in the number of Vitamin D tests ordered over the past few years, creating a significant backlog in its testing. To improve test utilization, a strategy for the optimization of Vitamin D testing was implemented in 2016. This included the use of a care-giver signed test-specific requisition and eight approved indications for which testing is allowed. The objective of this study is to evaluate utilization profiles of vitamin D testing post restricted ordering strategy. Specifically, the study aims to analyze the specialties ordering this test and the clinical indications.

**Methods and results:** A sample of 1500 requisitions covering a period of 2 months (Aug 2016 and Oct 2016) were manually screened to obtain information on the patients age and sex, the clinical indication for ordering the test, and the medical specialty of the ordering physician. After the implementation of the test-specific requisition, a significant change was observed with regards to the medical specially ordering the highest number of tests. Before test-specific requisition implementation, test requests made by family physicians made up 70 % of all requisitions, while after, family medicine only accounted for 44 % of the tests ordered. This is indicative of a more targeted and relevant use of Vitamin D testing. Analysis of the test-specific requisitions also demonstrated that the top two clinical indications for ordering Vitamin D testing are malabsorption syndromes (40 % of all Vitamin D tests) and suspicion of metabolic bone disease (30 % of all Vitamin D tests). Furthermore, greater than 75% of tests ordered by a particular specialty were for indications directly relevant to that specialty. For example, 88 % of all tests ordered by rehabilitation medicine corresponded to a clinical indication of metabolic bone disease. **Conclusions:** The introduction of a test-specific requisition for restricted clinical indications to improve test utilization for Vitamin D has proven to be a success. The strategy has enabled targeted vitamin D testing where needed. Information gathered from this study may help to further refine test optimization strategies, not only for Vitamin D, but for many other analytes that are currently plagued with over utilization.

## B-184

### Use of the Westgard Sigma Approach to Plan and Evaluate QC Rule Management in a New Automated Core Testing Line in an Integrated Regional Healthcare System

H. H. Harrison, E. Yu, J. Olson, M. Lo, E. Croall, M. Adler, B. Kerstetter, M. Knowles-Tuchman, K. Mankins, M. Sees, M. Sneidman, J. Watkins, J. Contreras. *Geisinger Medical Laboratories, Danville, PA*

**Background:** The Geisinger Medical Laboratories support an integrated healthcare system in Central PA which includes 7 hospitals, 3 specialty clinics, 8 regional clinic laboratories and a central reference laboratory. We have annually reported on enterprise-wide use of Westgard sigma statistics and a customized QC rules approach to routine Roche chemistry, Sysmex hematology and Stago coagulation instruments. We recently reported five years experience of monthly monitoring of sigma statistics for continuous verification of instrument performance (Clin Lab Med 2017; 37:207-41). A new central laboratory building with expanded core automation was opened in late 2015, and we herein report the sigma statistics for the first full year of operation of the new chemistry automation line.

**Methods:** The new automated laboratory line consists of a Roche Cobas 8100 automated preanalytical processing line with three Add-on buffers and a p701 robotic sample refrigeration unit. Attached to the 8100 are three Cobas 8000 chemistry lines, two Stago Star Evolution coagulation units, and a Sysmex 9000 hematology line. The three Cobas line configurations are 702-502-502-602, 702-502-602, and 602-602-602-602. The Cobas 702 and 502 analyzers are chemistry and the Cobas 602 analyzers are Immunoassay instruments. Control materials were from Bio-Rad with results data transmitted to Bio-Rad Unity Real Time through Roche DI middleware.

**Results:** Sigma values were calculated, tabulated, and averaged for twelve months for the instruments and control materials listed above using the equation Sigma = (TEa-bias)/CV, where TEa was determined by CLIA or CAP PT performance requirements, and bias was determined against the Bio-Rad peer group. The data for the new line were compared to Sigma data that was previously generated on Roche instruments replaced by the new automation. In addition to identifying many world class assays, we also identified a number of assays that had sigmas that varied substantially between low and high measurement ranges. These are mainly precision effects and represent targets for analytical improvement.

**Conclusion:** The new chemistry line incorporates some 80 analytes whereas the older line had 50. Although some of the assays had lower sigmas that required standard Westgard rule implementation, the majority of assays on the new line (44 of 80) had average Sigmas in excess of 6, and QC precision well within CLIA error limits. An additional 14 assays had sigma values between 4 and 6 and, as anticipated, this level of performance allowed us to use a 1-3s or 1-4s rule on more than two-thirds of assays. Our data also showed nine common assays with sigma values substantially higher than values cited by Marques-Garcia F, et al (Revista de Calidad Asistencial 2015; 30:302-9) for the 8000 c701 in 2015, perhaps reflecting assay or instrument changes.

## B-185

### Switching from MDRD to CKD-EPI - A Singapore Hospital Experience

L. Lam, J. Lee, H. Teo. *Ng Teng Fong General Hospital, Singapore, Singapore*

**Background:** Glomerular Filtration Rate (GFR) is used in the diagnosis of chronic kidney disease (CKD) and is an independent predictor of all-cause and cardiovascular mortality and kidney failure in a wide range of populations. Clinical guidelines recommend reporting estimated GFR when serum creatinine level is measured. Although the Modification of Diet in Renal Disease(MDRD) Study equation is recommended for estimating GFR, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) has recently proposed an alternative equation, which applies different coefficients to the same 4 variables used in the MDRD Study equation (age, sex, race, and serum creatinine level). The aim of this study was to assess the agreement between kidney function as estimated by the MDRD and CKD-EPI equations.

**Methods:** We retrieved all creatinine test results performed in our laboratory using Abbott Architect enzymatic assay from 01 January to 30 September 2016. **Results:** There were 93,343 tests performed with the following demographics and presentations: Age (21 - 91, 2.5th - 97.5th percentile respectively), Sex (Male 54.7%, Female 45.3%), Presentation (Inpatient 38%, Emergency 38%, Outpatient 24%). Estimated GFR was classified into 6 categories (≥90, 60-89, 45-59, 30-44, 15-29, and ≤15 L/min/1.73m2) by both equations. Compared with our current MDRD Study equation (IDMS traceable multiplier 175), 21.2% and 10.5% of participants from our study population were reclassified to a higher and lower estimated GFR category, respectively, by the CKD-EPI equation, and the prevalence of CKD stages 3 to 5 (estimated GFR ≤60 mL/min/1.73 m2) was reduced from 30.8% to 30.2%. In estimated GFR of 45 to 59 mL/min/1.73m2 by the MDRD Study equation, 11.2% of participants were reclassified to estimated GFR of 60 to 89 mL/min/1.73m2 by the CKD-EPI equation. **Conclusion:** The CKD-EPI equation classified fewer individuals as having CKD. We recognized that this simple exercise needs validation on overall diagnostic efficiency and associated clinical outcomes. It is hoped that this switch will mostly benefit people with mildly to moderately reduced GFR but who have otherwise no evidence of kidney disease, avoiding them from unnecessarily becoming "patients with a chronic disease" and allowing nephrology resources to be more concentrated on the patients that require it.

## B-186

### Computing a Risk Management Index: Correlating a Quality Control Strategy to Patient Risk

J. C. Yundt-Pacheco, C. A. Parvin. *Bio-Rad Laboratories, Plano, TX*

**Background:**

Managing the risk of patient harm from erroneous test results has become a focus for quality control strategy design in modern laboratories. Unfortunately, many of the solutions addressing this issue lack quantitative rigor.

Using the *CLSI EP23-A: Laboratory Quality Control Based on Risk Management* risk model, we present a method for using a quality control strategy, test method performance, and test method reliability to compute the predicted probability of patient harm from erroneous test results (erroneous results have measurement error that exceeds the allowable error, $TE_a$).

The predicted probability of patent harm is compared to the acceptable level of probability of patient harm to determine if the risk of patient harm has been adequately managed.

**Methods:**

The predicted probability of patient harm as a function of systematic error can be computed as:

$P_H(SE)=\{P_E(0)+E(N_{uf}(SE))/[MPBF+ANP_{ed}(SE)]\}*P_{h|u}$

Where:

$P_E(0)$ is the probability of producing erroneous results in the stable state.

$E(N_{uf}(SE))$ is the expected number of erroneous final results reported due to out-of-control condition, magnitude SE.

MPBF is the average number of patient results reported between out-of-control conditions.

$ANP_{ed}(SE)$ is the average number of patient results reported during out-of-control condition, magnitude SE.

$P_{h|u}$ is the probability that a reported erroneous result leads to patient harm.

Acceptable $P_H$ is derived from the risk acceptability matrix.

RMI=Predicted $P_H$/Acceptable $P_H$

**Results:**

Example calculation:

Glucose: CV=2.5%,$TE_a$=±10%.

QC Strategy: 2 QC levels, 1:3s/2:2s/R:4s Rule, evaluated every 50 results.

Mean days between test system failures=90.

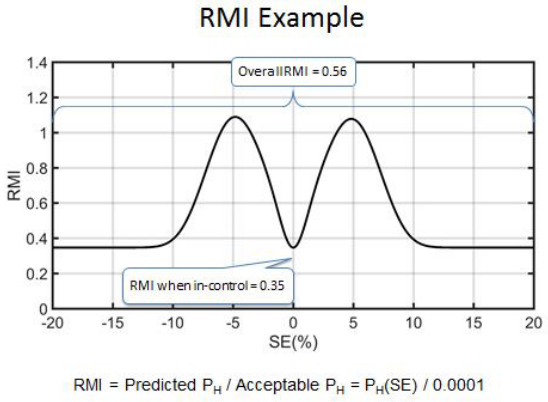
Average #patient results/day=100.

MPBF = 100*90=9,000.

Probability of harm given erroneous result, $P_{h|u}$=0.5.

Severity of Harm for Glucose=Minor

Acceptable frequency of harm=Occasional(1/10,000).




**Conclusion:** Computing a Risk Management Index (RMI) based on the ratio of predicted probability patient harm and the acceptable probability of patient harm makes it easy to assess acceptable risk management. An RMI ≤ 1 indicates managed risk. An RMI > 1 indicates unmanaged risk.

## B-187

### Application of Statistical Process Control (SPC) for Evaluation of Immunoassay Reagent Manufacturing

K. McMullen, S. Hauck, L. Shields, A. Aslamkhan, M. Kushner, L. Risbon, K. Smalley, C. Cifolelli, W. Covert, Z. Li, M. Harley. *Fujirebio Disgnostics, Inc., Malvern, PA*

**Background:** Statistical Process Control (SPC) is a statistical tool for the recognition of potentially assignable variations from random variations in a process (Statit Software, Inc., 2007). Fujirebio reported a preliminary application of SPC in monitoring of the manufacturing of one immunoassay product (He KL, et al, 2012). In the current study, SPC was applied to evaluate the manufacturing processes of six immunoassay reagent sets. **Methods:** All of the six assay reagent sets represent a chemiluminescent microparticle immunoassay (CMIA). Each set of the assay reagents is primarily composed of a bottled solid phase and a bottled conjugated antibody. Acceptable values (performance limits) of specific parameters, which include signal response for Calibrators and concentration response for Controls and Panels, were routinely reviewed from the Quality Control (QC) testing of the bottled reagents. SPC analysis was conducted to evaluate the process capability of the assay reagent manufacturing. Process capability index CpK represents the proportion of one side of the normal probability distribution curve of variables that will fall between the average and the nearest performance limit. In the current study, a CpK value was generated for each of the Controls, Panels and selected Calibrators for a two year period (n = 23 to 85) for each of the six assay reagent sets. **Results:** The distribution of the measurements for each of the specification parameters showed a normal distribution pattern for each reagent set. In total, 53 CpK values were obtained for the six reagent sets for up to nine specification parameters (Calibrator A and F signals, B/A ratio; Control L, M, H; Panel 1, 2, 3 and 4). 73.6% (39) of the 53 bottled reagent processes with a CpK value ≥ 1, demonstrated a process capability at acceptable 3-sigma or higher levels with at least 99.73% processes meeting requirements; 17.0% (9) with a CpK value ≥ 0.67 but < 1.00, demonstrated a 2-sigma of process capability with at least 95.45% processes meeting requirements; and 5.7% (3) with a CpK value < 0.67, indicated only a 1-sigma of process capability in which only 68.27% or more of the processes meet requirements. Assay reagent set wise, one set showed at least 99.73% of chances meeting requirements, and one set showed at least 95.45% of chances meeting requirements. One of the lowest CpK values at 0.487 was found to be attributable to a raw material switch. After the raw material switch issue was resolved, this CpK value at 0.487 was obviously improved to 1.68, demonstrating an improved process capability higher than 3-sigma level with greater than 99.73% processes meeting requirements. **Conclusion:** SPC allows for detection of variability of reagent manufacturing processes, provides a statistical tool for the detection of potential process trends, and identifies areas for improvement opportunities to support the commitment to Total Quality Management.

## B-188

### Urinalysis with Reflex Culture - Test Utilization Initiative and Quality Improvement Model

K. Bernhardt, S. E. Kahn, M. Saint Martin. *Loyola University Medical Center, Maywood, IL*

Practicing effective laboratory test utilization has become a necessity. Performing medically unnecessary lab tests increases operational expenses, results in staff burnout, leads to more abnormal results to be followed, more critical results needing notification and hospital-acquired anemia which can reduce patient safety as well as cause iatrogenic disease.

By the end of 2015, more than 3,000 urine cultures were performed in our Microbiology Laboratory per month. There were concerns that many urine cultures were ordered unnecessarily, yet still yielded positive results due to specimen contamination and not true infection. A potential best practice for ordering a urinalysis with reflex to culture was identified to decrease the performance of unnecessary urine cultures while improving utilization. We hypothesized that setting a result threshold for leukocytes for automated urinalysis could reduce the performance of these needless cultures which falsely elevated the rate of catheter-associated urinary tract infection (CAUTI). Furthermore, this could lead to better antimicrobial stewardship by reducing the number of patients exposed to unnecessary, expensive and potentially toxic antibiotics.

Staff and faculty from the lab were selected to establish a new process for urinalysis with reflex to culture. A project charter identified the sponsor (sets direction, allocates resources, removes barriers, makes decisions), manager (coordinates the project team), project team members (help define charter, provide input, complete assigned tasks), and other stakeholders affected. A business case with problem statement was created to ensure that team members had a common understanding of all aspects of the project. The overall project goals were to reduce urine cultures performed by 33% with a stretch goal of 50%.

With the support and involvement of our Chief Medical Informatics Officer as well as members of our EMR team (Epic) and LIS team (Sunquest), a new Urinalysis with Reflex Culture (UARC) order was developed to replace the previous stand-alone urine culture order. In the new process, urinalysis is performed prior to all urine cultures with reflex cultures only being performed when criteria for pyuria (≥6 WBC/hpf) are met unless one of six pre-determined clinical patient exceptions are met. EMR ordering allowed for these exceptions to be recorded at time of order in which case the culture was performed even if the urinalysis was negative for pyuria.

Successful implementation also necessitated buy-in and input from other stakeholders. Project team members met with clinicians from Infectious Disease, Urology, Obstetrics, Neonatal, Pediatrics and Maternal Fetal Medicine to ensure that the unique needs of patients managed by these departments were addressed. An extensive communication plan was developed, to ensure that all providers, caregivers and other stakeholders were knowledgeable regarding implementation of ordering urinalysis reflex to cultures. Effective communication and collaboration between multiple departments yielded impressive results. In the first thirty days after implementation, urine culture workload decreased to 1,713 (57 /day). Over the next six months, a 47% reduction in urine culture workload was sustained with substantial estimated annual savings in direct lab operational expenses while the quality metric target for the institution's CAUTI rate was reached.

## B-189

### An enzyme-linked immunosorbent assay for therapeutic drug monitoring of golimumab

C. Berger[1], A. Pilch[1], J. Ruppert[1], F. Armbruster[1], J. Stein[2]. *[1]Immundiagnostik AG, Bensheim, Germany, [2]Interdisciplinary Crohn Colitis Centre Rhein-Main, Frankfurt/Main, Germany*

Background: Golimumab is a therapeutic anti-TNF monoclonal antibody approved for use in moderate to severe ulcerative colitis (UC). The PURSUIT trials showed a significant exposure-response relationship of golimumab in UC. Interindividual differences in response to golimumab treatment may be explained in part by interindividual variability in pharmacokinetics.

Methods: Microtiter plates were coated with recombinant human TNF-alpha. Samples diluted at 1:100 were added to the microtiter plate for binding, and bound golimumab was detected using mouse anti-human immunoglobulin G1 (HRP-anti h IgG1). Performance characteristics of the assay were determined according to the European in-vitro diagnostic devices directive 98/79/EC.

Results: An enzyme-linked immunosorbent assay (ELISA) for the detection of golimumab drug concentration was developed. The limit of detection (LoD) for golimumab determination in human serum samples was 3.56 ng/mL. The measuring range of the assay was determined to be 0.415-22.5 µg/mL. Intra-assay variation (n=19) was ≤6.6%, while inter-assay variation (n=11) was ≤5.3%. Linearity testing was performed by analysis of three serially-diluted samples spiked with golimumab; golimumab concentrations measured by the new assay were within 98-129% of the expected concentrations. The assay detected no false-positive signals from samples taken from untreated patients. Due to the use of TNF-alpha as a capture reagent, other TNF-alpha blockers were detected.

Conclusions: This newly-developed ELISA method is rapid, accurate and reproducible. The use of monoclonal antibodies to golimumab could improve the specificity of the assay. The ELISA may be useful not only for pharmacokinetic/pharmacodynamic studies, but also in therapeutic drug monitoring of golimumab.

## B-190

### Evaluation of analytical performance and internal quality control procedure of an enzymatic method for measurement of Glycated albumin

T. Hu, D. Wen, D. Zhang. *Sun Yat-sen University affiliated Zhongshan hospital, Zhongshan, China*

**Background**: Strict control of plasma glucose levels is critical in the management of diabetes to avoid the potentially serious complications. The measurement of glycated albumin (GA) has been widely used in clinical practice as a new diabetes maker and intermediate glycation index for diabetes control. When monitoring the effects of therapy in patients with a varies of pathological state, such as gestational diabetes, unstable plasma glucose levels, varian themoglobins, and diseases that shorten the lifespan of erythrocytes, the GA level could provide useful information for the management of glycemic control. Lucica GA-L assay kit is a novel enzymatic diagnostic test for GA measurement and has been introduced for use in automated chemistry analyzers.The aim of our study is to evaluate the analytical performance and internal quality control procedure of an enzymatic method for measurement of Glycated albumin

**Methods**: The enzymatic GA assay was carried out in the ADVIA2400 chemistry system and the imprecision, accuracy, limit of quantification (LoQ), and linearity were evaluated according to the recommendation of CLSI documents (EP-15A2, EP-17A, and EP-6A). The internal quality control (IQC) data were collected from June to December 2016.The imprecision was calculated by accumulative CV, while the bias was calculated by the mean value of the IQC data across of global regions. Individual quality control scheme for GA analysis was developed combined with Function Power Graph and Six Sigma Chart based on 10% allowable total error (TEa) to improve the probability of error detection (Ped) and reduce the probability of false rejection (Pfr).

**Results**: The CV values of the within-run imprecision at low and high levels of the QC subjects were 1.6% , 1.0%, respectively. The CV values of the total imprecision at low and high levels of the QC subjects were 2.3% , 1.5%, respectively. The bias% was 0.8% when the manufacturer's standard material was used to assess the accuracy of the assay.The limit of quantification was 0.030 g/dL .Excellent linearity was observed in the range of 0.030[[Unsupported Character - Codename ]][[Unsupported Character - Codename ]]-3.563 g/dL($R^2$=0.9996). It was also demonstrated that the index of 90%Ped, 5%Pfr, and 7.5Sigma could be achieved using only 1-5s quality control rules with two levels of the QC in daily routine work (Figure1, Figure 2).

**Conclusion**: Function power graph and Sigma control chart can provide an objective assessment of the current analytical quality of laboratory examination procedures. Lucica GA-L kit, the enzymatic method with liquid reagents requiring no step of preparation, can be applied to general automated biochemical analyzers. In our study, we determined that the analytical performance of using Lucica GA-L kit in the ADVIA2400 analyzer was excellent and could meet requirements of the clinical application.

## B-191

### Effectiveness of Practices to Foster Quality Improvement Through Reaching Adequate Blood Volumes in Microbiological Tests in Taiwan: from Systematic Reviews to Validity Assessments

H. Ning[1], C. Wen[2]. *[1]Department of Laboratory Medicine, Linkou Chang Gung Memorial Hospital, TaoYan, Taiwan, [2]Department of Laboratory Medicine, Linkou Chang Gung Memorial Hospital, Tao-Yan, Taiwan*

**Background:** Blood cultures play a crucial role in the diagnosis of life-threatening bloodstream infection. The CLSI and many guidelines recommend an optimal volume of 20-30 mL for each set of blood bottles. However, in Taiwan, a lot of hospitals could not meet criteria of appropriate blood volumes due to difficulties in sampling process. In this way, we evaluate the effectiveness of increasing blood volumes to improve performances of blood cultures. First, we conducted a systematic review and meta-analysis to reassess the importance of blood volumes with positive rates. Due to positive correlations between the blood volumes and positive rates, we further used two strategies to foster quality improvement through reaching adequate blood volumes in blood cultures.

**Methods:** We searched MEDLINE from inception to March 2016 and all identified titles and abstracts were carefully examined by two independent reviewers. Of 531 studies, 4 papers were included and differences of positive rates between adequate volumes (8~10 ml) VS. low volumes (<3~5mL) were compared. For validity assessments, we used a series of assistive devices and educational programs for phlebotomy in our emergency department.**Results:**For the meta-analysis, the pooled estimates under the random effects model suggested the greater the blood volume, the greater the culture rate in adults. Between the two groups, each additional 1 ml volume of blood could almost lead to 0.5% increase in positive rates. Furthermore, after an evidence-based approach through two serial strategies were conducted in our emergency department in 12 months, the average of blood volume has increased form 1.5 mL to 4.5 mL, and the positive rates has also increased from 11.6 % to 13.6%, respectively. **Conclusion:** Reports concerning the importance of the volume of blood cultured with the new continuous-monitoring blood culture systems are scarce, and this is the first meta-analysis to evaluate the effectiveness of increasing the blood volume to improve the performance of blood culture between similar culture systems. However, the inconsistency may arise through study populations with different underlying diseases between different labs. In conclusion, to backup the lab practice, further analysis will need to assess direct relationships between blood volumes and positive rates and time to positivity.

## B-192

### Performance Evaluation of ADVIA Chemistry XPT and ADVIA Centaur XPT Immunoassay Systems in a University Hospital Laboratory

J. L. Bedini[1], N. Rico[1], E. Schneider[2]. *[1]Hospital Clinic de Barcelona, Barcelona, Spain, [2]Siemens Healthcare Diagnostics, Inc., Tarrytown, NY*

**Objective:** Hospital Clinic de Barcelona is a public university hospital in Barcelona, Spain. Within the hospital, the Core Laboratory (Core Lab) is a highly automated 24/7 facility that combines routine activities and emergency requests using Aptio® Automation. In 2015, its annual workload included about 3.4 million clinical chemistry tests and 347,000 immunoassay tests, among other testing disciplines. Core Lab sought to improve efficiency, productivity, service to physicians, and patient outcomes by upgrading its clinical chemistry and immunoassay testing capabilities. To achieve this goal, Core Lab added a new ADVIA® Chemistry XPT System and an ADVIA Centaur® XPT Immunoassay System (Siemens Healthcare Diagnostics Inc.). Both new analyzers were connected to the existing Aptio Automation track. The objective of this study was to evaluate the following performance parameters for the new analyzers compared to the legacy ADVIA 2400 Clinical Chemistry and ADVIA Centaur XP Immunoassay Systems: throughput, turnaround time (TAT), analytical precision of ISE results for chemistry testing, and operator intervention and hands-on time.

**Methods:** Assay performance was observed on routine samples over a 6-month period for the ADVIA Centaur XPT Immunoassay and ADVIA Chemistry XPT Systems. These analyzers ran in parallel with one ADVIA Centaur XP Immunoassay System and one ADVIA 2400 Chemistry System. ISE module stability was determined using BIO-RAD Liquid Assayed Multiqual 2 controls and BIO-RAD Unity Real Time software. To assess the impact on turnaround times (TAT) by incorporating both ADVIA XPT systems into the daily routine, Core Lab calculated the average turnaround (TATav) for determination of the glucose oxidase assay on the ADVIA Chemistry XPT System and the eHIV assay on the ADVIA Centaur XPT system. These assays were chosen because they are the tests most frequently performed on their respective instruments. Core Lab also assessed time savings for instrument maintenance, calibration, and reagent-loading processes.

**Results[1]:** For chemistry testing, average turnaround time improved by 15%, while throughput increased more than 5%. ISE analytical precision was excellent with a CV <2.5% for all analytes. The ADVIA Chemistry XPT System needed 58% fewer calibrations and 7% fewer quality control (QC) results than the ADVIA 2400 system. By reducing maintenance activities, the improved ISE module allowed 70 more hours of run time annually, and streamlined reagent loading saved about 42 hours annually. For immunoassay testing, average turnaround time was reduced by 6%. Automated daily controls saved 42 hours of labor per year, and automated water reservoir cleaning saved 18 hours of labor per year.

**Conclusions:** Significant improvements in throughput, turnaround time, and operator intervention and hands-on time were achieved for chemistry and immunoassay testing with the ADVIA Chemistry XPT and ADVIA Centaur XPT Immunoassay Systems. ISE module analytical precision for chemistry testing was excellent for all analytes.

[1] The outcomes obtained by Siemens' customer in this study were achieved in the customer's unique setting. Since there is no typical setting, there can be no guarantee that others will achieve the same results.

## B-193

### Performance Evaluation of Consolidated, Automated Chemistry and Immunoassay Testing in a Reference Laboratory

H. Lang, H. Wickert. *Labor Blackholm MVZ GmbH, Heilbronn, Germany*

**Objective:** Labor Blackholm MVZ is a private, independent reference laboratory in Heilbronn, Germany. Its annual workload includes about 6 million clinical chemistry tests and 1 million immunoassay tests. Labor Blackholm sought to improve efficiency, productivity, service to physicians, and patient outcomes. To achieve this goal, two existing laboratories were consolidated into one, and a high-capacity Siemens Aptio® Automation solution with connected ADVIA® Chemistry XPT Systems and ADVIA Centaur® XPT Immunoassay Systems was introduced to harmonize and standardize workflow. The objective of this study was to quantify the performance improvement obtained from the new Siemens Healthineers solution, primarily in terms of reduced turnaround time (TAT) for chemistry and immunoassay testing, compared to the laboratory's original equipment and configuration.

**Methods:** Labor Blackholm's existing facility comprised two laboratories on separate floors of a building. Key components of these labs were the Roche modular analyzer series configuration with connected clinical chemistry systems and the Siemens ADVIA® LabCell® Automation Solution with connected immunoassay systems. In this scenario, optimizing efficiency was a challenge, primarily because of complex tube-sorting and distribution processes for the two separate laboratories.

The new consolidated laboratory employs ADVIA Chemistry XPT Systems, ADVIA Centaur XPT Immunoassay Systems, and a variety of automated sample-handling modules connected to a single Aptio Automation track. Lab workflow for this new system was assessed during a single day of normal operation in January 2016. A total of 12,330 sample tubes of all disciplines were registered. Of these, 5701 sample tubes had test requests for the ADVIA Chemistry and ADVIA Centaur assay portfolio and went directly onto the automation system. Complete process control for Aptio Automation, including intelligent sample routing and workload balancing, was performed by the Siemens CentraLink® Data Management System (middleware) based on lab-defined rules. Total TATs, from order generation to result transmission to the LIS and including reruns, dilutions, and add-on tests, were assessed for chemistry and immunochemistry test results.

**Results[1]:** Tests performed on ADVIA Chemistry XPT Systems had a mean TAT of 22 minutes and a 95th percentile of 38 minutes, a 45% faster time compared to chemistry tests run on the original systems. Tests performed on ADVIA Centaur XPT Immunoassay Systems had a mean TAT of 34 minutes and a 95th percentile of 69 minutes, a 40% faster time compared to the previous immunoassay system configuration.

**Conclusion:** Significant improvements in processing times were achieved for chemistry and immunoassay testing with the combination of the Aptio Automation solution and connected ADVIA Chemistry XPT and ADVIA Centaur XPT Immunoassay Systems. Additional benefits included reduced manual hands-on time and easier training for operators, improved productivity and utilization of resources, reduced overtime labor costs, and improved physician satisfaction with the laboratory's services.

[1]The outcomes obtained by Siemens' customer in this study were achieved in the customer's unique setting. Since there is no typical setting, and many variables exist, there can be no guarantee that others will achieve the same results.

## B-194

### Failure to retrieve: a follow up study on unacknowledged send-out results

G. N. Winston-McPherson[1], J. A. Dickerson[2]. *[1]University of Washington, Seattle, WA, [2]Seattle Children's Hospital, Seattle, WA*

**Background:** A systematic study of send-out testing result retrieval was conducted in 2011 at a tertiary care pediatric hospital. The results of that study showed that over a period of one month 20% of all send-out tests were flagged as abnormal, yet only 45% of those abnormal results were acknowledged in the electronic medical record. Furthermore, a 1% harm rate was estimated directly related to unacknowledged results. Since that time, several interventions have been enacted to improve the rate at which send-out tests are acknowledged by the clinical team. These interventions included improvements to the HIS messaging system, patient portal with access to laboratory results, individual email notifications and increased discussion about failure to retrieve.

**Methods:** The goal of this study was to reevaluate the state of retrieval of send-out test results at a pediatric hospital in the western United States. One month of send-out tests were analyzed by volume, order type, normal/abnormal status and order-to-result turn-around time. All abnormal results were characterized as acknowledged or unacknowledged. Results were considered unacknowledged if there was no reference to, mention of, or change in care related to the result within 90 days of result verification. The Time to Acknowledge an Abnormal Send- out Result (TAASR) was also determined. Unacknowledged abnormal results were further analyzed by sending a questionnaire to the ordering provider and assessing possible patient harm. Send-out tests that were excluded from the study were nutritional and allergy testing as well as drug screens. Adult (21) patients were excluded. Deceased patients who expired prior to the close of the 90-day acknowledgement window were also excluded.

**Results:** During the study period, a total of 1991 send-out tests met the above criteria. The median order-to-result turn-around time was 3 days (mean 5 days). Seventeen percent of the results took longer than one week to return, with a maximum of 102 days. Of these results, 15% were abnormal. Of these abnormal results, 79% were acknowledged in the chart, and 21% were not. The TAASR had a median of 4 days (mean 8 days). Thirty-five percent of acknowledged results took greater than one week to acknowledge, with a maximum acknowledgement time of 57 days. Comparing current data to the 2011 study, we found that the total volume of send-out tests has increased by 59%. The median order-to-result turn-around time was consistent, however the average TAT decreased by 28% (7 days to 5 days). Current data shows that abnormal send-out test results are currently unacknowledged 21% of the time. This represents a 24% reduction ($p < 0.0001$) in the number of unacknowledged results. The median TAARS decreased (7 days to 4 days), but there was a 13% reduction in the number of abnormal results that took longer than 1 week to acknowledge.

**Conclusions:** The findings of this study suggest that interventions aimed at reducing the number of unacknowledged send-out results have made an impact at this institution leading to a 24% decrease in unacknowledged results.

## B-195

### Should critical values be repeated prior to release?

M. M. Zimmer, A. B. Muenzenmeyer, S. Wang. *Cleveland Clinic Foundation, Cleveland, OH*

**Background:** Certain laboratory results are deemed as critical to patient care, and immediate action may be needed. In our laboratory, upon obtaining a critical value the test was often repeated to confirm analytical accuracy before notifying the care team. This step resulted in delayed result reporting and additional lab resource expenditure. With the current state of high precision technologies and sophisticated quality assurance programs employed in modern laboratories, the continuance of this practice

was questioned. **Objective:** The purpose of this study was to investigate the need to retest for a critical value and assess the time savings if the repeat is not performed. **Methods:** Randomly selected critical values and the repeat results were collected for glucose and potassium tests in our clinical chemistry laboratory. These two tests were performed on Cobas c 702 (Roche Diagnostics, Indianapolis, IN). The time savings were estimated by calculating the difference in timestamps from the first and second result posting times. **Results**: Initial and the corresponding retesting values for glucose (n=100) and potassium (n=100) were closely matched with a maximum difference of 2mg/dL for glucose results and 0.2 mmol/L for potassium. The differences were well within the CLIA criteria for assay precisions (glucose target: ±6mg/dL or ±10% and potassium target: ± 0.5mmol/L). The mean elapsed time between the initial and retesting results posted in the lab information system was assessed for January 2016, and was found to be 14 minutes (ranging from 11-49 minutes) for glucose (n=31) and 4 minutes (ranging from 0-37 minutes) for potassium (n=197). **Conclusions:** This study demonstrated that repeat testing for critical values prior to release is not necessary due to the high precision of the modern technologies and sophisticated quality assurance programs employed in the clinical laboratory. Eliminating the unnecessary step has allowed our laboratorians to reach out to our clinicians at a more rapid pace thus enabling them to provide a quicker response to the critical values. In addition, eliminating unnecessary analytical runs improves operation efficiency and overall turnaround time for all patient resulting in our high volume laboratory.

## B-196

### Managing test results disagreement complaints: the experience of a large clinical laboratory

P. Araujo, A. Bispo, J. Duarte, L. Hasselman, D. Rodrigues, L. Santos, A. Salvador, M. Freire. *DASA, RJ, Brazil, Rio de Janeiro, Brazil*

**Background**: Accurate laboratory tests are vital to ensure that healthcare professionals provide the best care for their patients. Therefore, it is essential that the laboratory offers a complaint handling service which is easily accessible, trustworthy and transparent. The aim of this study was to access the dimension and efficiency of the established process for managing outpatient and physician's disagreement complaints about tests results. **Methods**: To understand the performance of the current process of management of disagreement complaints, we analyzed the records from a large clinical laboratory Quality Management System (QMS) from January to December of 2016. Complaints from all contact points are recorded (drawing facilities, website, email, social media and phone calls), and managed by the medical staff, remotely based from the central lab. The first feedback to the claimant is performed within 12 commercial hours after the complaint is recorded on the QMS, in order to better understand the questioning. Parallel to that, medical team analyzes the client's report, previous and concomitant tests results in the laboratory database, investigate the equipment analytical performance, and post analytical information from the central lab, using either the LIS and/or QMS. **Results**: During the study period, 27.4 million laboratory tests were performed. A total of 521 cases of complaints of result disagreements were recorded, distributed as follows: biochemistry (130, 24.9%), hematology (106, 20.34%), hormonology (86, 16.5%), microbiology (70, 13.4%), and others (129, 24.8%). The claimant was a patient in 82% (n=427) and a physician in 18% (n=94) of recorded cases. After medical staff analysis, 22% (n=115) of complaints were judged as laboratory errors, representing 4.19 defects per million opportunities (DPMO). The process performance evaluation showed that 97.7% of first feedbacks took less than 12 hours to be concluded and 85.8% of complaints were solved and closed within 48 commercial hours. In 30 (0.06%) cases the report result was rectified. **Discussion**: Results obtained by different laboratories may vary for several reasons. Even results from the same laboratory may vary, especially if there is a time gap between collections. Properly managing the disagreement complaints, under the supervision of the medical staff, improves the relationship between patients, physicians and lab, generating greater transparency and, consequently, confidence in the future results. Our data show that only the minority (22.0%) of complaints could not be explained after deep investigation. It is of notice that the current management of complaints is fast and efficient, taking less than 48 hours for conclusion in the majority of cases, leading to a Net Promoter Score (NPS) of 69.4% by the end of 2016. **Conclusion**: The medical process of investigation and discovery of causes for discordant results take some time and technical expertise. However, this process should proceed with the utmost transparency and respect, since pursuing quality should be the main goal of a clinical lab.

## B-197

### Use of external quality assessment summary statistics to produce comparative precision profiles of common hemoglobin A1c methods

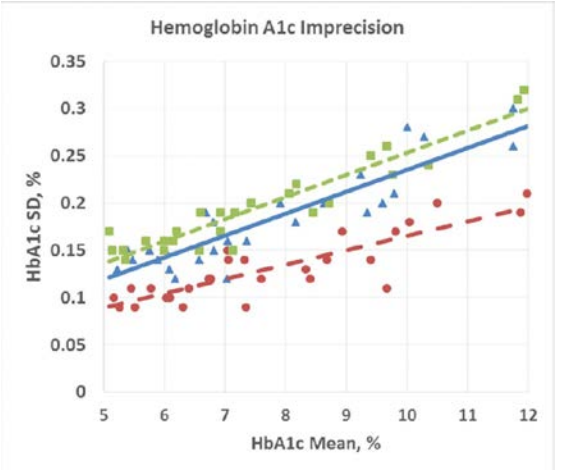
G. S. Cembrowski[1], T. Higgins[2]. *[1]Alberta Health Services, Edmonton, AB, Canada, [2]DynaLife, Edmonton, AB, Canada*

**Background:** When a series of quality control products are repeatedly analyzed over short or extended durations, the standard deviations (imprecisions) of the quality control products are generally linear when graphed against the level of the analyte contained in the control product. An instrument's targets for imprecision are not only important for quality control purposes but also are very useful in instrument comparison and selection. Generally, the instrument with the lowest imprecisions will be the most desirable system for specimen analysis and result reporting. Inter-analyzer imprecision provides an excellent indicator of instrument variation as it provides a measure of average system operation. Different instruments will display unique standard deviation (imprecision) profiles which can be used to simplify instrument selection and acquisition. These precision profiles provide a measure, over time, of analytical imprecision over a range of mean analytical values.

**Methods:** College of American Pathologist proficiency test (PT) summaries were obtained for two hemoglobin A1c (HbA1c) PT surveys, GH2 (2 cycles yearly [2013-2014] with 3 unknowns per cycle) and GH5 (3 cycles [2015] with 5 unknowns). When summary statistics were available for a minimum of 100 like analyzers, the analyzer model, the HbA1c group mean and standard deviation were abstracted for each unknown. Standard deviation vs mean (precision profile) graphs were generated for each analyzer model.

**Results:** The precision profile graphs are largely linear with the HPLC analyzers generating tighter HbA1c values compared to immunoassay. The Figure compares the precision profiles of a representative immunoassay (top line) and two HPLC HbA1c analyzers (lower lines).

**Conclusions:** These precision profiles facilitate the identification (and acquisition) of HbA1c analyzers with the best reproducibility. The availability of these comparative profiles should motivate the manufacturer to relentlessly improve their products.




## B-198

### Beckman Coulter AU Chemistry Method Performance Three Years Later - Any Improvements?

N. Fleming[1], D. D. Koch[2]. *[1]Grady Memorial Hospital, Atlanta, GA, [2]Emory University, Atlanta, GA*

**Background**: Clinical chemistry laboratories should know the analytical performance of their laboratory methods. Six Sigma quality metrics are an excellent way to quantify the precision and accuracy of analytical methods. Three years ago at the 2014 AACC Annual Meeting, we presented data from 26 clinical chemistry analytes on an AU 5800 platform, which were used to calculate sigma metrics. Some of the methods failed to achieve Six Sigma quality. Since then, we deployed two new AU 5800 instruments. We wanted to discover if any improvements occurred compared with our observations in 2014. **Quality Goals**: Quality goals to evaluate the performance of the tested methods were generally derived from CMS CLIA PT goals, but also included criteria from the Biologic Variation Database and from RCPA. **Collection**

**of data**: Precision and accuracy were evaluated for each chemistry analyte. Within-instrument precision was calculated by measuring quality control materials over one month. Accuracy was estimated by calculating observed bias (mean of the actual data minus an average mean). **Results**: The AU 5800 results for 12 methods exhibiting Sigma metric values of 4 or less in 2014 are summarized in the Table. Seven of these methods showed noticeable improvement from the 2017 data, several now achieving Six Sigma performance. These methods should now be easy to control with cost-effective QC practices. In contrast, a few analytes continued to not achieve desired status and therefore require more rigorous QC practices. **Conclusions**: The great majority of clinical chemistry methods function at world-class specifications using the Beckman Coulter AU5800. The methods that fail to achieve such performance demand analytical improvement from the method research community. The data presented here help the clinical laboratory profession again to emphasize certain analytes to focus on for method improvement. Additionally, optimal QC practices for laboratory production are suggested.

| Sigma Metric Results | | | | | |
|---|---|---|---|---|---|
| analyte | 2014 | 2014 | 2017 | 2017 | 2017 |
| level | 1 | 3 | 1 | 2 | 3 |
| Alk Phos | 3.5 | 16 | 2.5 | 10.6 | 13.7 |
| ALT | 3.8 | 14 | 5.1 | 14.9 | 19 |
| BUN | 3.1 | 3.4 | 3.3 | 3.5 | 3.4 |
| chol | 3.2 | 6.4 | 4.1 | 5.0 | 5.4 |
| Cl | 4.0 | 4.7 | 3.8 | 3.1 | 3.8 |
| $CO_2$ | 1.9 | 1.7 | 2.0 | 1.8 | 1.9 |
| d bili | 1.4 | 3.6 | 3.6 | 10.3 | 13 |
| Fe | 3.0 | 7.1 | 2.4 | 5.9 | 8.4 |
| glucose | 3.3 | 6.8 | 5.9 | 7.8 | 6.3 |
| lipase | 1.9 | 2.7 | 7.1 | 8.5 | 12.3 |
| Na | 1.6 | 1.4 | 3.7 | 2.7 | 2.2 |
| $PO_4$ | 2.2 | 3.7 | 8.1 | 8.4 | 12.1 |

## B-199

### Serum Indices for Monitoring Pre-Analytical Interferences

A. Vekich, B. Schmidt, J. Cole. *Bio-Rad Labs, Irvine, CA*

Background: Pre-analytical monitoring of instrumentation performance is an important step in the assessment of patent samples. Human samples are often compromised in ways that may interfere with assay's analytical functions. Three of the most common interferences in clinical chemistry that contribute to pre-analytical variation are hemolysis, icterus, and lipemia.

Automated instrument detection of serum indices is more accurate than visual assessment because it is based on absorbance measurements rather than subjective appearance. These systems are typically calibrated and maintained by instrument manufacturer field support However, until recently, commutable, human sera commercially produced materials were not available to monitor this critical pre-analytical test module.

Methods: Liquichek Serum Indices is a four "level" liquid material, specifically developed to assist in monitoring instrumentation serum indices detection modules. The product is prepared by spiking true human serum with hemoglobin, bilirubin, and lipids. The product was tested on several instrument platforms to determine performance.

Results: After preparation of this quality product, accelerated stability studies indicate a closed vial shelf life of 3 years when stored at -20 to -70 °C, Open vial stability is 14 days at 2-8 °C. Thawed and unopened vials are stable for 30 days at 2-8°C, while frozen aliquots are for 28 days at -20 to -70 °C.

Table 1 shows recovery results of Liquichek Serum Indices on six instruments by different manufacturers.

Table 1. Liquichek Serum Indices - Instrument commutability

| | Instrument A | Instrument B | Instrument C | Instrument D | Instrument E | Instrument D |
|---|---|---|---|---|---|---|
| Hemolysis Level | 7 | 6 | 7 | Suppressed OIR HI* | 164 | 67 |
| Icterus Level | 7 | 6 | 5 | Suppressed OIR HI* | 69 | 58 |
| Lipemia Level | 8 | 6 | 7 | Suppressed Rx ABS HI* | 1890 | 1211 |
| Non-Interfered Level | 1 | 1 | 1 | 1 | 1 | 1 |

Conclusion: The results of this investigation demonstrate that Liquichek Serum Indices is commutable across multiple platforms and can be used as a third party quality product to monitor the ability of laboratory pre-analytical testing procedures to detect interferences, improve analytical results, and ultimately improve patient safety.

## B-200

### Rapid serum tubes to facilitate specimen processing and improvement of cost savings at patient service centers

D. Orton[1], L. Gervais[2], B. Frandle[2], B. Strange[2], A. Chin[2]. *[1]Surrey Memorial Hospital, Surrey, BC, Canada, [2]Calgary Laboratory Services, Calgary, AB, Canada*

Objectives: To compare the numerical result from the analytical instrumentation between the rapid serum tubes (RSTs) and serum separator tubes (SSTs) in order to evaluate potential bias and to determine the amount of savings that have been realized when RSTs are used for the final hour of operations at community patient service centers (PSCs) following definition of the potential bias. Relevance: Traditional SSTs require an approximate 30 minute incubation to clot prior to being separated from cells and sent to the laboratory for testing. When patients arrive at community PSCs to undergo collections for laboratory testing in the last hour of the day, at least two medical laboratory assistants commonly have to stay overtime for safety reasons and to allow these samples to clot before they can be sent to the lab. This results in an enormous added cost for staff time with little benefit. The purpose of this study is to evaluate a new type of blood collection tube called RSTs which reduce the clot time to less than 5 minutes as compared to routine SSTs. These tubes could be implemented for use in the final hour of daily operations at PSCs, allowing the medical laboratory assistants to separate serum from blood cells sooner, thereby saving overtime and significant cost savings. Methods: This study was split into two separate parts. For the first phase, 20 healthy volunteers were enrolled for evaluation of routine lab tests 25-hydroxyvitamin D, sex hormone binding globulin, apolipoprotein B, tissue transglutaminase (tTG) , serum folate, anti thyroid peroxidase, alpha 1 anti trypsin, lithium, cyclic citrullinated peptide antibodies (CCP), hepatitis A antibody IgG, hepatitis A antibody IgM, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis B core antibody IgM, hepatitis Be antigen, hepatitis Be antibody, anti nuclear antibody, protein electrophoresis, neutrophil cytoplasmic antibody (ANCA), and extractable nuclear antigen antibodies (ENA). These tests were selected according to test volumes during the final hour of daily operations. For the second part of the study, patients were consented and enrolled at a designated community PSC for evaluation due to the rare nature of positive results from the first phase of the study such as lithium for therapeutic drug monitoring and other serological tests for infectious disease and autoimmune conditions. All blood samples were collected from subjects with a de-identified matched set of RST and SST tubes. The numerical results for each test from the two tube types were compared and analyzed for significant differences according to total error. Results: When compared to SSTs, RSTs were acceptable to the majority of the tests except for tTG, anti-CCP, ANCA, and ENA due to the lack of samples with positive results. The collection strategy for these analytes will be altered to address this issue. Overtime costs at PSCs have significantly decreased and further studies will be initiated to expand RST collections on other tests. Conclusions: Implementation of RSTs for use in the final hour of daily operations at community PSCs led to reduction of overtime hours and significant cost savings.

## B-201

### The impact of TEa selection on patient risk

Z. C. Brooks[1], G. Sweeney[2]. *[1]AWEsome Numbers Inc., Worthington, ON, Canada, [2]M.O.R.E. Quality Consultants, Atlanta, GA*

**Objectives**

1. To quantify the impact of setting Acceptable Risk Criteria as the acceptable number of medically unacceptable results/year, rather than statistical metrics

2. To demonstrate how Margin for Error (ME) - the number of standard deviations the mean can shift before risk becomes unacceptable - responds to acceptable risk criteria while sigma metrics remain unchanged

3. To illustrate how Mathematically-OptimiZed Risk Evaluation can evaluate risk and design QC processes to vary with defined acceptable risk criteria

**Background:**

To evaluate risk, laboratories must "compare the estimated risk against given risk criteria to determine the acceptability of the risk" (CLSI EP 23-A). Acceptable risk criteria are implied in acceptable sigma values or error rates. Selection of Acceptable Risk Criteria as the number and cost of medically-unreliable results drives the perceived and factual acceptability of patient risk. Acceptable risk criteria set standards of acceptable analytical process quality and QC process effectiveness.

**Methods:**

1. We created a calcium data set with of recently 1. measured mean; 2. measured SD; 3. Peer Mean equivalent to a sigma values or z-value of 5.7 based on the CLIA limit

3. We used CatalystQC software (AWEsome Numbers Inc.) to evaluate analytical process quality relative to to Acceptable Risk Criteria of 5% allowable error; 2 sigma, 3 sigma and 1 Medically-Unreliable Result/year

**Results:**

Below is a table of the results of the study. The data used is the same within each case, only the Acceptable Risk Criteria is changed within each case.

**Discussion**

Risk management and IQCP present new challenges and new opportunities. CLSI EP 23-A states that risk evaluation is the "process of comparing the estimated risk against given risk criteria to determine the acceptability of the risk." Laboratories must "Evaluate the potential costs both in terms of the patient's well-being and in terms of financial liability of the treating parties vs known benefits to the patient." Laboratory methods have been previously considered acceptable if QC charts showed no rejects and summary results passed generally accepted standards if 5% allowable error, or had an acceptable sigma metric of 2 or 3 sigma at a regular/monthly review. To meet risk management standards, laboratories must measure and evaluate risk as the number and clinical/legal cost of results that exceed TEa limits. However, z-values (sigma values) do not vary with either patient volume or the acceptable risk criteria. Here we present the Margin for Error (ME) - the number of standard deviations the mean can shift before risk becomes unacceptable.

We see that by only changing acceptable risk criteria there is a significant change in the amount of patient risk and clinical cost allowed before QC will alert staff to stop and take action.

**Conclusion**

1. Comparing the results of evaluation with statistical and clinical acceptable risk criteria demonstrates that clinical acceptable risk criteria will play a major role in clinical acceptability, patient risk, and cost due to medically unacceptable errors.

2. The process of Mathematically-OptimiZed Risk Evaluation should be further evaluated

## B-202

### Managing risk with Acceptable Risk Criteria and Mathematically-OptimiZed Risk Evaluation

Z. C. Brooks[1], K. Przekop[2], G. Sweeney[3], J. Hopkins[4]. *[1]AWEsome Numbers Inc., Worthington, ON, Canada, [2]Crosswayz Consulting, Miami, FL, [3]M.O.R.E. Quality Consultants, Atlanta, GA, [4] eDRUID, Clinical Mass Spectrometry Consulting Ltd., Blackpool, United Kingdom*

**Objectives**

1. To quantify the impact of setting Acceptable Risk Criteria as the acceptable number of medically unacceptable results/year, rather than statistical metrics

2. To present the Margin for Error (ME) - the number of standard deviations the mean can shift before risk becomes unacceptable -

3. To illustrate how Mathematically-OptimiZed Risk Evaluation can evaluate risk and design QC processes to vary with defined acceptable risk criteria

**Background:**

To evaluate risk, laboratories must "compare the estimated risk against given risk criteria to determine the acceptability of the risk" (CLSI EP 23-A). Acceptable risk criteria are implied in acceptable sigma values or error rates. Selection of Acceptable Risk Criteria as the number and cost of medically-unreliable results drives the perceived and factual acceptability of patient risk. Acceptable risk criteria set standards of acceptable analytical process quality and QC process effectiveness.

**Methods:**

1. We created a calcium data set with of recently 1. measured mean; 2. measured SD; 3. Peer Mean equivalent to a sigma values or z-value of 5.7, based on a TEa CLIA

2. The QC data were compared in CatalystQC software (AWEsome Numbers Inc.) to Acceptable Risk Criteria of 5% allowable error; 2 sigma, 3 sigma and 1 Medically-Unreliable Result/year

3. We set the clinical/legal cost of each MUR at $100 based on the NIST study

4. We compared the interpretation of acceptability of quality and probable action based on the selected Acceptable Risk Criteria

**Results:**

Below is a table of the results of the study. The data used is the same within each case, only the Acceptable Risk Criteria is changed within each case.

**Discussion**

Risk management and IQCP present new challenges and new opportunities. CLSI EP 23-A states that risk evaluation is the "process of comparing the estimated risk against given risk criteria to determine the acceptability of the risk." Laboratories must "Evaluate the potential costs both in terms of the patient's well-being and in terms of financial liability of the treating parties vs known benefits to the patient."

Laboratory methods have been previously considered acceptable if QC charts showed no rejects and summary results passed generally accepted standards if 5% allowable error, or had an acceptable sigma metric of 2 or 3 sigma at a regular/monthly review. To meet risk management standards, laboratories must measure and evaluate risk as the number and clinical/legal cost of results that exceed TEa limits. However, z-values (sigma values) do not vary with either patient volume or the acceptable risk criteria. Here we present the Margin for Error (ME) - the number of standard deviations the mean can shift before risk becomes unacceptable.

**Conclusion**

1. Comparing the results of evaluation with statistical and clinical acceptable risk criteria demonstrates that clinical acceptable risk criteria will play a major role in clinical acceptability, patient risk, and cost due to medically unacceptable errors.

2. The process of Mathematically-OptimiZed Risk Evaluation should be further evaluated

## B-203

### The Lab Quality Continuum & The Current State of Lab Quality

J. Dawson. *Proove Biosciences, Irvine, CA*

Building on existing models, distinct phases of laboratory quality were identified and organized into phases comprising the Lab Quality Continuum(LQC), a tool to aid laboratories in determining their current level of quality. Collaborating with Roche Lab Leaders, a self-assessment was developed to assist labs in understanding their current phase of quality and to provide improvement recommendations (www.lableaders.com/quality).

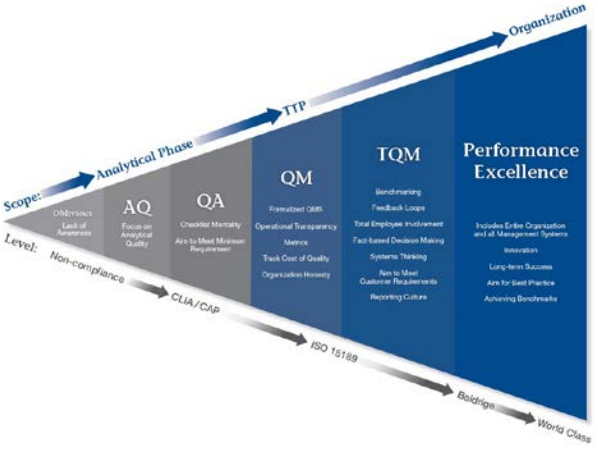
Objective: The objective of this study was to assess the current state of clinical laboratory quality utilizing the LQC Self-Assessment.

Methods: An anonymous online survey captured demographics and self-assessment results. LinkedIn was utilized to identify potential respondents and was administered utilizing convenience sampling. Data were extracted into a spreadsheet and analyzed for trends.

Results: 117 respondents participated in the study. Most respondents are employed by hospital and independent laboratories spanning a wide variety of titles. Respondents' laboratories represent all CLIA specialties, all U.S. regions with widely varying annual volumes and organizational sizes. The distribution of the self-assessment results: Oblivious 5.13%, Analytical Quality (AQ) 11.11%, Quality Assurance (QA) 52.14%, Quality Management (QM) 18.80%, Total Quality Management (TQM) 7.69%, Performance Excellence 2.56%, Pinnacle of the Lab Quality Continuum 2.56%. 59.83% of respondents reported that their laboratory employed at least one full-time quality professional (55.00% QA and below, 70.27% QM or higher). 70.09%

reported that they felt their lab's quality program was adequate (62.82% QA and below, 86.49% QM or higher, 93.33% TQM or higher).

Conclusions: The current state of lab quality is that 68.38% are at QA or lower on the LQC (study ongoing), suggesting that most labs are currently focusing on merely meeting minimum regulatory requirements. Positive correlations exist between employment of a full-time quality professional and perception of an adequate quality program and progression along the continuum.



# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Molecular Pathology/Probes

## B-204

### SHOX2 and SEP9 genes hypermethylation as biomarkers for plasma-based discrimination between malignant and nonmalignant colorectal lesions

M. K. Ahmed[1], A. M. Hashem[2], A. F. Mostafa[3], H. M. Shams[4]. *[1]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt, [2]Infectious disease and endemic hepatogastroentrology department, Faculty of medicine, Cairo University, Cairo, Egypt, [3]Pathology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt, [4]Clinical and Chemical Pathology Department, National Cancer Institute, Cairo, Egypt, Cairo, Egypt*

**Background:** Colorectal cancer (CRC) is a common malignancy and the fourth leading cause of cancer deaths worldwide. It results from the accumulation of multiple genetic and epigenetic changes leading to the transformation of colon epithelial cells into invasive adenocarcinomas. In CRC, epigenetic changes, in particular promoter CpG island methylation, occur more frequently than genetic mutations. Most cases of CRC are curable if diagnosed early enough. There is a variety of procedures for CRC screening that may be divided into invasive (colonoscopic and sigmoidoscopic examination) and non-invasive methods (fecal occult blood testing (FOBT)). Significant efforts have been invested to develop biomarkers that identify early adenocarcinomas and adenomas with high-grade dysplasia. methylated SEPT9 has been proved to be assay for CRC detection by many studies. However, different analysis methods used currently in data interpretation led to variation in test sensitivity. The aim of the present study was to determine the sensitivity and specificity of SEPT9 and SHOX2 genes hypermethylation as biomarkers for colorectal cancer (CRC). Furthermore, usefulness of these circulating methylated genes will be compared to colonoscopy which is considered as the gold-standard investigation of CRC screening.

**Methods:** A total of 106 selected individual (25 CRC negative and 81 CRC positive; 50 to 78 years old; 71 male and 35 female) undergoing screening by colonoscopy were included in the study. Circulating DNA was extracted from 3.5 mL plasma samples using Abbott mSample preparation system DNA kit automated on Abbott m2000sp instrument, treated with bisulfite using Abbott Real-time Bisulfite Modification Kit , purified, and assayed by real-time polymerase chain reaction for assessment of DNA methylation of (SHOX2) and (SEP9) genes, these assays were validated, optimized and evaluated before processing of patient samples. A multiplex polymerase chain reaction combining either SHOX2 or SEP9 and the reference gene beta gene (ACTB) was performed in triplicate for all specimens.

**Results:** SHOX2 and SEP9 genes methylation was significantly higher in patients with malignant colorectal lesions than those with nonmalignant lesions (P<0.001). *In detecting malignant colorectal lesions,* SHOX2 showed higher sensitivity (97.5% vs. 88.8%) and specificity (92.6% vs 73.5%) than SEP9. SHOX2 *revealed a better sensitivity than SEP9 in detecting stage I (92% vs. 72%) and II (100% vs. 93%) CRC, while both markers showed similar sensitivity (100%) in detecting stages III and IV CRC.*

**Conclusion:**

*SHOX2* and *SEPT9* are frequently methylated in CRC patients. Promoter hypermethylation of *SHOX2* and *SEPT9* may therefore serve as minimally invasive biomarkers for detection CRC. SHOX2 methylation was found to be more sensitive than SEP9 in detecting stages I and II of malignant CRC lesions.

## B-205

### Genomic DNA extraction from whole blood: A comparative study between modified salting out technique and spin - column based method

O. Elgaddar, L. Desouky, R. Bedair. *Medical Research Institute, Alexandria University, Alexandria, Egypt*

**Background and Objective:**

DNA extraction from different sources is the essential primary step in any genomic research. Methods used for extraction are evaluated based on the duration, feasibility and cost - effectiveness. Generally speaking, all methods used in DNA extraction should involve disruption of cells / tissues, denaturation of nucleoprotein complex, inactivation of DNase in the sample, and removing contaminants from the extracted DNA. The used method is judged based on the quantity and quality of the yielded DNA. The traditional salting - out technique remained the standard DNA extraction method for years. Researchers started to develop several modifications to this method in order to improve the DNA yield, decrease the extraction time and minimize the cost. Over the past years, spin - column DNA extraction kits became widely used in molecular biology labs. This could be attributed to the simplicity of the technique and the possibility of producing a better quality DNA. In the present study we aimed at comparing the extracted genomic DNA using a modified salting out technique versus that produced from the same peripheral blood samples using a commercially available spin - column DNA extraction kit.

**Methods:**

Peripheral blood was collected from 100 volunteers, in standard EDTA tubes, and DNA extraction from leukocytes was performed using both a modified salting out technique and a commercially available spin - column kit. In this in - house modified salting out technique, proteinase K was not used, 1% sodium dodecyl sulfate was used as a detergent in the white blood cells lysis solution, and protein precipitation was performed using ammonium acetate in high concentration. The concentration of the resulting DNA from both methods was measured using Nanodrop spectrophotometer, and the 260 / 280 ratio was checked for all samples.

**Results:**

Comparing the DNA extracted from peripheral blood leukocytes using both mentioned techniques showed a significantly higher concentration using the spin column kit ($p = 0.003$) than the resulting DNA from the modified salting out technique. On the other hand, the effective deproteinization of both methods (Using the 260 / 280 ratio) did not show any significant statistical difference ($p = 0.134$). Correlation was tested between the resulted DNA concentration using both methods but it was insignificant ($p = 0.7$).

**Conclusion:**

Using spin - column based genomic DNA extraction method from peripheral blood results in a yielded DNA with higher concentration than that produced from salting out technique, although the quality (purity) of DNA resulted from both methods is comparable. Further work is needed to assess whether the difference in concentration is cost effective or not taking into consideration that the spin - column technique is more expensive especially when used on a large scale.

## B-206

### Multi-variant Genetic Panel for genetic risk of opioid addiction

K. Donaldson, K. Taylor. *Prescient Medicine, Hummelstown, PA*

**BACKGROUND**

Over 116 million people worldwide have chronic pain and prescription dependence1. In the US, opioids account for the majority of overdose deaths, and in 2014, almost 2 million Americans abused or were dependent on prescription opioids2,3. Genetic factors may play a key role in opioid prescription addiction.

**OBJECTIVES**

Describe genetic variations between opioid addicted and non-addicted populations and derive a predictive model determining risk of opioid addiction.

**METHODS**

Design:

Case cohort study comparing the frequency of sixteen single nucleotide polymorphisms involved in the brain reward pathways in patients with and without opioid addiction. Data were modeled with TreeNet 10-fold cross validation (Salford Systems, San Diego, CA), and used to generate a weighted score.

Setting:

Thirty-seven patients with prescription opioid or heroin addiction and thirty age and gender matched controls were used to design the predictive score. Generalizability of the prediction score was tested on an additional 138 samples.

**RESULTS** Method Derivation: Observed data: ROC statistic=0.92 ,

sensitivity=82%(95% CI: 66-90), specificity=75% (95% CI: 56-87). TreeNet "learn" data: ROC statistic=0.92, sensitivity=92%, specificity=90%, precision=92%, and overall correct=91%. Generalizability: Sensitivity=97% (95% CI: 90 to 100), specificity=87% (95% CI:86 to 93), positive likelihood ratio=7.3 (95% CI: 4.0 to 13.5), and negative likelihood ratio=0.03 (95% CI:0.01 to 0.13).

**CONCLUSIONS**

The NeurR score can be used for opioid addiction risk assessment. By identifying patients with a lower risk for opioid addiction, our model may inform therapeutic decisions. Further studies are needed to evaluate additional populations and settings.

## B-207

### Preclinical validation of fluorescence *in situ* hybridization assay for detection of 5p deletions associated to Cri-Du-Chat syndrome

F. K. Marques[1], W. G. Texeira[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Cytogenetic Division), Vespasiano, Brazil*

**Background:** Cri-du-chat syndrome is a chromosomal disorder caused by a deletion of the short arm of chromosome 5, which may be visible or submicroscopic. The most important clinical features are a high-pitched cat-like cry, facial dysmorphism, microcephaly, severe psychomotor and mental retardation. The patients with 5p deletion show a high variability phenotypic and cytogenetic. A critical chromosomal region involved in the cat-like cry is mapped to proximal 5p15.3, while the region involved in the remaining features of the syndrome mapped to 5p15.2. The CTNND2 gene, mapped in this region, is potentially involved in cerebral development and their deletion may be associated with mental retardation. The first test to perform is karyotype analysis, which will confirm the diagnosis. In doubtful cases, when there is a conflict between the clinical suspicion and an apparently normal karyotype result, Fluorescence *in situ* Hybridization (FISH) analysis should be performed. Although the performance of FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of assay. **Objective:** To validate a FISH assay for detection of 5p15.2 and 5p15.31 deletions associated to Cri-Du-Chat syndrome. **Methods:** We used Cri-Du-Chat and Sotos probe combination manufactured by Cytocell®. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its sensitivity and specificity. To establish a normal cutoff was estimate the false positive rate from 10 cultured normal blood samples. Two analysts score 200 cells (100 per analyst). All probe signal patterns were recorded. The cutoffs were calculated using the beta inverse (BETAINV) function. FISH analysis was also performed with a sample whose 5p15 deletion previously detected by the karyotype. **Results:** The Cri-Du-Chat probe presents the FLJ25076 (5p15.31) and CTNND2 (5p15.2) probes labelled respectively with green and red fluorophores. The SOTOS probe (NSD1gene) is labelled in green and is used as a control. In the normal cell, there should be fusion of the red and green signals (2F) and two green signals (2G), whilst a deletion of FLJ25076 probe results in 1F1R2G signal pattern, a deletion of CTNND2 results in 1F3G**.** The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of 10 normal blood samples were did not observe false positive cells. The normal cutoff for the positive signal pattern was 1.5%. The FISH analysis performed in a sample with 46,XY,del(5)(p15.2) karyotype showed the 1F1R2G signal pattern in 100% of the cells. In this case there was only loss of FLJ25076 probe (5p15.31). **Discussion:** FISH analysis confirmed the previously identified 5p15 deletion, allowing more accurate detection of the deleted region. The occurrence of mosaicism is a very rare finding. Although we did not observe false positive cells, resulting in a 1.5% cutoff, a case with low number of positive cells should be carefully evaluated. The probe specificity and sensibility was higher than recommended by the ACMG. This FISH assay showed high quality and reproducibility and was approved for use in our laboratory.

## B-208

### Validation of dual-color, dual-fusion fluorescence *in situ* hybridization assay for the detection of PML-RARA translocation

F. K. Marques[1], V. D. Fonseca[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Cytogenetic Division), Vespasiano, Brazil*

**Background:** Acute promyelocytic leukemia (APL), comprises 5% to 8% of cases of acute myeloid leukemia (AML). It is typically characterized by neoplastic proliferation of cells in the bone marrow with a promyelocytic phenotype and presence of the fusion gene PML/RARα created by the t(15;17)(q24;q21) translocation. The detection of this translocation by conventional cytogenetic can be hampered by low quantity and quality of metaphases. In this context the fluorescence in situ hybridization (FISH) is applied as an usual and rapid diagnostic tool. FISH can be performed in dividing and nondiving cells, which is important when dealing with leukemia cell with low proliferation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, each clinical laboratory must individually validate its FISH. **Objective:** To validate a fast FISH assay for detection of translocation PML/RARα following recommendations from the American College of Medical Genetics (ACMG). **Methods:** We use the *FAST* PML/RARα translocation, dual fusion probe manufactured by Cytocell®. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a normal cutoff) were estimate the false positive rate from 10 uncultured normal bone marrow samples and 10 uncultured normal blood samples. Two analysts score 500 interphase cells (250 per analyst). All probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function. The probe hybridization time was only one hour. **Results:** The *FAST* PML/RARα probe presents the PML (15q24) and RARα (17q21) probes labeled respectively with red and green fluorophores. A normal result should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicate the presence of the classical translocation. The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of bone marrow and blood samples were identified six atypical signal patterns, but we did not observe false positive cells with the typical positive signal pattern (2F1G1R). The normal cutoff for the 2F1G1R signal pattern was 1.5%, both bone marrow and blood samples. The cutoffs obtained with BETAINV function were validated for counting 200 cells. The analyses of normal and abnormal samples by FISH were in agreement with the karyotype results. **Discussion:** Immediate treatment of patients carrying the t(15;17) translocation is critical due to the risk of early death. *FAST* PML/RARα FISH probe allows rapid detection of the rearrangement, with only one hour of hybridisation required. The probe specificity and sensibility was higher than recommended by the ACMG. Adopt a protocol without cell culture using the *FAST* PML/RARα probe will allow optimizing the process and reducing the release time of the result with the same quality and reliability obtained with the conventional probe. This FISH assay showed high quality and reproducibility and was approved for use in our laboratory.

## B-209

### Accelerated Telomere Shortening in Chinese Parkinson's Disease Patients

Y. Wu[1], W. Cui[1], Y. Pei[1], Z. Yang[2], Y. Guo[1]. *[1]Department of Clinical Laboratory, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*

Background: Telomeres are the repeated sequences which protect the ends of chromosomes. The shortening of telomere length is regarded as an indicator of cellular aging and enhanced by oxidative stress and inflammation. Parkinson's disease (PD) is a long-term degenerative disorder of the central nervous system characterized by both inflammation and oxidative stress. The telomere length in PD is assumed to be shortened, however, the results of telomere length in Parkinson's disease are inconsistent.

Methods: We performed a hospital-based case-control study of 288 cases (137 women, 151 men) of PD patients and 301 (144 women, 157 men) of healthy normal controls, with sex and age matched, from the Peking Union Medical College Hospital, China.

The mean age of the patients at sample collection was 64.0 ± 12.8 years (range 26-89 years). All patients fulfilled the UK PD Society Brain Bank criteria for clinical

PD. The healthy subjects were recruited from those visiting the hospital for a health examination free from neurological disorders. Circulating leukocytes were collected for DNA extraction. LTL was measured by a quantitative PCR method. Biochemical variables, including total protein (TP), albumin, prealbumin, GLU, total cholesterol (TC), triglycerides (TG), high-sensitivity C-reactive protein (hs-CRP), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and homocysteine (hcy) were measured by use of an automatic analyzer. Linear regression was used to analyze the relationship between clinical characteristics and LTL. Logistic regression was used to determine the risk of PD associated with LTL. To further explore telomere length in PD, we searched relevant articles using the same method to detect LTL in Medline, Embase, Web of Science and Cochrane Library to conduct a meta-analysis. All analyses were conducted using STATA 12.0 (StataCorp, College Station, TX, USA) or SPSS 16.0. A two-sided P<0.05 indicated statistical significance.

Results: LTL was significantly shortened in PD comparing with controls (1.09±0.45 vs. 1.69 ± 0.79, P<0.001) and decreased steadily with age in both controls and PD, respectively (r=-0.474, P<0.001; r=-0.187, P<0.001). Meanwhile, the PD group had relative low levels of TP, albumin, TC, HDL-C, LDL-C but high of prealbumin. Through multi-adjustment, only age (P<0.001), hcy (P=0.016) were stably negatively related with LTL. The age and sex adjusted odds ratio (OR) for PD was 12.96 [95% confidence interval (CI) 7.23-23.23, P<0.001] comparing the lowest to the highest quartile of LTL. After search, 6 studies using qPCR to detect telomere length were found, meta-analysis indicated telomere length was significantly shortened in PD (random SMD=0.63, 95% CI 0.03-1.24, P=0.041).

Conclusion: This is the first study exploring the relationship between telomere length and PD in Chinese. Our study indicated telomere length is shortened in Chinese PD patients, and the result is consistent with the pooled result of meta-analysis. These observations suggest that telomere is accelerating shortened in PD patients in comparison to the normal population and shorter telomeres were associated with increased PD risk.

## B-210

### Multiplex Ligation-Dependent Probe Amplification (MLPA) as a diagnostic tool for detection of a large deletion on the *MECP*2 gene in Rett Syndrome.

J. O. Rodrigues[1], T. M. Santos[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil*

**Background:**Rett syndrome (RS; MIM #312750) is a neurodevelopmental progressive disorder, X-linked that occurs almost exclusively in females. It is caused by mutations in the *MECP2* (MIM #300005), *gene* located on chromosome Xq28, which comprises four exons. The prevalence of RS has been estimated to be between 0.25 and 1 per 10 000 female live births. The disorder is characterized by arrested development between 6 and 18 months of age, followed by developmental regression with loss of acquired skills. The patients first lose purposeful hand movements and an interest in the surrounding world, along with speech. They develop apraxia with characteristic stereotypical hand-wringing movements that resemble hand washing, autistic behaviour and learning disabilities. With increasing age, they can also develop additional complex neurologic findings. The most of the pathogenic mutations described in *MECP*2 gene are located in exons 3 and 4. About 5-10% of the pathogenic mutations described are large deletions spanning whole exons of the *MECP*2 gene. Multiplex Ligation-Dependent Probe Amplification (MLPA) has been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as gene large duplications and deletions. **Aims:** Validate the MLPA kit P015 in Brazilian patients with RS. **Methods:** All of the patients include in this study had a consistent clinical diagnosis for this disorder. Thirteen patients with RS were tested using the commercial MLPA kit P405 version A1 (MRC-Holland), following manufacturer's instructions. Three patients were also tested with Sanger sequencing and one patient were tested by array-comparative genomic hybridization (CGH). The analysis was performed using the Coffalyser v.140721.1958 software. **Results:** The MLPA results were concordant in all patients tested with same kit. Three patients presented MECP2 mutations. One of them presented deletion in exons 1 and 2. Another patient presented deletion in exon 3 and one of them presented duplication in exons 1, 2, 3 and 4. Two patients tested by Sanger sequencing presented pathogenic *MECP*2 point mutations. The same patients not presented alterations in MLPA because the test detect large deletions or duplications. Array-CGH identified duplication in *MECP*2 gene in one patient. This finding was also confirmed by MLPA. **Conclusions:** Until recently, no suitable screening method for detecting whole-exon deletions was available. MLPA has become available for the detection of a large deletion on the MECP2 gene allowing genetic confirmation of previously unconfirmed cases of clinical Rett syndrome.

## B-211

### Serological performance of AESKU.Seven-up compared to DiaSorin and Euroimmun ELISA Borrelia kits

T. Matthias[1], J. Blecken[1], A. Platzgummer[2], K. Prager[1], A. Joos[2], A. Lerner[1]. *[1]AESKU. Diagnostics GmbH&Co KG, Wendelsheim, Germany, [2]Clinical Pathology Laboratory, Merano, Italy*

Lyme borreliosis, caused by spirochaetes of the Borrelia burgdorferi genospecies complex, is the most commonly reported tick-borne infection in Europe and North America.

Lyme disease is expanding and its latency, multi-phenotypes and complications deserve a quick, reliable serological diagnosis. Since neither gold standard, nor diagnostic standardized serology exists, 3 commercial ELISA kits were compared for diagnostic accuracy.

Combined IgM+IgG anti-Borrelia antibodies were checked using AESKU.SEVEN-UP, DiaSorin, Liaison® and Euroimmun ELISA kits, on 236 adults suspected to be infected. History and clinical presentation and specific serology distinguished between 61 positive and 177 negative patients. The 61 infected individuals presented early or late, cutaneous, neurological, musculoskeletal, cardiac or ocular manifestations. The table below summarizes the main results:

| Parameters | Liaison IgM & IgG | AESKU.SEVEN-UP IgM & IgG | Euroimmun IgM & IgG |
|---|---|---|---|
| **Sensitivity%** | 70.5 | 57.4 | 63.9 |
| **Specificity%** | 78.5 | **91.5** | 85.9 |
| **PPV** | 0.53 | **0.70** | 0.61 |
| **NPV** | 0.88 | 0.86 | 0.87 |
| **Negative likelihood ratio** | 0.38 | 0.47 | 0.42 |
| **Positive likelihood ratio** | 3.38 | **6.77** | 4.53 |
| Diagnostic odds ratio | 8.74 | **14.54** | 10.78 |

It is concluded that all kits can detect anti-Borrelia antibodies, however, the AESKU.SEVEN-UP outperformed DiaSorin and Euroimmun diagnostic performances in specificity, positive predicted value, positive likelihood and diagnostic odds ratio. Hence, positive predictive values in combination with specificity values indicated that the exclusion of these infections was more relevant than its confirmation, thus, avoiding

unnecessary false alarms and therapy. Taking together, AESKU.SEVEN-UP outperforms DiaSorin and Euroimmun performances in ruling out the Borrelia infection.

## B-212

### Cytogenetics findings in a brazilian male population with infertility

A. M. da Silva, J. M. Fernandes, M. P. Migliavacca, G. A. Campana, J. R. Alves, R. Kuhbauche. *DASA, Barueri, Brazil*

**Background:**

The causes of infertility are diverse, and can occur in men, women, or both. We can mention among the genetic causes of male infertility the aneuploidies, such as Klinefelter Syndrome, translocations between the pseudo-autosomal regions of sex chromosomes, additions or deletions of chromosomal material, mosaicism and inversions.

**Objectives:**

To report the incidence of cytogenetic findings in men referred for karyotype to investigate infertility or gestational loss in a brazilian clinical laboratory.

**Methods:**

Men between the ages of 18 and 50 who performed karyotype examination between 2014 and 2016, with clinical diagnosis of: infertility, miscarriage and complications related to the male reproductive system; hormonal dysfunction; or Klinefelter syndrome.

**Results:**

In this period, 4,843 cases were referred to the laboratory. We identified 108 altered karyotypes (2.2%), 46 cases (0.9%) of Klinefelter's Syndrome (47, XXY), followed by translocations in several chromosomes in 0.6% (27 cases) and inversions in 0.26% (4 cases with inv(Y) and 7 cases of varied inversions). Mosaicism was present in only

4.6% (5 cases) and all presented alterations in the sexual chromosomes, being 2.8% mosaicism for the Klinefelter Syndrome (3 cases).

**Conclusion:**

The study for chromosomal alterations is indicated in cases of complications in fertility, when anatomical and physiological causes have been investigated. In most cases, the patient is unaware of being a carrier for a chromosomal alteration until reaching the reproductive age and experiencing difficulty to conceive. The percentage of altered karyotypes it is still low when compared to the number of exams requested, we can explain this discrepancy by the incorrect indication of the exam, by the technical limitation itself (by not visualizing micro deletions) and finally by the very low incidence of structural rearrangements and aneuploidies. However, the importance of the karyotype examination is maintained for the confirmation of chromosomal alterations as a possible cause of infertility/abortion, as well being able to direct the patient to a possible genetic counseling in case of discovering other cytogenetic alterations.

## B-213

### An evaluation of Roche cobas MRSA/SA test on the Roche cobas 4800 system

B. Lee, J. Tan, S. Chai, G. Wee, A. Omar, J. Murugesh, R. Deepak, M. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

**Introduction**

Routine screening of patients for methicillin-resistant *Staphylococcus aureus* (MRSA), an important nosocomial or hospital-acquired pathogen, followed with cohorting of patients tested positive for MRSA, is one of measures to prevent and control spread of MRSA in healthcare institutions.

We evaluated cobas® MRSA/SA test, a qualitative polymerase chain reaction (PCR) assay for detection of MRSA and *S. aureus* (SA) on cobas 4800 system (Roche Diagnostics, Switzerland), against MRSA*Select*™ II chromogenic agar plates (Bio-Rad Laboratories, USA).

The cobas 4800 system consists of software-driven fully automated sample processing and pre-PCR preparation module and PCR thermo-cycler.

**Materials and Methods**

Total of 53 anonymised nasal, axillary and groin swab samples that have been suspended into Mswab™ liquid media (Copan Diagnostics, USA) were tested for MRSA concurrently using PCR and chromogenic culture methods. Data was analysed using binary matrix.

Limit of Detection (LOD) study was conducted using serially diluted liquid media that was spiked with ATCC MRSA strain. LOD was defined as lowest concentration detectable by method.

Within-run precision studies were conducted using assay quality control material and variance of MRSA, SA and Internal Control (IC) cycle threshold values (Ct) were analysed.

For interference studies, liquid media with MRSA concentration 3 times the LOD was spiked with *P. aeruginosa* and *E.coli* at up to 1 McFarland equivalent concentrations, methicillin-susceptible SA (MSSA) at greater than 4 McFarland equivalent concentration and 0.1 g/L haemoglobin. Difference in Ct as well as the obtained response of test and control samples were evaluated.

Cefoxitin-resistant *S. epidermidis* and *S. lugdunensis* at up to 1 McFarland equivalent concentrations were used to assess analytical specificity.

**Results**

cobas MRSA/SA test yielded diagnostic sensitivity and specificity of 100.00% and 96.43% respectively compared with chromogenic culture. Concordance between both methods was 98.11%. Discrepant results, when investigated, agreed with the PCR findings. Assay LOD was assessed at 1950 CFU/mL.

Precision studies gave coefficients of variation of 0.821%, 0.690% and 0.707% respectively for positive control MRSA, SA and IC Ct and 1.208% for negative control IC Ct.

Compared with control sample, difference of 0.5, 0.3 and 0.3 Ct for MRSA were observed with test samples with haemoglobin, *E. coli* and *P. aeruginosa* respectively.

In presence of high MSSA concentration, assay was able to detect MRSA in test samples. Difference of 0.1-0.3 Ct between test and control samples was obtained. The assay did not yield any false negative results. All tubes of cefoxitin-resistant *S. epidermidis* and *S. lugdunensis* yielded 'not detected' for both SA and MRSA.

**Conclusion**

Our data suggests that cobas MRSA/SA test correlates well with chromogenic culture method with good sensitivities and specificities. MRSA detection in samples was also not affected by common interferences such as haemoglobin, high concentration of MSSA and commonly isolated organisms such as *P. aeruginosa and E.coli*. No cross reactivity was observed with cefoxitin-resistant coagulase negative staphylococcus. As the cobas MRSA/SA test is largely automated and has high throughput, laboratory productivity is increased which translates to cost savings. cobas MRSA/SA test therefore can play an important role in the epidemiological control of MRSA in hospitals.

## B-214

### Application of Matrix Assisted-Laser Desorption Ionization Time-of-Flight Mass Spectrometry for *CYP2D6* Genotype and Copy Number Analysis

F. Wu[1], R. Melis[2], G. A. McMillin[1]. *[1]Department of Pathology, University of Utah, Salt Lake City, UT, [2]Arup Laboratories, Salt Lake City, UT*

**Background:** Cytochrome P450 (CYP) 2D6 enzyme activity is known to affect individual responses to pharmacological treatments, particularly variation in drug levels and risk of dose-related adverse reactions. The prediction of *CYP2D6* phenotype from genotype is complicated by more than 100 single nucleotide variants (SNV), copy number variations (CNV), presence of pseudogenes and hybrid rearrangements. Recently, matrix assisted-laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) has been used to analyze *CYP* genes including *3A4, 3A5, 2C9*, *2C19 and 2D6*. The objective of this study was to use MALDI-TOF/MS to identify *CYP2D6* SNVs and CNVs in samples collected from venlafaxine treated patients. Of note, venlafaxine is a serotonin-norepinephrine reuptake inhibitor used for the treatment of anxiety and major depressive disorders. SNVs and CNVs of *CYP2D6* have been reported to associate with venlafaxine pharmacokinetics and optimal dose.

**Analytical Methods:** seventeen whole blood samples from venlafaxine treated patients were collected pre-dose at steady state from consenting patients. Concentrations of venlafaxine and metabolites were determined and therapeutic doses were recorded in a previous study. Genomic DNA was extracted (PureGene) and samples were de-identified according to institutional protocols. The MALDI-TOF/MS based MassARRAY® System combined with iPLEX® *CYP2D6* panel assay (Agena) was used for *CYP2D6* SNV and CNV analysis. The iPLEX® *CYP2D6* assay includes a panel of 35 pre-designed SNV assays and 5 CNV assays. Each DNA sample was subjected to a multiplexed PCR amplification followed by shrimp phosphatase treatment to neutralize unincorporated dNTPs. Subsequently, samples were subjected to the iPLEX reactions. In an automated Chip prep module the extension products were desalted, and a nanoliter of each reaction was dispensed onto a SpectroCHIP® Array (Agena). Mass of each allele was detected via MALDI-TOF/MS. Data were analyzed using the Typer™ software (Agena). Report tables were generated with presumed haplotypes to predict allele calls and copy number. *CYP2D6* genotypes identified by MALDI-TOF/MS were compared to those obtained previously using the Luminex Bioscience Tag-It Assay.

**Results:** We identified one poor metabolizer (PM) with two copies of nonfunctional alleles (*3/*4) and 4 intermediate metabolizers (IM) with one copy of nonfunctional allele and one copy of decreased functional allele (*5/* 41, *4/*41, and *5/*9). Furthermore, 12 out of 17 samples were identified as extensive metabolizer (EM) with two copies of functional alleles (neg/neg,*35/neg) or one copy of functional allele and one copy of decreased allele (*41/neg, *35/*41, *2A/*41, and *2A/*9) or one copy of functional allele and one copy of nonfunctional allele (*5/neg, *4/neg, and *2A/*4). Among seventeen samples five samples were detected with only one copy of CYP2D6 and one sample was detected with more than 2 copies. MALDI-TOF/MS results were found to be 100% concordant with the findings obtained by Luminex technology.

**Conclusion:** The MassARRAY® System combined with iPLEX® *CYP2D6* panel assay reported here is a suitable and reliable platform for *CYP2D6* SNV and CNV analysis.

## B-215

### Comparison of the *Realtime*HPV HR-S Detection with the Cobas4800 HPV test for the detection of high-risk types of human papillomavirus

H. Yu, S. Lim, S. Lee, H. Woo, H. Park, M. Kwon. *Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of*

**Background:** The *Realtime*HPV HR-S Detection (SEJONGMEDICAL, Paju, Korea) is one of the recently developed assays, which is a real-time PCR based test designed for detecting 14 types of high-risk (HR) HPVs (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). This study was to compare the performance of *Realtime*HPV HR-S Detection to Roche Cobas4800 HPV test (Roche Molecular Systems, Branchburg, NJ, USA) for the detection of high risk (HR) HPV. In addition, we analyzed the distribution of HPV genotypes in the patients with abnormal pathologic test result.

**Methods:** A total of 305 cervical swab specimens were retrospectively collected from patients whose mean age was 43.3 years (22-90 years) between June and September 2016. We tested all the specimens by *Realtime*HPV HR-S Detection and Cobas4800 HPV test. HPV DNA sequencing was subsequently analyzed to confirm the discordant results. HPV distribution by age, results of cytology for 286 patients and biopsy for 43 patients was also analyzed.

**Results:** Cobas 4800 detected one of 13 HR HPV types in 58.7% of specimens, while *Realtime*HPV HR-S detected in 59.0% of specimens. The overall agreement rate between the assays was 96.1% with 0.947 kappa coefficient. One of the discordant sample was revealed that the result from Cobas was equal to sequencing and the rest 11 samples were revealed that the results from *Realtime*HPV were equal to sequencing. Sensitivity and specificity of 16, 18 and other high HPV detections were high enough (Cobas: 95.9%-100% and 97.8%-100%, and *Realtime*HPV: 98.0-100% and 100.0-100.0%). At the distribution by age, 31-40 age group showed 76.7% (69/90) positive rate while 51-60 age group showed 40.8% (20/49). Upon cytological examination, HPV positive rate of the patients in high-grade squamous intraepithelial lesions (HSIL), LSIL, and atypical squamous cells of undetermined significance (ASCUS) was 72.7% (8/11), 82.4% (14/17), and 82.8% (24/29), while in normal cytology 51.6% (112/217). The patients who were reported CINI, II, III, carcinoma in situ, and invasive cancer by cervical biopsy showed 78.6% (22/28) of HPV positive rate but normal and benign patients showed 33.3% (5/15) of positive rate.

**Conclusion:** Considering the high agreement rate with Cobas 4800 HPV test, more than 95.9% of sensitivity and specificity, and ability to differentiate HPV 16/18 from other HPV types, *Realtime*HPV HR-S Detection could be a reliable laboratory testing method for the screening of HPV infections. Moreover, as HPV detection rate is markedly higher in patients with precancerous lesion than normal or benign patients, early detection of HPV is very important to screen for early detection of cervical cancer.

## B-216

### Comparison study of manual and automated extraction systems for cell-free circulating DNA

J. Shaw[1], C. Cowley[1], H. Joos[2], C. Jurinke[2]. *[1]Department of Cancer Studies & Molecular Medicine, University of Leicester, Leicester, United Kingdom, [2]Stratec Molecular GmbH, Berlin, Germany*

Background:

Cell-free circulating DNA (cfDNA) is of vastly growing diagnostic interest in cancer treatment and prognosis therefore there is a need for automation of the respective extraction methods in order to standardize procedures. Since cfDNA can be present at low concentrations in plasma, the method needs to maximize the possible output whilst maintaining a high enough purity and quality to work optimally in downstream analyses such as qPCR, digital PCR or Next Generation Sequencing (NGS).

Methods:

For this study, an automated extraction method (InviMag Circulating DNA Kit / Stratec ) was compared with a manual method (QIAamp Circulating Nucleic Acid Kit/ Qiagen). Both methods isolated the DNA from a starting volume of 4 ml of plasma into an elution volume of 80 µl. 8 ml of 68 plasma samples from: No Cancer (4), Breast Cancer (3), NSCLC=Non-small cell lung cancer (16), SCLC = Small cell lung cancer (3), and Metastatic Melanoma Patients (42) were aliquoted in two portions of 4 ml and processed by each method. To determine the potential influence of storage duration, three groups of samples were used: short term stored (less than 12 months; 16), mid-term stored (up to 5 years; 45) and long term stored (>7 years; 7). After extraction, all DNA samples were measured by fragment analysis on a TapeStation 4200 (Agilent) and by qPCR concentration measurement (InviQuant GeneCount - Stratec). Digital droplet PCR (BioRad) was performed for BRAF V600K mutation (8 samples – Melanoma), BRAF V600E mutation, (28 samples – Melanoma) and EGFR T790M mutation (5 samples, NSCLC).

Results:

Fragment analysis by TapeStation showed the characteristic cfDNA fragments of 170 bases in most of the extractions from both methods. Nevertheless some QIAamp extractions showed additional signals of fragments above 500 bases. Quantification by TapeStation showed a lower mean concentration of cfDNA with the automated method than with the manual method, whereas with qPCR mean concentration values, InviMag extracted samples were slightly higher than QIAamp samples. In general, cfDNA concentrations measured in InviMag samples by TapeStation showed a stronger correlation with concentrations measured by qPCR, than those isolated with the manual QIAamp method. Nevertheless, both methods delivered comparable cfDNA yield, especially measured by qPCR.

No differences between the short term stored, mid-term stored and long term stored samples could be observed. We conclude, that storage at -70°C can stabilize cfDNA in plasma samples for years.

Digital PCR results were comparable in both methods in terms of accepted droplet counts and fractional abundance of detected mutations. Excluding invalid samples, both methods displayed 100% correlation for the detected mutations in this study.

Conclusion:

We conclude that the automated method is appropriate for cfDNA extraction.

## B-217

### Non-invasive fetal sex determination using cell-free fetal DNA isolated from maternal capillary blood obtained by fingertip puncture: the elimination of exogenous male DNA from the collection site is crucial.

T. H. Santa Rita, C. S. Nobre, R. H. Jácomo, L. F. A. Nery, G. B. Barra. *Laboratório Sabin, Brasília, Brazil*

Background:

Noninvasive fetal sex determination is a test capable to indicate the baby's sex at the beginning of pregnancy. This assay searches the Y-chromosome in a maternal blood sample drawn by venipuncture. Its presence indicates the gestation of a boy and its absence indicates a girl. Despite its clinical indications, the greatest demand is from mothers eager to know the sex of their unborn child. With the intention of bringing comfort to the mothers and evaluate the presence of fetal DNA in maternal microcirculation, we idealized to perform it in the capillary blood. Thus, the aim of the present study was to investigate whether fetal sex determination performed on plasma isolated from capillary blood is comparable to venous blood. The latter is a well-established method in our laboratory.

Methods:

This study enrolled 101 pregnant volunteers. The gestation weeks ranged from 8 to 20 weeks, and the median was 11 weeks. After asepsis with isopropyl alcohol, venous and capillary bloods were collected at the same occasion in appropriated EDTA tubes by cubital fossa venipuncture and fingertip puncture, respectively. EDTA-plasma was isolated within two hours (150 uL for capillary and 1mL for venous bloods) and submitted to an automated DNA extraction. The multicopy sequence DYS-14 was assessed in quadruplicate by qPCR. RNAse P gene was co-ampified in all instances. After testing the first 27 volunteers, it was noted that exogenous Y-chromosome is present on women's fingertips, what results in a strong false-male (positive) signal in almost half of the samples from mothers bearing female fetuses by the reference method. Thus, a fingertip's asepsis with diluted sodium hypochlorite solution was implemented and the volunteers were divided in 3 groups: the isopropyl alcohol (n=27; 15 males, 12 females), the 0.5% buffered sodium hypochlorite twice (n=39; 20 males, 19 females) and 1% buffered sodium hypochlorite once (n=35; 17 males, 18 females). The total, male (positive) and female (negative) agreements between the results for each specimen were computed in all groups. The degree of agreement was also quantified by kappa statistics.

Results:

For the isopropyl alcohol group, the total, male and female agreements between capillary and venous bloods were 81%, 100% and 58%, respectively (Kappa = 0.6, good). For the 0.5% buffered sodium hypochlorite twice group they were 100%, 100%, and 100%, respectively (Kappa = 1, perfect). For the 1% buffered sodium hypochlorite once group, the total agreement was 100%, the male agreements was 100% and the female agreement was 100% (Kappa = 1, perfect).

Conclusion:

Fetal DNA is present in the maternal microcirculation allowing the execution fetal sex determination on the capillary blood. The capillary blood collection is much less invasive than venipuncture bringing comfort to the mother. Moreover, it offers the possibility of home self-sampling. However, exogenous male DNA could be present at the women's fingertips and for a reliable fetal sex determination by using the above-described method, the elimination of exogenous male DNA from the collection site is critical. Furthermore, the knowledge gained in this study can also impact the forensic sciences, specifically, the touch DNA field.

## B-218

### Simplified workflow for BCR-ABL1 e14a2/e13a2 fusion quantification in whole blood by semi-automated nucleic acids extraction, multiplex one-step RT-qPCR and delta-delta Cq method.

P. G. Mesquita, T. H. Santa Rita, R. H. Jácomo, L. F. A. Nery, G. B. Barra. *Laboratório Sabin, Brasília, Brazil*

**Background**:

Molecular testing for the BCR-ABL1 fusion gene by RT-qPCR is the most sensitive approach for monitoring the response to therapy of patients with chronic myeloid leukemia. The most frequent fusions subtypes are e13a2 and e14a2 (found in 97-98% of the patients). Moreover, there are guidelines describing consensus best practices for monitoring these transcripts. These documents report multi-steps workflows that are not routine-friendly. Thus, the objective of the present study was to validate simplified e14a2/e13a2 quantification in whole blood by RT-qPCR.

**Methods**:

Positive samples were constructed by the spiking of known amounts of k562 cells into the negative peripheral blood (5 mL) or in-vitro transcribed e14a2 or e13a2 RNA into the samples during the nucleic acid extraction. Red blood cells were removed by using PharmLyse (BD Biosciences). Nucleic acid was extracted by using EasyMag (Biomerieux). e14a2/e13a2 and ABL1 mRNA were co-amplified with the Europe Against Cancer primers/probes in a duplex one-step RT-qPCR reaction performed with QuantiNova RT-qPCR master mix (Qiagen) on light cycler 480 II (Roche). For quantification, standard curves were constructed with ssDNA oligos. Amplification efficiencies were retrieved from these curves and the applicability of the ΔΔCq method was evaluated. The absolute and relative quantification were compared using samples spiked with K562 (n=28, $1.39x10^6$ to 15 cells) by linear regression and Bland-altman statistics. The limits of detection for e14a2, e13a2 and K562 RNAs were determined applying probit regression analysis to a serial dilution of each target. Precision was evaluated by testing samples spiked with $10^5$, $10^3$, and $10^2$ k562 cells in triplicate during 6 days. The accuracy was investigated comparing the proposed method with Xpert BCR-ABL Monitor IS G2 (Cepheid) (n=53, with K562 cells from $1.39x10^6$ to 0) using linear regression and Bland-altman. Experiments with K562 were calibrated to international scale (IS) using calibrator panel e14a2 (Asuragene).

**Results**:

The median (Max-Min) of ABL1 achieved by the proposed workflow was $1.1x10^6$ ($1.75x10^7$-$4.3x10^5$) copies/sample. e14/a2, e13/a2 and ABL1 RT-qPCR efficiencies did not differ in all tested occasions, meaning that the ΔΔCq method is applied (absolute and relative quantification comparison reveled: $R^2$ of 0.99 and bias of -0.0073 log). The limits of detection were 0.138 (95%CI 0.041-3.7), 0.017 (95%CI 0.004-1.23) and 0.093 IS (95%CI 0.075-0.131) percent of BCR-ABL1/ABL1 for e14/a2, e13/a2 and K562 RNAs, respectively. In the precision assay, the medians (Max-Min) were 32.8% (33.9-31.6), 0.52 (0.66-0.42) and 0.059 (0.022-0.075) for the samples with $10^5$, $10^3$, and $10^2$ k562 cells, respectively. Qualitatively, the total agreement between the proposed method and expected results was 98.1% (95%CI 90-99%) and between Xpert and expected result was 86.8% (95%CI 75-93%). Quantitatively, the $R^2$ and bias for the proposed method *versus* Xpert were 0.89 and 0.57 log, respectively.

**Conclusion**:

We described a reliable and routine-friendly workflow for BCR-ABL e14a2/e13a2 fusion quantification in whole blood that reduces the number of steps proposed by the current guidelines. The proposed workflow showed acceptable sensitivity, precision and accuracy. The assay reached the molecular response 5 ($MR^5$) based on the ABL1 molecules extracted per sample and $MR^3$, $MR^4$ and $MR^3$ for 14/a2, e13/a2 and K562 RNAs, respectively.

## B-219

### Incidence of Turner syndrome in a brazilian female population with infertility

A. C. M. Romera, A. M. da Silva, M. P. Migliavacca, J. M. Fernandes, G. A. Campana, J. R. Alves, R. Kuhbauche. *DASA, Barueri, Brazil*

**Background:** Infertility affects approximately 10% of women between the ages of 15 and 44 and the female factor accounts for 30% of the causes of infertility. Some of the causes of female infertility are: anatomical abnormalities, genetic alterations and age. Among the genetic alterations, we can mention Turner syndrome, translocations between the pseudo-autosomal regions of the sex chromosomes, mosaicism and inversions. For every 2,500 girl born, 1 is a carrier of Turner syndrome, often discovered when there are recurrent miscarriages or amenorrhea.

**Objectives:** To report the incidence of cytogenetic findings in women referred for karyotype to investigate infertility or gestational loss in a brazilian clinical laboratory.

**Methods:** Analysis of the cytogenetic findings of female karyotype between the ages of 18 and 40, referred for the investigation of infertility, abortion and amenorrhea during the years of 2015 and 2016.

**Results:** 3,330 cases were referred to the laboratory. We identified 60 altered karyotypes (1.8%), with 24 cases (0.7%) related to the X chromosome, 4 cases (0.1%) of women with karyotype 46,XY and 32 cases (0.9%) with other changes such as inversion, translocation, robertsonian translocation or the presence of a marker chromosome. Among the related to X, 9 cases were 45,X0 (0.27%), 8 cases of mosaicism (0.24%), 2 cases with a deletion on Xq (0.06%), 1 derX (0.03% ), 1 isoX (0.03%), 1 dupX (0.03%), 1 translocation (X;5) (0.03%) and 1 mosaic of Turner with a derX (0.03%).

**Conclusion:** The karyotype is essential for the discovery of chromosomal alterations related to infertility, such as Turner syndrome. Genetic counseling becomes a possibility for those patients with the presence of mosaicism for Turner syndrome. Only 2-10% of syndromic women achieve spontaneous pregnancy, but anomalies on the X chromosome can be transmitted to offspring, increasing the risk of miscarriages, congenital malformations and chromosomal abnormalities.

## B-220

### *PRKAR1A* Sanger Sequencing and Deletion/Duplication Analysis for the Clinical Diagnosis of Carney Complex

S. A. Lagerstedt, L. J. Train, J. J. Maleszewski, L. M. Baudhuin. *Mayo Clinic, Rochester, MN*

**Background:**

Carney Complex (CNC) is an autosomal dominant condition, characterized by spotty skin pigmentation, endocrine hyperactivity, and myxomas (both cardiac and non-cardiac). Cardiac myxomas may result in an obstruction of blood flow, embolism, and/or heart failure. The phenotype overlaps with Cushing Disease, Primary Pigmented Nodular Adrenocortical Disease (PPNAD) and other adrenal hyperplasias. The majority of CNC is due to pathogenic variants in *PRKAR1A*, coding for the type 1-alpha regulatory subunit of protein kinase A. More than 125 pathogenic variants in *PRKAR1A* have been identified. The estimated prevalence is 1:1,000,000 with 100% penetrance and approximately 160 index cases reported hitherto. Approximately 70% of individuals diagnosed with CNC have an affected parent, and approximately 30% have a de novo pathogenic variant. Sequence analysis of *PRKAR1A* will detect a pathogenic variant in 60% of probands, while an additional 10% of probands may harbor a large deletion or duplication variant. While immunohistochemical (IHC) staining analysis may be used to screen for CNC, identification of a *PRKAR1A* pathogenic variant can confirm diagnosis.

**Materials and Methods:** We have developed and validated a clinical assay for *PRKAR1A* variants using Sanger sequencing and qPCR for Deletion/Duplication. In this procedure, genomic DNA is first extracted from whole blood, followed by polymerase chain reaction (PCR) amplification of all exonic regions and intron/exon boundaries of the gene. Following enzymatic digestion to purify the PCR product and remove it from unincorporated primers and nucleotides, bi-directional Sanger sequencing is performed using universal primers and fluorescent-dye terminator chemistry. Sequencing products are separated on an automated sequencer and trace files are analyzed for variations in the exons and intron/exon boundaries of all exons using Mutation Surveyor™ software and visual inspection. Deletion/Duplication Analysis consists of a very short (80-150 bp) PCR amplification in a reference gene and in all *PRKAR1A* exons. During PCR, SYBR Green intercalates into the double stranded DNA structure and the fluorescence increases greatly. The fluorescent signals are used to calculate a relative copy number.

**Results:** To validate the accuracy of the assay, we compared our Sanger sequencing results with results reported in the 1000 Genomes Project for 4 Corriell samples, and we compared our Deletion/Duplication results with array-CGH results for 3 samples that were previously identified with large deletions or duplications. Assay imprecision was also assessed.

**Conclusions:** In summary, we have developed and validated a clinical assay to detect *PRKAR1A* variants including large deletions and duplications. This test will aid in the clinical diagnosis of CNC in individuals, specifically in confirmation of those cases that exhibit loss of immunohistochemical PRKAR1A expression.

## B-221

### Cytogenetic findings in Brazilian patients investigating for autism spectrum disorders

A. P. Debei, M. P. Migliavacca, J. M. Fernandes, G. A. Campana, J. R. Alves, M. Conchon, R. Kuhbauche. *DASA, Barueri, Brazil*

**Background:** The array-CGH technics utilize both molecular and the cytogenetics approach to perform the whole genome analysis with a higher resolution than previous technics such as the karyotype. Now it is possible to detect microdeletions or microduplications (loses or gain of genetic material) previously not visualized by karyotype. These changes are often found in patients with behavioral disorders such as autism. Therefore, the array analysis was established as a first line test to investigate autism spectrum disorders However, there are still many requests for karyotype analysis for these patients.

**Objective:** To identify the cytogenetic findings in patients referred to investigate autism spectrum disorders.

**Methods:** Statistical analysis between the years 2014 and 2016 from all cases referred to perform a peripheral blood karyotype with a autism diagnostic hypothesis.

**Results:** 488 patients were analyzed in this period, 478 without any abnormalities, 5 cases with chromosome 9 inversion; 1 case with chromosome 9 duplication; 2 patients with a marker chromosome; a Robertsonian translocation involving the chromosomes 14 and 21; and finally a chromosome 22 ring.

**Conclusion:** In the evaluation of the 488 patients, only one had a relevant alteration for autism (the chromosome 22 ring). This is justified because most of the alterations are undetectable using the karyotype. Studies have shown that array-based tests make it possible to diagnose 15 to 20% of patients with autism spectrum disorders and other behavioral disorders, while the G-banding karyotype detects changes in about 3% of cases not associated with clinically recognizable chromosomes syndromes. We can explain the still high demand for karyotypes by the particular scenario of health insurance companies that require a normal karyotype to perform the array, medical education to request and interpret a new test, and the higher cost than conventional karyotype. We expect that addressing this issues we will help change this scenario.

## B-222

### Cytogenetics findings in Jacobsen Syndrome

A. C. M. Palumbo, M. P. Migliavacca, J. M. Fernandes, G. A. Campana, J. R. Alves, R. Kuhbauche. *DASA, Barueri, Brazil*

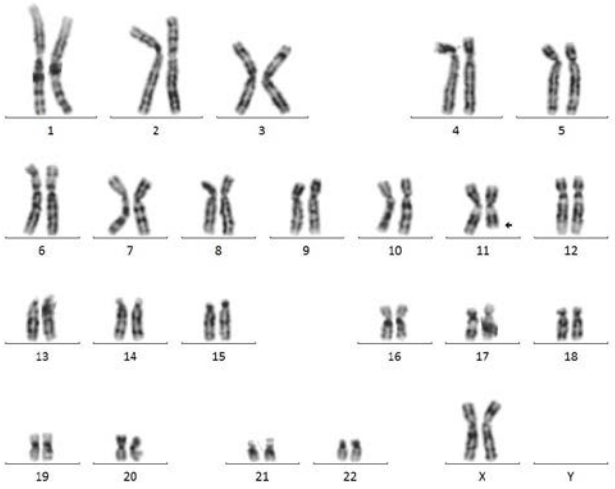
**Background:** Jacobsen syndrome is rare and caused by the terminal deletion of the long arm of chromosome 11, more specifically in the 11q23 band. This region is home to a hereditary folate-sensitive site. The first time this disorder was observed was in 1973 by Jacobsen. This syndrome results in a complex phenotype characterized by delayed neuropsychomotor development, craniofacial anomalies, varied cardiac defects, and blood dyscrasias. The diagnosis is initially based on karyotype analysis when the deletion involving the q23 band is identified. It is also possible to obtain confirmation through molecular studies (FISH), where the absence of the FLI-1 and JAM-3 genes is visualized. Studies confirm that only 15% of these alterations may have been inherited and 85% occur *de novo*.

**Objective:** To report the efficiency of cytogenetics in the diagnosis of Jacobsen Syndrome in a Brazilian population.

**Methods:** A survey of karyotypes performed at DASA Cytogenetic Laboratory of São Paulo from January 2014 to January 2017, with a deletion in the 11q23 region visible by microscopy.

**Results:** During this period 6,286 constitutional karyotypes of patients up to 20 years old were performed, with the diagnostic hypothesis compatible with some of the features found in Jacobsen Syndrome. Of these karyotypes, only 3 cases presented the 11q23 deletion.

**Conclusion:** The results demonstrate the small incidence of Jacobsen Syndrome. We can conclude, due to the size of the deletion, that it is possible to be visualized through classical cytogenetics in the analysis of the karyotype with resolution of at least 400Mb, confirming the Jacobsen Syndrome, and guaranteeing the efficacy of the analysis method. However, deletions less than 7.5 Mb which involve the genes for the development of the syndrome may not be identified in the cytogenetic study, being necessary the complementation with the analysis by FISH.




## B-223

### IL28B genotipe and liver fibrosis in patients with chronic hepatitis C

I. Cebreiros López, J. A. Noguera Velasco, F. Guzmán Aroca, J. D. D. Berna-Senra, A. Vargas Acosta, F. Carballo Álvarez. *Clinical University Hospital Virgen de la Arrixaca, Murcia, Spain*

**Background:** Interleukin (IL)28B polymorphisms are strongly associated with spontaneous clearance of hepatitis C virus (HCV) infection and response to therapy, but whether IL28B genotype affects liver fibrosis severity is unclear. Our aim was to study the relationship between IL28B genotype and markers of liver fibrosis.

**Methods:** We studied a population of 120 chronic hepatitis C patients. Liver fibrosis was assessed by shear wave velocity (Vc) determined by Arfi technique. In all patients was determined IL28B polymorphism, biomarkers of fibrosis hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP-1), tissue inhibitor of metalloproteinase 1 (TIMP-1), platelets, AST and ALT. Moreover algorithms for estimating the degree of fibrosis ELF, Apri, Forns, Fibrotest, Fibrometer, Fib-4, Fibro-Q and Hepascore were calculated. An abdominal ultrasound was also performed to evaluate liver disease.

**Results:** Patients had a mean age of 49.5 years and 64% were men. Prevalent viral genotype was 1 (79.5%) and distribution of IL28B genotypes was as follows: 22% CC, 64.7% CT, and 13.3% TT. Patients with CT genotype had significantly lower concentrations of PIIINP ($p=0.006$), TIMP-1 ($p=0.039$) and higher platelet count ($p=0.001$) compared to those with CC and TT. Apri, Forns, Fibrotest, Fibrometer, Fib-4 and Fibro-Q also were significantly lower in CT patients ($p<0.05$). AST, ALT, ELF and Hepascore were also lower in CT patients but differences were not significant. Furthermore CT patients have lower degree of liver fibrosis according to a lower Vc ($p=0.039$) and lower presence of ultrasound findings of liver disease ($p=0.008$).

Several authors have suggested that CC genotipe is associated with a state of enhanced immunity that can promote viral clearance but alternately can increase the liver damage. Other authors have noted that T allele is more prevalent in patients who develop cirrhosis. According to this and based on our results, the combination of the two alleles (CT) could be the most favorable option in relation to the severity of fibrosis.

**Conclusions:** The genotype CT of IL28B is associated with a lower degree of liver fibrosis determined by biomarkers, imaging techniques (ultrasound) and elastography techniques (Arfi).

## B-224

### Minimal Residual Disease Monitoring in AML by RT-qPCR of NPM1 mutations

S. T. Campbell, L. A. L. Bazydlo, M. S. Mahadevan. *University of Virginia, Charlottesville, VA*

**Background:** While many markers exist for cytogenetically abnormal AML, standard-risk AML offers a limited number of markers with which to base treatment decisions. In order to offer physicians at our hospital a better tool for prognostication and treatment, we recently investigated the use of a newly published method to track NPM1 mutation status in peripheral blood as a marker of residual disease. **Methods:** An RT-qPCR method was devised based on work by Ivey et al published last year. We modified the protocol by analyzing various PCR conditions on positive and negative cell line DNA to determine the best conditions for the RT-qPCR reaction. This was then followed by patient samples with various mutational statuses confirmed by the Illumina TruSight Myeloid Sequencing Panel. **Results:** We determined that the RT-qPCR reaction run with primers designed by Ivey et al were able to determine the presence of mutated NPM1 transcripts in peripheral blood of positive patients, though it was not able to distinguish Type A (TCTG insertion) from Type B (CATG insertion). Efficiency of the PCR reaction was determined to be 102% and 97% for the NPM1 and Abl reactions, respectively, with linear correlations of greater than .99 across over 1000-fold dilution of RNA. Limit of detection was determined to be .01% mutated RNA in wild-type RNA background, with potential for lower limits. Concordance with the TruSight Myeloid panel was 100% between wild-type and Type A mutation patients, with an N of 5 and 2, respectively. In addition, we determined that the RT-qPCR reaction could be run at a single annealing temperature for both the NPM1 and control ABL reactions as well as with a simpler intercalating dye reaction instead of the costlier quenched probe assay without sacrificing specificity or sensitivity. A larger cohort study is now underway in our institution. **Conclusion:** We now have a successful pre-validation this assay for use in determining minimal residual disease in NPM1-mutated AML patients. While it is unfortunate that the assay cannot distinguish Type A and Type B mutations, the prognostic information is currently identical for both types, allowing this assay to be used for additional patients. We have also successfully simplified the assay through several means from the original paper, reducing necessary tech time and potential mistakes.

## B-225

### Association between angiotensin-converting enzyme gene insertion/deletion polymorphism and risk of recurrent miscarriage in Middle Eastern population

M. K. Ahmed[1], H. M. Shams[2]. *[1]Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt, [2]Clinical and Chemical Pathology Department, National Cancer Institute, Cairo, Egypt*

**Background** Angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin-aldosterone system, converts angiotensin I to angiotensin II. Recurrent pregnancy loss (RPL) had said to be related to the angiotensin converting enzyme insertion/deletion gene polymorphisms (ACE I/D). But the conclusions were controversial. This study was conducted to investigate the real association in ACE I/D polymorphisms and RPL.

**Method** A total of 786 women (496 with history of RPL and 290 with no history of RPL; 21 to 36 years old) from 4 different Middle Eastern countries (Egypt (213), Saudi Arabia (297), Qatar (173) and United Arab Emirates (103)) whose know to be wild type for both factor V Leiden and factor II (prothrombin) genes mutation included in the study. Genomic DNA extraction was performed using Qiagen DNA blood extraction kit, ACE I/D genotype was assayed using validated reverse hybridization polymerase chain reaction (PCR) kit from ViennaLab Diagnostics GmbH Vienna, Austria.

**Results** We found that the genotype frequencies of ACE I/D (24.1% vs. 18.3%) and D/D (59.7& vs. 4.5%) were more seen in women with RPL compared with the women with no RPL history, the difference between the two groups is statistically significant ($p$<0.001).

**Conclusion:** Our data shows a significant association between ACE I/D polymorphism and recurrent miscarriage risk. ACE polymorphic D allele contributes to increased risk of recurrent miscarriage.

## B-226

### Confirmation of CGG Repeats in Brazilian Women Tested for Fragile X Syndrome

L. T. Galindo, P. Y. Nishimura, P. G. C. Trojano, L. C. Scarpelli, D. Moratori, M. P. Migliavacca, J. Reis, G. Campana. *DASA- DIAGNOSTICOS DA AMERICA, BARUERI, Brazil*

**Background:** Fragile X syndrome (FXS) is a common genetic condition that causes intellectual disabilities, including deficits in development. It affects both males and females of all ages, but the frequency of the disease is higher in males. The repetition of the trinucleotide CGG in the 5´UTR region and the methylation status of *FMR1* (Fragile X Mental Retardation 1) gene are the major causes for this disorder. Clinical interpretation is based on the number of CGG repeats and alleles with more than 200 CGG characterizes a full mutation with *FMR1* gene inactivation, 55-200 CGG is considered a premutation, 45-54 CGG is considered a grey-zone and in healthy people, this segment is repeated around 5-45 times. Diagnostic testing for FXS usually relies on *FMR1* gene PCR and conventional sequencing. The *AmplideX™ FMR1 PCR kit* (Asuragen, Austin, USA) enables the conversion of the size of PCR products to number of CGG repeats. After sequencing, the electropherograms are analyzed and it is possible to identify gene-specific product peaks, however in some female samples it is difficult to observe both alleles (two peaks) due to the very similar number of CGG repeats.**Objective:** Here we report the incidence of the identification of just one allele in blood samples from female patients submitted to FXS diagnostic test in a large Brazilian private laboratory.

**Methods:** 199 samples collected during the year of 2016 from women from zero to 66 years old were evaluated using the *AmplideX™ FMR1 PCR kit*. Briefly, a PCR was performed using specific primers that spun the CGG repeat region. PCR products were sequenced at the 3730xl DNA analyzer and the electropherograms analyzed using the GeneMapper v4.0 software. The archives originated in the 3130xl were analyzed using GeneMapper and the sizes of the alleles were plotted in an excel file that converted sizes to number of CGG repeats using a specific formula.

**Results:** From all 199 samples, 40% (79) presented only one peak, which represented only one allele; 54% (107) were considered normal; 4% (8) were classified as premutaded; 2% (3) grey-zone and 1% (2) fully mutated.

**Conclusion:** Since it was not possible to define whether the other allele was fully mutated or the difference between the amounts of CGG in both alleles were so small making it impossible to distinguish the difference between then, we established a routine to solve this issue. The injection time during sequencing was decreased from 5 to 2.5 seconds, in order to avoid signal saturation, and after decreasing injection time, it was possible to confirm that the samples that showed only one peak in fact presented signal saturation and looked like CGG repeats of only one allele. After the decrease of injection time, it was possible to observe a small but clear difference of CGG repeats between the two alleles with the separation of the peaks and ensuring the correct result.

## B-227

### Development and Validation of a Quantitative Digital Droplet PCR Assay for Detection of *KRAS* Mutations in Codons 12, 13, 61 and 146 in Plasma cfDNA

D. Milosevic, J. R. Mills, N. Vidal-Folch, W. E. Highsmith, M. Liu, K. C. Halling, B. R. Kipp, S. K. Grebe. *Mayo Clinic and Foundation, Rochester, MN*

**Introduction:** Approximately 30-50% of colorectal cancers (CRC) have mutations in *KRAS*. Most occur in hotspot regions in codons 12, 13, 61 and 146. These mutations lead to constitutive activation of the RAS/MAPK pathway downstream of EGFR, limiting the effectiveness of anti-EGFR therapies, such as cetuximab and panitumumab, which inhibit ligand-mediated activation of EGFR. Therefore, identification and quantitation of these mutations is critical in selecting the appropriate therapy. Liquid biopsies might provide a less invasive and cost effective alternative to tissue biopsies to establish KRAS status. **Objective:** To assess the analytical performance of three commercially available KRAS assays: A screening KRAS12, 13 assay for detection of mutations in codon 12 and 13 (G12A, G12C, G12D, G12R, G12S, G12V, G13D), a screening KRAS 61 assay for detection of mutations in codon 61 (Q61L, Q61H, Q61R, Q61K ) and a targeted assay for detection of KRAS A146T mutation ( BioRad, Hercules, CA) in patient plasma. **Materials and methods**: We used *KRAS* reference standards (Horizon Discovery), nucleosomal *KRAS* cell line DNA (ATCC H747, SW948, SW48), formalin-fixed paraffin embedded (FFPE) tissue DNA, and cell free DNA (cfDNA) from patients and healthy volunteers. Blood

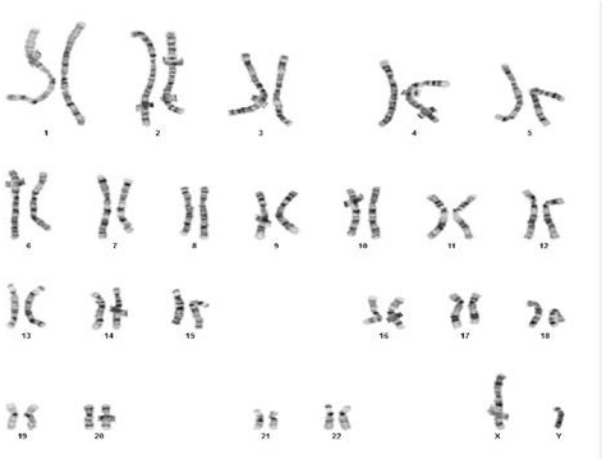
samples were collected into Streck Cell-Free DNA BCT® tubes. DNA was extracted using Qiagen's QIAmp Circulating Nucleic Acid kit. Simultaneous quantitation of the wild type (WT) and KRAS mutants (MT) was performed using the AutoDG and QX200 ddPCR multi-well BioRad system (Hercules, CA).We assessed sample stability, limit of detection (LOD), limit of quantitation (LOQ), analytical precision, dilution linearity, analytical specificity, and cross reactivity. For accuracy assessment, we performed spike recovery and re-testing of genotyped FFPE DNA samples (n=60) and Horizon Discovery reference standards (n=10). **Results:** Plasma stability was acceptable up to 21 days at -80°C. For all three KRAS assays the LOD of plasma DNA was established at 5 positive droplets (15 copies/ml plasma), based on CLSI guidelines. Intra- (n=20) and inter -assay (n=20) imprecision were <20% CV at 42 copies/ml plasma. Based on this observation, the LOQ was established at 42 copies/mL plasma. Serial dilutions of KRAS-positive cell lines and KRAS-positive plasma-derived DNA were linear down to 34 copies/mL for KRAS 12, 13 assay, 31 copies/mL for KRAS 61 assay, and 27 copies/mL for KRAS A146T assay. Analytical specificity was assessed by adding increasing amounts of WT DNA (up 10 times the normal level) in a mutant sample (0-5772 copies/ml plasma). There was no significant change in MT copies measured. Cross-reactivity between KRAS 12/13, KRAS 61 and KRAS A146T assays was undetectable using KRAS reference standards. Recoveries for spike in experiments were 100% for KRAS 12/13 (range 85-118%), 109% for KRAS 61 (range 97-117%) and 105% for KRAS A146T (range 92-121%). Plasma DNA from 60 normal donors was negative for each KRAS mutation tested, while all FFPE and reference standards were KRAS mutation positive. **Conclusions:** The tested KRAS assays are fast, reliable and accurate in detecting and quantitating KRAS mutations in liquid biopsies for therapy selection and might also prove useful in assessing patients' responses to treatment.

## B-228

### FISH Microdeletion in the Diagnosis of Complete Androgen Insensitivity Syndrome

G. Ilyas, D. B. Laskar, A. Ahmad, A. Zuretti. *SUNY Downstate Medical Center, Brooklyn, NY, 11203, Brooklyn, NY*

Complete androgen insensitivity syndrome occurs when the body cannot use androgens at all. Individuals with this condition have the external sex characteristics of females, but do not have a uterus and therefore do not menstruate and are unable to conceive a child (infertile). Affected individuals have male internal sex organs (testes) that are undescended, which means they are abnormally located in the pelvis or abdomen. Undescended testes have a small chance of becoming cancerous later in life if they are not surgically removed. Laboratory diagnosis is rendered by conventional karyotyping (46, XY) and confirmed if androgen receptor gene sequencing reveals a mutation. Our patient is a four-year old phenotypical female who was brought to the outpatient clinic by her mother for evaluation of a painless right inguinal swelling. On physical examination, she had a normal vaginal introitus and urethral meatus with absence of clitoromegaly. The right inguinal swelling was reducible. Pelvic ultrasound showed bilateral inguinal hernias, absence of the uterus, and presence of bilateral gonads in the inguinal canals. Total testosterone was 6.5 ng/dl (normal range 1-3 ng/dl). Karyotype was 46, XY. FISH Microdeletion showed an SRY hybridization signal Yp11.3(SRY+). AR gene sequencing detected no mutation. A conventional karyotyping or fluorescent in situ hybridization (FISH) probes for either the SRY region of the Y chromosome or a subtelomeric Y chromosome probe are essential to differentiate an undermasculinized male from a masculinized female. FISH microdeletion can detect upwards of 98% of the SRY signal hybridization for complete androgen insensitivity syndrome and partial androgen insensitivity syndrome. The analysis is performed on DNA obtained from buccal swabs. Mutation analysis of the androgen receptor gene is now available. Hence, chemistry and genetics analysis play a pivotal role in the diagnosis of androgen insensitivity syndrome.



## B-229

### Determination of apoE isoforms in young with Alzheimer's disease in the family and its association with cognitive tests and dyslipidemias

M. C. Feres[1], K. M. M. Sousa[1], D. Ramadan[2], G. S. Silva[2], R. Piovezan[1], B. B. Perez[3], R. Bini Jr.[1], J. T. T. Torrecilhas[2], A. A. Lino de Souza[1], D. Poyares[1], S. Tufik[2]. *[1]Universidade Federal de Sao Paulo- Brazil, Sao Paulo, Brazil, [2]Associação Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil, [3]Associacção Fundo de Incentivo a Pesquisa- AFIP, Sao Paulo, Brazil*

**Background:** The association with Alzheimer disease (AD) reinforces the need for faster and cheaper methods for Apolipoprotein E (ApoE) genotype and their isoforms E2, E3 and E4 as well as early cognitive methods. Many studies in this area are being developed but are still in inconclusive phase regarding the risk factors that lead to the establishment of AD, so the authors of the study aims to study the lipid profile and the determination of ApoE and its isoforms in active young A community, comparing with cognitive tests in two groups: control individuals without AD in the family and a group of individuals with AD in the family.

**Methods:** Initially we selected 56 volunteers from the community, with a mean age of 39.7 ± 11.5 years, composed of active youngsters, the majority of whom were women (n = 44). N = 19 individuals had a history of AD in the family and n = 37 stated they did not. All will sign the TCLE. The project was sent to CEP/UNIFESP. Laboratory Analyzes: Venous blood was collected for basic laboratory test dosages, including the lipid profile (Enzymatic/Colorimetric method in Beckman-Coulter® AU5800 equipment) and Apo E gentrification (PCR method with equipment-7500 Real Time After blood collection the volunteers did Cognitive Tracking Instruments: The Rey Auditory Verbal Learning Test (RAVLT), Verbal Span of Digits, Stroop Test of Colors and Words, Trail Making Test, Verbal Fluency, and Categories, Codes and BECK Depression Scale

**Results:** In our study of ApoE and its alleles, we identified the following groups: Apo E2/E3 (n = 6, 10.7%), Apo E3/E3 (n = 34, 60.7%) , E3/E4 (n=15, 26.8%) and E4/E4 (n=1, 1.8%). In the logistic regression, the group of individuals with E2/E3 genotype (p = 0.014, 0.027) and the E3/E3 group (p=0.065 and 0.093) presented a slight significance in the Trail Making Test and Stroop Test tests respectively. The groups that had the E4 allele did not present significant difference between the cognitive tests until the moment. Regarding dyslipidemia, the groups E2/E3, E3 E3, E3/E4 and E4/E4 presented respectively; 33.3%, 23.5%, 13.3% e)% of dyslipidemias and 50%, 35.3%, 26.7% and 0% of family history.

**Conclusion:** Initially The authors concluded that in our sample, the E2/ E3 group had a higher number of dyslipidemics and a family history despite being a smaller group. The E3/E3 group presented 58.8% of cases with one of the two risk factors and the E4/E4 group, which despite being the most involved allele in AD, had neither dyslipidemias nor family history. Obviously, it will be necessary to increase our casuistry.

## B-230

### Performance characteristics study comparing Roche Cobas AmpliPrep/Cobas TaqMan and Cephied GeneXpert Real-Time PCR-based Hepatitis C Virus (HCV) Assays

M. K. Ahmed[1], H. M. Shams[2], R. N. Bedair[3]. *[1]Molecular Diagnostics Unit, Al Borg Medical Laboratories, Jeddah, Saudi Arabia, [2]Clinical and Chemical Pathology Department, National Cancer Institute, Cairo, Egypt, [3]Chemical Pathology Department, Medical Research institute, Alexandria, Egypt*

**Background:** Hepatitis C virus (HCV) RNA detection and quantification are the key diagnostic, prognostic and monitoring tools for the management of hepatitis C. The accurate and sensitive measurement of HCV RNA is essential and critical for the clinical management and treatment of infected patients and as a research tool for studying the biology of HCV infection. The aim of this study was to evaluate the performance characteristics and manufacture claims of the new Cepheid GeneXpert HCV assay in comparison with the FDA approved Roche Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) assay for HCV RNA quantification.

**Method:** Accuracy study between the two HCV assays CAP/CTM and the GeneXpert were performed on 167 available samples covering the 6 known HCV genotypes to determine whether the methods are equivalent within Allowable Total Error 20%, specimens were compared over a range of 0.00 to 6.75 Log IU/mL. Commercial HCV controls (genotypes 1, WHO fourth International standard) were used to evaluate linear range, Limit of detection (LOD), Limit of Quantification (LOQ) and precision, analytical specificity (Exclusivity) was evaluated by adding potentially cross reactive organisms with different concentrations into HCV negative EDTA plasma.

**Results:** The comparison (accuracy) study has been passed; difference between the two methods was within allowable error for 167 of 167 specimens (100%). The average Error Index (Y-X)/TEa was 0.00, with a range of -0.02 to 0.03. The largest Error Index occurred at a concentration of 5.27 Log IU/mL. The reportable range was verified near the manufacturer claimed range and found to be acceptable. The LOD evaluation was performed according to CLSI guideline E17-A2 and HCV RNA concentration that can be detected with a positivity rate of greater than 95% was determined to be 5.0 IU/mL. The LOQ analysis demonstrates that the HCV VL Assay can determine 10 IU/mL (1.0 log10) with an acceptable precision. The precession study passed (SD: 0.082, 95% confidence for SD: 0.057 to 0.150, CV: 1.5) within allowable total error of 20% and allowable random error of 10%. The linearity of the assay was accepted over a measured range of 0.0 to 6.9 log IU/mL within allowable systematic error (SEa) was 10.0%.

**Conclusion:** *Cepheid GeneXpert HCV assay shows excellent correlations and produces highly comparable results if compared to the FDA approved Roche CAP/CTM HCV assay. All performance characteristics of Cepheid GeneXpert HCV assay was verified and found to be within manufacturer claim. Cepheid assay is much easier and time saving than Roche assay. GeneXpert instrument modules should be considered as a separate Real-time PCR machines and the validation process should include all modules before testing patient samples.*

## B-231

### Incidence of inversion of Chromosome 1 as a cause of Infertility

M. M. de Almeida, J. M. Fernandes, M. P. Migliavacca, G. A. Campana, J. R. Alves, R. Kuhbauche. *DASA, Barueri, Brazil*

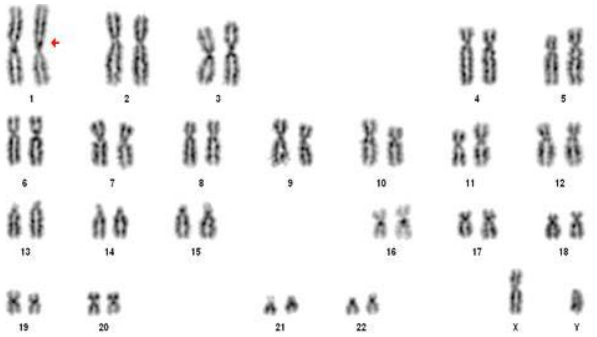
**Background:** Infertility can be defined as the inability to achieve a pregnancy within one year or the repeated failure to bring a pregnancy to term, most cases of infertility and abortion are not related to chromosomal abnormalities, but in absence of another causal factor, cytogenetic studies of the couple are indicated and may demonstrate infertility caused by changes in sex chromosomes not investigated and/or manifested during puberty or a balanced structural rearrangement.

**Objective:** To verify the incidence of cases with inversion of chromosome 1 in Brazilian patients with infertility and referred to perform a karyotype in a clinical laboratory.

**Methods:** Statistical survey of cases from 2014 to 2016 with diagnostic hypothesis of infertility and abortion.

**Results:** A total of 8,166 normal patients with only 165 altered were analyzed, and only 3 of them present the inversion of chromosome 1.Two male patients and one female patient with diagnostic hypothesis of infertility and repetitive abortion.

**Conclusion:** The inversion of chromosome 1 corresponds to a very low percentage of the alterations found, being less common than the inversion of chromosome 9. The importance of cytogenetics and the increase of the research on infertility help in the identification, for example, of the polymorphisms of the regions of constitutive heterochromatin, considered normal variations in the population, but that can be decisive for the determination of the cause of reproductive incapacity. The enormous variation of heterochromatin observed among the homologous pairs led several researchers to consider the idea that this difference could hinder the pairing or non-disjunction of the chromosomes, thus predisposing the carriers to alterations in the reproductive capacity.



## B-232

### Detection of an atypical 22q11.2 deletion not including the critical region related to the DiGeorge and Velocardiofacial syndrome

F. K. Marques[1], G. C. Lopes[2], S. D. L. Cueva[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil*

**Background:** Deletions of chromosome 22q11.2 comprise the most common microdeletions in humans; it is associated with a highly variable phenotypic spectrum including Velocardiofacial and DiGeorge syndromes. The majority of patients (~97%) with the 22q11.2 microdeletion have either a recurrent 3 Mb deletion from LCR22-A to LCR22-D that contain at least 30 genes, or a less frequent smaller 1.5 Mb deletion that occurs between LCR22-A and LCR22-B. Both deletions include TUPLE1 and TBX1 as candidate genes to the major syndrome phenotypes. The presence of several highly homologous low copy repeats (LCRs) at the proximal end of the long arm of chromosome 22 predisposes to these rearrangements. However, a limited number of patients with atypical deletions have been described. **Objective:** To report a case of atypical distal 22q11.2 deletion detected by array-based comparative genomic hybridization (array-CGH) using the Agilent platform. **Case report:** We report a three-year-old girl with neuropsycomotor development delay, malformation of the central nervous system and typical dysmorphic features. The karyotype analysis at 450 band resolution shows 46,XX normal result. Fluorescence *in situ* hybridization (FISH) analysis was negative using the commercially available TUPLE1 probe. Array-CGH revealed a 369 Kb interstitial microdeletion: arr 22q11.2(19,425,275-19,794,119)x1, and a ~28 Kb deletion in 11q12.2 that could be classified as VOUS (Variant of Unknown Significance). In this study we reported an atypical deletion between LCR22-C and LCR22-D that includes 10 genes, this kind of deletion could be considered an atypical one. It is described in the literature as related to a variable phenotype that includes mild developmental delay (54%) behavioral disorders (30%), growth retardation and dysmorphic features. Among the present genes in this deletion, LZTR1 related to Noonan syndrome 10; AD. (OMIM 616564), and CRKL an oncogene that mediates Fgf8 interactions, responsible for part of the DGS and VCFS phenotype (OMIM 602007) are especially interesting. The haploinsufficiency of these genes, especially the LZTR1, must be the cause of the proband phenotype, furthermore, in contiguous gene deletions syndromes, the deletion composition is critical to the phenotypic presentation. The aCGH was capable to identify a critical deletion that could explain the proband phenotype, filling the lack of resolution of Karyotyping and the specificity of FISH probes. Distal deletions on 22q11.2 were described as inherited from healthy parents in 58% of the cases, which suggests an incomplete penetrance or variable expressivity so it is necessary to expand the investigation to the proband's parents. That investigation can be made using less expensive methodology like MLPA.

## B-233

### Assessment of six susceptibility variants of LRRK2 on the risk of Parkinson's disease: a case control study in China

Y. Pei[1], Y. Wu[1], Z. Yang[2], W. Cui[1]. *[1]Cancer Hospital Chinese Academy of Medical Sciences, BeiJing, China, [2]Peking Union Medical College Hospital, BeiJing, China*

**Background:** Leucine-rich repeat kinase 2 (LRRK2) is identified as a candidate gene linked to both familial and sporadic Parkinson's disease (PD). A number of variants of LRRK2 have been reported to affect the risk of PD but the results were not always consistent in Chinese.

**Methods:** In this study, we conducted a case-control study comprised 296 PD patients and 297 matched controls to investigate the prevalence of three well-known pathogenic variants (R1441C/G/H, G2019S, I2020T) and three Asian-prevalent (R1398H, R2385R, R1628P) variants, and to assess their roles in the susceptibility to PD. All the patients were recruited from the Department of Neurology of Peking Union Medical College Hospital with standard diagnosis. Controls were age and gender-matched healthy people with no history of neurodegenerative diseases. DNA samples were extracted from peripheral blood and amplified for sequence analysis. Chi-square test was performed to compare frequency distribution of genotypes and alleles, and Hardy-Weinberg equilibrium was verified.

**Results:** The results showed that all the three pathogenic variations were absent in our study, indicating they were not common pathogenic SNPs in Chinese. PD patients carried a higher frequency of variant R2385R than control subjects (10.8% vs. 5.7%; AA+AG vs. GG OR=2.0, 95%CI 1.08-3.68, P=0.027; A vs. G OR=1.89, 95%CI 1.05-3.39, P=0.033). However, no significant difference was found in the prevalence of variant R1398H (15.5% vs. 16.2%) and R1628P (2.4% vs. 1.0%) in PD patients and controls. In addition, we found a patient carrying both R1398H and R1628P variants.

**Conclusion:** Our study demonstrated that R2385R was a risk factor associated with increased PD susceptibility in Chinese, and called for larger samples or comprehensive systematical reviews for the other two Asian-prevalent variations for further confirmation.

**Table 1.** Genotype and allele distribution of Asian-prevalent variants and the association with PD.

| Genetic variant | Geno-type | Dominant model | | | | Al-lele | Allele model | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pa-tients n=296 | Con-trols n=297 | OR (95%CI) | P value | | Pa-tients n=592 | Con-trols n=594 | OR (95%CI) | P value |
| R1398H | AA | 2 | 2 | 0.95 (0.61-1.48) | 0.836 | A | 48 | 50 | 0.96 (0.63-1.45) | 0.847 |
| | AG | 44 | 46 | | | G | 544 | 544 | | |
| | GG | 250 | 249 | | | - | - | - | - | - |
| R2385R | AA | 1 | 1 | 2.00 (1.08-3.68) | 0.0268 | A | 33 | 18 | 1.89 (1.05-3.39) | 0.0334 |
| | AG | 31 | 16 | | | G | 559 | 576 | | |
| | GG | 264 | 280 | | | - | - | - | - | - |
| R1628P | CC | 0 | 0 | 2.37 (0.61-9.27) | 0.214 | C | 7 | 3 | 2.37 (0.61-9.22) | 0.212 |
| | CG | 7 | 3 | | | G | 585 | 591 | | |
| | GG | 289 | 294 | | | - | - | - | - | - |

## B-234

### What is the yield of whole exome sequencing after unsuccessful standard-of-care genetic investigation?

Y. Giguère[1], N. Bernard[1], C. Lindsay[1], J. Blais[1], K. M. Boycott[2], F. Rousseau[1]. *[1]Chu de Québec-Université Laval, Québec, QC, Canada, [2]Children's Hospital of Eastern Ontario Research Institute,University of Ottawa, Ottawa, ON, Canada*

Rationale and Objective

In patients with a phenotype suspected to be due to a mendelian (single-gene) genetic disorder, after known single-gene candidates have been eliminated from consideration or when a multigene testing approach is prohibitively expensive, whole exome sequencing (WES) is currently used for the detection of rare variants. However, it is important to establish its diagnostic yield after an inconclusive initial standard-of-care diagnostic investigation. We thus performed a systematic review aiming to estimate the diagnostic yield of WES for suspected monogenic disorders when standard-of-care investigations have failed to provide a diagnosis.

Methods

We performed an electronic search of PubMed and Embase bibliographic databases from inception to February 1st 2015. We applied a comparable search strategy with adaptations for each database. The search strategy consisted of MeSH (medical subject heading) or Emtree terms and keywords related to "exome sequencing". To be eligible for inclusion in the systematic review, a genetic standard-of-care investigation must have been conducted in patients of the original studies. The different steps of the search and the selection process were in line with PRISMA recommendations. The diagnostic yield of exome sequencing was calculated as the percentage of patients with a suggestive molecular diagnosis after WES analysis following unsuccessful initial genetic standard-of-care molecular investigation.

Results

We retrieved 1104 potentially eligible original studies for detailed evaluation and inclusion in the review. Detailed evaluation led to the exclusion of 1003 publications where WES was performed on unaffected or affected parents, unaffected or affected relatives; or was performed on only one case. Overall, 101 studies that included at least two unrelated patients were considered relevant and were included in the review. Between 2 to 172 (median = 6) patients of all ages were enrolled in the selected studies, for a total of 1838 cases (77 diseases). Among the 77 diseases studied, the most common conditions were related to nonsyndromic or syndromic retinal dystrophies (n=6), neurologic disorders (intellectual disability, autism spectrum disorders, progressive cerebellar atrophy, primary microcephaly, brain atrophy and other malformations, and myopathy) (n=5), Noonan syndrome (n=4), and mitochodrial encephalomyopathy (n=3). A suggestive molecular diagnosis was found for 551 of cases (0 to 105/study; median: 3). When calculated within individual studies, the median diagnostic yield was 67%, while the overall molecular diagnostic yield calculated on pooled cases from all studies was 30% (95%CI 28-32%). WES did not provide a molecular diagnosis in a total of 1112 patients (61%), while 174 patients (9%) had a molecular diagnosis of uncertain or partial significance.

Conclusion

We observed an overall diagnostic yield of 30% after unsuccessful initial standard-of-care molecular investigation, suggesting that additional molecular investigation by WES is relevant for identifying pathogenic mutations linked to rare genetic conditions after failure of standard-of-care investigation.

## B-235

### Disease targeted NGS coupled with homozygosity mapping improves theefficiency of mutation identification in patients with epidermolysis bullosa

H. Vahidnezhad[1], A. Saeidian[1], L. Youssefian[1], S. Zeinali[2], S. Sotoude[3], H. Mahmoudi[4], M. Daneshpazhooh[4], M. Hamid[5], M. Zahabiyon[1], A. Touati[1], A. Ertel[1], J. Uitto[1], P. Fortina[1]. *[1],Thomas Jefferson University, Philadelphia, PA, [2],Pasteur Institute of Iran, Tehran, Iran, Islamic Republic of, [3],Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [4]Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [5]Pasteur Institue of Iran, Tehran, Iran, Islamic Republic of*

**Background:** Epidermolysis bullosa (EB) comprises a phenotypically and genotypically heterogeneous group of blistering disorders with the clinical hallmark of skin and mucosal fragility. The lack of definitive clinical findings suggestive of a candidate gene for molecular diagnosis of EB makes patient skin biopsies for electron microscopy and/or immune-epitope mapping necessary as a screening method before mutational analysis. However, antigen mapping is a complicated and demanding technique in terms of expertise and cost and is restricted to a few referral EB centers.

**Methods:** To circumvent these problems, we developed an EB-targeted next generation sequencing (NGS) panel consisting of 18 genes reported to be causative of EB (*COL17A1, COL7A1, DSP, DST, EXPH5, FERMT1, ITGA3, ITGA6, ITGB4, JUP, KRT5, KRT14, LAMA3, LAMB3, LAMC2, PKP1, PLEC1 TGM)* and 3 (*CD151, CDSN, CHST8)* causing a skin fragility disorder in the differential diagnosis. In addition, to find the appropriate candidate genes for sequencing, in consanguineous pedigrees we applied homozygosity mapping with genome-wide single nucleotide polymorphism (SNP) arrays consisting of 550,000 markers.

**Results:** A total of 94 patients with clinical diagnosis of EB (and two controls) were sequenced. In 50 patients, pathogenic variants were found in one candidate gene and for the others several variants of unknown significance were found in more than one gene. SNP-based homozygosity mapping identified runs of homozygosity of ≥2 Mb, and in

43 probands there was at least one candidate gene identified by co-alignment of the gene with a homozygosity block. Using this approach pathogenic variants were found in 86 out of 93 families (detection rate of 94.6%) in 17 different EB-associated genes. All mutations were confirmed by Sanger sequencing. The most common EB mutated genes were *COL7A1, COL17A1*, *PLEC* and *LAMB3* (23%, 16%, 13% and 10% of all EB cases, respectively); and, collectively they comprise about 62% of mutated genes. A previously unreported splice junction mutation, the second pathogenic variant in *CD151*, was found in a patient with pretibial EB and nephropathy. The pathogenic splice mutation leads to exon skipping and was confirmed by RT-PCR from whole skin and Sanger sequencing. The other 5 mutations were located in *LAMA3* (5%), *LAMC* (4%), *EXPH5* (2%) and *FERMT1* (1%). Collectively, genome-wide SNP-based homozygosity mapping facilitates identification of candidate genes in EB families.

**Conclusion:** The specific mutation information forms the platform for prenatal testing and preimplantation genetic diagnosis, as well as for development of allele-specific therapies in the realm of precision medicine for this group of currently intractable disorders. We developed a disease-targeted next generation sequencing approach which is rapid, minimally invasive and cost-effective in identifying mutations in patients with EB.

## B-236

### Clinical Utility of Genetic Testing for Mitochondrial Disorders in Adult Patients and the Importance of Tissue-Specific Analyses

L. Hasadsri, S. E. Roellinger, E. Selner, W. E. Highsmith. *Mayo Clinic, Rochester, MN*

*Background and Objectives:* Mitochondrial diseases are a group of clinically heterogeneous disorders, typically of childhood onset, caused by mutations in mitochondrial DNA (mtDNA) or in nuclear genes encoding mitochondrial proteins. DNA sequencing is considered the gold standard for diagnosis and, historically, the preferred specimen was a muscle biopsy. Over time, however, practitioners have come to favor less invasive screening. Whole mitochondrial genome analysis by next generation sequencing (NGS) was launched one year ago by the Mayo Clinic Molecular Genetics Laboratory and, to date, 110 unique patient samples have been received for clinical testing. Surprisingly, nearly 70% of our specimens were received from adults (≥18 years old). Here we present our data on the clinical utility of mtDNA testing in adult patients, and highlight two unique cases in which a diagnosis could only be made when tissue biopsies were analyzed rather than blood.

*Methods:* The mitochondrial genome was amplified by long-range PCR, and NGS was performed on the PCR products using a TruSeq Nano library preparation sequenced on an Illumina MiSeq (primary) and an Ion Plus Fragment library preparation sequenced on an Ion Torrent PGM (confirmatory). Large deletions detected by the MiSeq were further confirmed by gel electrophoresis. A retrospective study of all patient results and clinical information, when provided, was then performed. Samples received for verification or proficiency testing were excluded from our analyses. All variants detected were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines. A positive diagnosis was defined as having one or more likely pathogenic or pathogenic mtDNA mutations as per ACMG guidelines.

*Validation:* Accuracy was demonstrated for our NGS assay by testing 127 DNA samples extracted from blood, cultured cells, and muscle biopsies that had been previously genotyped using established methods. Results were 100% concordant. Precision was assessed using eight samples run in triplicate both on the same run and across three separate runs. All results were 100% concordant among replicates down to the limit of detection (LOD). The LOD was determined to be 1.875ng input of DNA for the long-range PCR and 25ng input PCR product for library preparation. The minimum detectable variant frequency was 6% for single nucleotide variants and insertions/deletions, and 20% for large deletions.

*Results:* Our diagnostic yield was approximately 15% (16/110). Eighty-eight percent (14/16) of these positive cases were adults and, interestingly, 43% (6/14) of these were above age 60. Of note, two patients who were negative for mtDNA mutations in blood were found to be homoplasmic and 62% heteroplasmic for pathogenic variants in their muscle and renal biopsies, respectively. One case has the oldest known onset of Kearns-Sayre syndrome, and the other expands the phenotypic spectrum of MT-ND5 mutations beyond Leigh syndrome, MELAS, and optic atrophy.

*Conclusions*: Next generation sequencing of the whole mitochondrial genome may be especially useful in adult patients suspected of having a mitochondrial disease. Due to heteroplasmy, patients with negative testing on blood may benefit from additional testing of other tissues.

## B-237

### Pathogenic genomic findings revealed by aCGH in a boy with Klinefelter Syndrome (47, XXY) and loss of Xq28: Rett Syndrome or inactive X chromosome?

J. O. Rodrigues[1], P. D. R. Cirillo[1], G. C. Lopes[2], S. D. L. Cueva[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil*

**Background:** Klinefelter Syndrome is a common chromosomal disorder affecting 1/500 live birth males affecting physical and cognitive development. Rett Syndrome (RTT) is a X-linked condition that affects almost exclusively females showing learn and motor skills delay. This condition is mainly caused by loss of functional copy of *MECP*2 gene (Xq28). The probability for the simultaneous occurrence of Klinefelter and RTT events is very low (about 1/10 to 15,000,000 live births). Here, we described a boy (9y) previously diagnosed by karyotyping with Klinefelter Syndrome, showing chromosome 1 long-arm duplication (19.54Mb) and an extra chromosome X with Xq28 deletion (3.26Mb). Familial investigation showed that grandmother and mother had apparently balanced translocation between chromosomes 1 and X. In addition, a younger sister (4y) has chromosomes 1 partially duplicated and Xq28 deletion. This girl show neuropsychomotor development delayed, intellectual deficiency and strabismus. **Objective:** The aim of this study was to investigate genomic alterations at the proband and his sister using a higher resolution methodology microarray-based comparative genomic hybridization (aCGH) to improve the clinical diagnosis and management in a putative RTT syndrome. **Methodologies:** DNA was extracted from both proband and his sister after informed consent term. The aCGH was performed using Cytochip ISCA 180K V.2. Data were analyzed using the BlueFuse Multi v3.3 software and Cytochip v2 algorithm. The classification of each change followed the recommendations of the American College of Medical Genetics. **Results and Discussion:** aCGH findings for proband were: arr[hg19]1q42.13q44 (229,676,306-249,212,666)x3; arr[hg19] Xp22.33q28(0-151,906,593)x2; arr[hg19] Xq28(151,974,942-155,232,894)x1. Sister genomic findings were: arr[hg19] 1q42 .13q44(229,676,306-249,212,666)x3; arr[hg19] Xq28(151,974,942-155,232,894) x1. Few cases of duplications in 1q are known but poorly characterized. Almost all patients with 1q42-qter duplications present craniofacial dysmorphisms but a quite normal psychomotor development. However, Xq28 deletions including *MECP2* gene is lethal in men and clinically relevant in women. The present case has an extra X chromosome that allows its compatibility with life. To our knowledge, this event is uncommon in the literature. All these findings lead us to hypothesize that proband must present RTT phenotypic characteristics. If not, is possible to assume that extra X chromosome with Xq28 deletion must be preferentially inactivated. **Conclusion:** Based on these results it is necessary follow-up proband´s endophenotype and family members to investigate eventual familial common alteration. The next step is to investigate X inactivation to confirm our hypothesis that suggests a preferential X inactivation as important modulator factor of the severity of the RTT phenotype in these patient. The use of aCGH allows the identification of new genomic findings and helps clinicians at diagnosis of rare genetic syndromes. The familial study is essential for genetic counseling and a better understanding of neurological disorders.

## B-238

### Validation of a Quantitative Digital Droplet PCR Assay for Assessment of EGFR T790M Mutation Status using Plasma cfDNA

J. R. Mills, D. Milosevic, M. Campion, M. C. Liu, K. C. Halling, E. W. Highsmith, B. R. Kipp. *Mayo Clinic, Rochester, MN*

**Background:** The release of tumor-derived cell-free DNA (cfDNA) into circulation allows for rapid and non-invasive plasma-based tumor genotyping. Plasma-based genotyping provides a solution to some of the limitations of traditional tissue-based genotyping, which include slow turnaround time (TAT), limited tissue biopsy material, and the risk of failure due to sampling bias. In the case of non-small-cell lung cancer (NSCLC), determining the EGFR p.T790M mutation status is critical for treatment decision-making. Two-thirds of NSCLC patients who carry activating EGFR mutations and experience disease progression after being treated with an EGFR-TKI have developed the T790M resistance mutation. Early detection of the T790M mutation may provide benefit by allowing an earlier switch to an alternative therapy without necessity of a repeat biopsy. The objective of this study was to evaluate the analytical performance of the Bio-Rad EGFR T790M digital droplet PCR (ddPCR) assay for the identification and quantification of EGFR p.T790M mutations in plasma cfDNA. **Methods:** A high-throughput semi-automated ddPCR assay was developed using commercial reagents from Bio-Rad. Plasma cfDNA was extracted

using Qiagen's QIAmp Circulating Nucleic Acid kit. ddPCR was performed in a 96-well plate format using an automated droplet generator (AutoDG™, Bio-Rad), a standard thermocycler and a fluorescence droplet reader (QX200™, Bio-Rad). A Bio-Rad multiplexed TaqMan probe-based assay, designed to detect both the *EGFR* c.2369C>T (p.T790M) mutation and the corresponding wild-type nucleotide, was utilized. Analytical performance was assessed using reference standards (Horizon Discovery), cell-line derived EGFR p.T790M nucleosomal DNA (ATCC #H1975), tissue-derived tumor DNA, and cfDNA from patients and healthy donors. Using these materials, the following parameters were measured: specimen stability, intra- and inter-assay imprecision, linearity, accuracy, carryover, analytical specificity and cross reactivity, LOD and LOQ, analytical turnaround time and reference intervals. **Results:** Plasma specimens were stable up to 21 days stored at -80C. Intra- and inter-assay imprecision of twenty replicates were measured at five different concentrations of mutant DNA (EGFR T790M) spanning the anticipated analytical measurement range. Probit regression analysis of this data determined that the LOD was five mutant droplets (15 copies/mL of plasma). CVs were <20% at concentrations as low as 40 mutant DNA copies/mL of plasma. Dilution linearity was demonstrated from 3750 to 30 copies/mL of plasma. The lowest reportable copy number (LOQ) was determined to be 40 mutant DNA copies/mL of plasma. Increasing wild-type DNA (over two orders of magnitude) had no impact on the accuracy of mutant DNA concentrations. A reference interval study failed to find any detectable mutant DNA copies in 60 healthy individuals. A blinded method comparison study was performed using tumor-derived DNA and demonstrated 100% concordance between the Bio-Rad ddPCR assay and the FDA-approved Roche Cobas EGFR v2 plasma cfDNA assay (n=20). **Conclusion:** The Bio-Rad EGFR T790M ddPCR assay performed using the AutoDG/QX200 system is a robust, economical and rapid method for determining EGFR T790M status from plasma cfDNA.

## B-239

### Polymorphisms on *MTOR* and *FOXP3* genes are associated with impaired renal function at one-year post-transplant in kidney recipients

A. B. Campos-Salazar[1], F. D. V. Genvigir[1], C. R. Felipe[2], H. Tedesco-Silva[2], J. Medina-Pestana[2], M. H. Hirata[1], A. Cerda[3], R. D. C. Hirata[1]. *[1]School of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil, [2]Nephrology Division, Hospital do Rim, Federal University of Sao Paulo, Sao Paulo, Brazil, [3]Center of Molecular Biology and Pharmacogenetics, Universidad de La Frontera, Temuco, Chile*

**Background:** Monitoring of immunosuppressive drugs, such as calcineurin and mTOR inhibitors, is essential to avoid undesirable kidney transplant outcomes. Polymorphisms in pharmacokinetics-related genes have been associated with variability in blood levels of immunosuppressive drugs and adverse effects, but influence of pharmacodynamics-related genes remains to be elucidated. The aim of this study was to investigate the relationship of polymorphisms in *MTOR* e *FOXP3* genes with renal function in kidney transplant recipients within the first-year post-transplant.

**Methods:** Two-hundred seventy-five kidney transplant recipients were recruited at a kidney transplant center in São Paulo city, Brazil. The patients were randomized in three groups of immunosuppressive regimens containing tacrolimus (TAC), everolimus (EVR) and mycophenolate sodium (clinical trial NCT01354301). Clinical and laboratory data, including renal function parameters and TAC and EVR blood levels, were recorded. Genomic DNA was extracted from blood samples to analyze gene polymorphisms (*MTOR* rs1057079, rs1135172, rs1064261; *PPP3CA* rs3730251; *FKBP1A* rs6033557; *FBKP2* rs2159370 and *FOXP3* rs3761548, rs2232365) by real-time PCR.

**Results:** *MTOR* rs1057079 (c. 1437T>C), rs1064261 (c.2997C>T) and *FOXP3* rs3761548 (c.-23+2882A>C) were associated with variability on serum creatinine and estimated glomerular filtration rate (eGFR), but not with cellular rejection episodes at month 12. *MTOR* c.1437CC and c.2997TT, and *FOXP3* c.-23+2882GG genotypes were associated with high levels (upper tercile) of serum creatinine (OR=2.44, 95%CI=1.27-4.69, p=0.008; OR=1.97, 95%CI=1.06-3.65, p=0.032; OR=2.35, 95%CI=1.19-4.63, p=0.013, respectively), at month 12. *MTOR* c.1437CC and c.2997TT, but not *FOXP3* c.-23+2882GG genotypes, were also associated with low values of eGFR (lower tercile) (OR=2.47, 95%CI=1.28-4.77, p=0.007; OR=2.06, 95%CI=1.10-3.85, p=0.023). Patients carrying high genetic score (three risk genotypes) of *MTOR* and *FOXP3* variants showed higher serum creatinine than carriers of lower genetic score (up to two risk genotypes) (p=0.018) at month 12. *MTOR* and *FOXP3* polymorphisms were not associated with rejection episodes within the first year post-transplant (p>0.05).

**Conclusions:** Polymorphisms in *MTOR* and *FOXP3* genes are associated with increased serum creatinine and reduced eGFR, suggesting that individuals carrying risk genotypes are more susceptible to graft dysfunction within the first-year post-transplant.

## B-240

### Performance of a multi-level, multi-analyte external assayed quality control developed for the detection of Healthcare-associated infections (HAIs).

J. Liu, K. Barnecut, V. P. Luu, T. Cheever, M. Zeballos, R. Stahl, B. Fukunaga. *Bio-Rad laboratories, Irvine, CA*

**Background:** Bio-Rad recently obtained FDA clearance for Amplichek II as an external assayed quality control material to monitor the performance of the Cepheid GeneXpert assays targeted for HAI detection. The Amplichek II quality control product consists of 4 levels targeted for HAI assays manufactured by Cepheid: Level Negative containing Methicillin sensitive *S. epidermis* (MSSE), Level 1 containing Methicillin Sensitive *S. aureus* (MSSA), Level 2 containing low concentrations of Methicillin Resistant *S. aureus (MRSA)*, *C. difficile (Cdiff)*, and Vancomycin resistant *Enterococci* (VRE), and Level 3 containing high concentrations of MRSA, Cdiff, and VRE. The performance of the product was tested on the appropriate Cepheid assays over 24 months, on multiple control and reagent kit lots. The Amplichek II product was also tested on four additional HAI testing platforms.

Although the Cepheid assays provide qualitative assessments for the presence of HAIs, we used the data from a precision study to assess whether the semi-quantitative $C_t$ values could be used to track assay performance. Here, we show an example of the potential use of $C_t$ values to track performance over a period of 24 months on the Cepheid Cdiff assay.

**Methods:** The Amplichek II product, stored at 2-8°C for the length of the study, was tested on the appropriate Cepheid assays at the following time points: 4, 8, 12, 16, 20, and 24 months. The qualitative data results were analyzed as % agreement with the expected results for each of the time points. Additional platform testing of Amplichek II was assessed for Level Negative and Level 3 only. The additional platforms in the study were the BD GeneOhmTM/Max (VRE and MRSA), Nanosphere Verigene (Cdiff), Luminex xTAG (Cdiff), and Meridian Illumigene (Cdiff), for their respective analytes. The results were analyzed for % agreement with the expected results. A precision analysis was performed by monitoring $C_t$ values on the Cepheid assays according to recommendations provided by CLSI document EP5 (2 replicates tested twice a day, over a period of 10 days, 40 total replicates). The data was analyzed using Microsoft excel's Analyze-IT software package. The Cepheid assays' %CV analyzed using semi-quantitative Ct values is <6%. Based on this finding, the semi-quantitative $C_t$ data from the Real Time stability study (for a representative analyte, Van A, with 6 %CV ), was assessed using a Levey Jennings plot.

**Results:** Amplichek II was in 100% agreement of expected results throughout the 24 month shelf life of the product for all levels and all analytes. Additional platform testing shows that Level Negative and Level 3 were in 100% agreement for all analytes, on all platforms.

**Conclusion:** Bio-Rad's Amplichek II control provides an independent assessment of the performance of in vitro laboratory nucleic acid testing procedures developed for the detection of HAIs.

## B-241

### Performance of a bi-level, multi-analyte external unassayed quality control across multiple sample type configurations, for molecular diagnostic platforms detecting sexually transmitted infections.

V. P. Luu, T. Cheever, K. Barnecut, J. Liu, M. Zeballos, R. Stahl, B. Fukunaga. *Bio-Rad laboratories, Irvine, CA*

**Background:** The CDC estimates that the incidence of sexually transmitted infections (STIs) is 20 million new cases every year in the United States, accounting for almost $16 billion in health care costs. Part of the prevention and treatment plan includes the surveillance of STIs through the use of numerous FDA cleared molecular diagnostic tests. Unlike molecular diagnostic tests of other diseases, tests targeted for STIs utilize multiple patient specimen types ranging from swab to cytology specimens, which require additional precaution during validation and quality assurance. Bio-Rad's Amplichek STI external unassayed quality control was developed to monitor the performance of these assays in the various specimen or sample types, to add confidence in the reliability of test results. The use of quality control materials is

indicated as an objective assessment of the performance of methods and techniques in use and is an integral part of good laboratory practices. Amplichek STI consists of a negative level (negative for CT, NG, and HPV, while containing HPV negative human cells) and a positive level (positive for CT, NG, and HPV), developed for use across multiple STI molecular diagnostic assays and various specimen type configurations (e.g. swab, urine, PreservCyt).

**Methods:** A comprehensive performance evaluation study was conducted on the Amplichek STI product, in order to assess its performance across the various specimen types validated for use by the assay manufacturer, for 8 commonly tested STI assays for the detection of CT, NG, and HPV. The study was conducted using 3 separate testing sites, with 2 replicates per day, over a period of 5 days (n=10 replicates per site, for a total of 30 replicates).

**Results:** The results were within 100% agreement for both the Level negative and Level positive, on all assays, across all sample type configurations.

**Conclusion:** Bio-Rad's Amplichek STI is the first molecular control formatted for use across multiple sample type configurations on common molecular assays used in the detection of STIs, providing an independent assessment for laboratories to maintain good laboratory practice.

## B-242

### Precision of a multi-level, multi-analyte external assayed quality control for molecular diagnostic platforms monitoring viral load of blood borne pathogens.

W. Chiu, T. Cheever, V. P. Luu, K. Barnecut, J. Liu, M. Zeballos, R. Stahl, B. Fukunaga. *Bio-Rad Laboratories, Irvine, CA*

**Background:** Monitoring the viral load for the blood borne pathogens, HIV, HCV, HBV, facilitates in assessing the prognosis and viral response to treatment. The Abbott RealTime and Roche COBAS AmpliPrep/COBAS TaqMan are FDA cleared to monitor the viral load of these blood borne pathogens. Bio-Rad Laboratories' Amplichek I is an external independent assayed quality control developed to monitor the performance of these assays to add confidence in the reliability of test results obtained for unknown specimens. The routine use of assay agnostic quality control materials is indicated as an objective assessment of the precision of methods and techniques in use and is an integral part of good laboratory practices.

**Methods:** Precision tests were performed for Amplichek I based on the guidelines recommended by CLSI document EP5, in which two replicates were tested twice a day, over a period of ten days (n=40 total). Amplichek I Level Negative (negative for HIV, HCV, and HBV), Level 1 (low positive for HIV, HCV, and HBV, targeted for Roche) and Level 3 (high positive for HIV, HCV, and HBV, targeted for both platforms), were tested on the Roche assays for the respective analytes. Amplichek I Level Negative (negative for HIV, HCV, and HBV), Level 2 (low positive for HIV, HCV, and HBV, targeted for Abbott) and Level 3 (high positive for HIV, HCV, and HBV, targeted for both platforms), were tested on the Abbott assays for the respective analytes. Precision analysis was performed using the Microsoft Excel "Analyze-it" tool package for precision measurements.

**Results:** The precision results were within the expected assay performance documented in the respective manufacturers' package inserts, for all the analytes tests.

**Conclusion:** Bio-Rad's Amplichek I provides an independent assessment of the performance of in vitro laboratory nucleic acid testing procedures for the quantitative detection HIV-1, HBV and HCV on the Abbott RealTime and Roche COBAS molecular diagnostic platforms.

## B-243

### Electrochemically Modified Sensitive Nitric Oxide Sensors for Detecting Nitric Oxide at the Level of Single Cells.

T. Bose, N. Maheshwari, M. Bayachou. *Cleveland State University, Cleveland, OH*

**Background:** Nitric oxide (NO) is an important biological molecule that has diverse functions in human physiology. The concentration of NO in tissues and cells are of vital importance and the presence of too low NO concentration is the source of a variety of diseases. Measurement of NO in biological samples is a challenging task because of its rapid chemical reactions with a wide range of biomolecules, its nano-molar concentrations in tissues and its very short half-life of approximately a few seconds. In general, NO measurement techniques can be classified as direct (measuring NO itself) and indirect methods. Analytical techniques for direct measurement of NO include electrochemistry; fluorometry and electron paramagnetic resonance (EPR). Amongst them electrochemical tools are most promising because they allow fabrication of miniaturized probes with electro-catalytic surfaces which greatly enhances the sensitivity and selectivity for direct, real time and accurate measurement of NO in cell lines and tissue samples along with enabling very low limit of detection.

**Methods:** In this work, we fabricated combined reference/working carbon fiber electrodes with 7-micron diameter tips for direct placement near the cell lines. The exposed surface of the fiber tip was electrochemically modified with ruthenium oxide and Poly(3,4-ethylenedioxythiophene) (PEDOT). To improve the selectivity of our sensors we coated the surface with an ionic liquid composite and measured the response to NO using Differential Pulse Amperometry to differentiate NO response from other interferents present in biological samples. Madin-Darby Canine kidney (MDCK) epithelial cell lines were used to detect NO release with our sensor. To get a detailed analysis of the temporal and spatial resolution of the NO release, simultaneous monitoring by fluorescence and electrochemical method was performed.

**Results:** With our method of sensor modification we attained a normalized sensitivity of 2.82E-4 pA/nM/$\mu m^2$ and 4.31E-4 pA/nM/$\mu m^2$ towards NO in the linear range of 0.1 -3.2 μM and 2-16 nM (biologically relevant range) respectively, with a $R^2$ value of 0.993. The limit of detection (LOD) of our modified sensor to NO with a signal to noise ratio of 3 was as low as 250 pM. Such an enhanced LOD is unique to our method of sensor modification in comparison to other work done in this field. In terms of selectivity our sensors could effectively discriminate amongst the major biological interferents that are present near NO producing systems. The interference (%) was 0.0003 for 1.0 mM L-Arginine; 0.0002 for 1.0 mM $NO_3^-$; 0.11 for 0.1 mM $NO_2^-$ and 0.015 for 0.1mM ascorbic acid.

**Conclusion:** The relevance of our work to clinical laboratory medicine is the development of a highly sensitive and selective NO sensor capable of direct and accurate measurement of very low concentration of NO at the level of single cells and biological samples due to its excellent linearity in a relatively wide concentration range as well as selectivity towards NO in the presence of interferents. Thus, our sensors can be used to study clinical pathology of diseases where NO release is significantly decreased.

## B-244

### Microparticles derived from tissue factor, leukocyte, endothelium and neuron are associated with Alzheimer's Disease

C. A. Magalhães[1], M. G. Carvalho[1], C. M. Loures[1], A. C. Chaves[1], M. T. G. Cintra[2], M. A. Bicalho[2], V. G. Fraga[1], L. P. Sousa[1], P. Caramelli[2], F. M. F. Campos[1], K. B. Gomes[1]. *[1]Pharmacy Faculty, Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Medicine Faculty, Federal University of Minas Gerais, Belo Horizonte, Brazil*

**Background:** In the last few years, microparticles (MPs) have been studied as new specialized structures for intercellular communication. MPs are important messengers in cell-cell communication and contribute to the induction of endothelial damage, inflammation, and angiogenesis, carrying signaling molecules, such as chemokines, cytokines, enzymes, growth factors, receptors, adhesion molecules, mRNAs and microRNA. This study aimed to evaluate the levels of MPs derived from platelets, leukocytes, endothelium, tissue factor and neuron in Alzheimer's Disease (AD) patients compared with cognitively healthy individuals.

**Methods:** 54 individuals were recruited and classified as probable AD (29 patients - 15 men and 14 women, age 72.9±7.0 years) and cognitively healthy individuals (25 controls - 8 men and 17 women, age 73.2±7.7 years). Blood samples (citrate plasma) were collected and MPs were isolated by ultracentrifugation and measured by flow cytometry. Trucount control tubes were included as a quality control. Statistical analyses were performed using Mann-Whitney test on SPSS program version 13.0. Values of $p < 0.05$ were considered significant.

**Results:** The median (interquartile range) levels of MPs (MPs/μL) derived from tissue factor [78.8 (82.2)], leukocytes [109.9 (86.9)], endothelium [40.9 (76.5)] and neuron [200.3 (362.4)] are significant higher in AD group than in control group [37.4 (13.4); 39.0 (27.3); 21.8 (20.8) and 41.4 (72.4), respectively; all $p<0.05$]. MPs derived from platelet did not differ between the groups ($p = 0.167$).**Conclusion:** The results suggests that MPs derived from tissue factor, leukocytes, endothelium and neuron could be associated with the physiopathology of AD and, in the future, the MPs may be included as diagnostic biomarker for AD in the clinical routine.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Pediatric/Fetal Clinical Chemistry

## B-245

### Correlation between CD4 counts and Total Lymphocyte Counts in newly diagnosed HIV positive children at Korle Bu Teaching Hospital in Ghana.

C. E. Lekpor[1], W. Ababio[2], F. A. Botchway[1]. *[1]Korle-Bu Teaching Hospital/ University of Ghana/Department of Pathology, Accra, Ghana, [2]KorleBu Teaching Hospital/ University of Ghana, Accra, Ghana*

**Background:** HIV infects T helper lymphocytes, replicates within them and lyses the cells as the replicated virions are released extracellularly to infect yet other CD4 cells. Consequently CD4+ T-helper lymphocytes are gradually depleted and the immune system crippled. Given that HIV induced immunodeficiency is largely due to infection and gradual depletion of CD4+ T-helper cells, CD4 count has become a useful indicator of immune function in infected patients. CD4 count is said to be the most reliable prognostic indicator of immune response to therapy, and is thus a major criterion in the CDC/WHO classification of HIV. Few clinical settings in Low and middle Income countries perform CD4+ T-lymphocyte counts required as a baseline test for antiretroviral therapy. **Methods:** We investigate CD4 count using BD Fascount analyzer in newly diagnosed HIV-infected children attending the HIV clinic at the Child health Department, Korle Bu Teaching Hospital and evaluated suitability of total lymphocyte count (TLC) using Mindray BC 5300 Hematology analyzer as a surrogate marker for CD4+T-lymphocyte count required as a yardstick for initiating antiretroviral therapy. Usefulness of TLC as a surrogate marker for CD4+T-Lymphocyte counts<200, ≤350 and <500cells/ µL for HIV-positive children in our facility was evaluated by 85 pairs of TLC and CD4 counts from 85 newly diagnosed HIV-infected children and results were compared by linear regression and Spearman's correlation analytical tools. **Results:** Approximately 61.7% of our patients were diagnosed late as revealed by CD4 count ≤350cells/µL. An overall good correlation was noted between TLC and CD4+T-cell counts (r=0.54, slope=0.58), mean total lymphocyte count of1.03 ± 0.79, 1.28 ± 1.04 and 1.46 ± 1.02 x $10^9$/L correspond to CD4 lymphocyte counts of <200, ≤350 and < 500cells/µL respectively. **Conclusion**: When considering initiating ART for HIV-infected Ghanaian Children, TLC can be considered as an inexpensive and easily accessible surrogate marker for predicting CD4+T -lymphocyte at two clinically important CD4 thresholds of CD4 count of ≤350 cells/µL and <500cells/µL.

## B-246

### Serum iron reference intervals for brazilian children

B. S. Moura, M. D. Farace, L. G. Assunção, L. Moutinho. *Lab Rede, Belo Horizonte, Minas Gerais, Brazil*

**Background:** Pediatric-specific reference intervals remain inadequate or unavailable for many analytes. Published at 2009, the Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) established reference intervals for children metabolically stable, from five age groups; 0-12 months, 1-5 years, 6-10 years, 11-14 years and 15-20 years. Reference intervals were established according to CLSI/IFCC C28-P3 guidelines by the Robust statistical method. The ranges reflect the central 95% confidence intervals for the population tested. Samples were analyzed for 24 chemistries and 15 immunoassays. These intervals were determined in the Abbott ARCHITECT System®, but could be validated in other analytical immunoassay platforms and local populations, as recommended by the CLSI. Since iron deficiency is the most common cause of anemia, this study aims to assess the applicability of serum/plasma iron CALIPER reference intervals for pediatric Brazilian population, using laboratory database, Lab Rede® - Minas Gerais, Brazil.

**Methods:** Plama/serum samples were collected from 2698 children, with normal ferritin (7-140 ng/mL), from December 2014 to November 2015 and stored at 2-8°C. The ARCHITECT i2000 platform (Abbott Park, IL, USA), chemiluminescent microparticle immunoassay, was used. The results were distributed by age as the CALIPER data: 1-5 years old (n: 1289); 6-10 years (n: 647); 11-14 years (n: 403) and 15-18 (n: 359). Data were submitted to EP Evaluator® program for reference interval verification and statistical analysis (C28-A CLSI). **Results:** The results for groups (central interval of 95%) and their CALIPER reference intervals were approved for all groups.

**Conclusion:** It is a challenge to obtain reference intervals for pediatric population, therefore the use of database sampling constitutes a viable option for checking the ranges proposed at scientific literature. The iron CALIPER 2009 data are applicable to our population.

## B-247

### Heads up! Fetal scalp lactate misclassification error rates based on simulation modelling for the Lactate Pro meter

A. R. Andrews[1], A. W. Lyon[2], M. E. Lyon[2]. *[1]Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Pathology and Laboratory Medicine, Saskatoon Health Region, Saskatoon, SK, Canada*

**Background: :** Fetal scalp lactate is a surrogate measure of fetal metabolic acidosis. The most common cause of fetal metabolic acidosis is intrapartum hypoxia, which can lead to poor neonatal outcomes including low Apgar score and hypoxic ischemic encephalopathy (HIE). Point of care testing (POCT) is used in some centers to measure fetal scalp lactate as an adjunct to electronic fetal monitoring (EFM). The goal of implementing this technique in modern obstetric practice is two-fold: to identify fetuses with abnormal EFM experiencing significant hypoxic distress that require immediate delivery; and to identify stable fetuses who do not require intervention, despite abnormal EFM. Kruger et al. established 4.8 mmol/L as a threshold for the Lactate Pro meter for predicting poor outcome. Wiberg and Källénin used the Lactate Pro meter in their 2017 study to establish a normal reference range for fetal scalp lactate. The aim of this study is to establish the rates of misclassification of fetuses at 4.8 mmol/L using the Lactate Pro meter in populations with both normal and abnormal EFM results.

**Methods:** Descriptive statistics from the Wiberg and Källénin data for fetuses with abnormal EFM and or Apgar scores < 9, and those with normal EFM and Apgar scores ≥ 9 were used. The distributions of lactate were skewed so data was logarithmically transformed and then simulated datasets of 1000 were created for each group. A Monte Carlo simulation model was performed to assess the levels of misclassification at the 4.8mmol/L cut off with various levels of bias (+/- 1mmol/L) and imprecision (CV 0-20%) for both groups.

**Results:** The misclassification rates were affected far more by bias than imprecision in both groups. Furthermore, imprecision had a smaller impact on misclassification rates when associated with positive bias than with negative bias. In the abnormal EFM group, simulation with biases of 0 to +1mmol/L resulted in false positive misclassification rates of 10-70%, while negative biases up to 1mmol/L lead to false negative misclassification rates from 5-20%. In the normal group, false positive misclassification rates were also 10-70% for positive biases of up to 1mmol/L, while the false negative misclassification rates ranged from 1-5% with biases of 0 to -1mmol/L.

**Conclusion:** Simulation models predicted that up to 70% of lactate readings below 4.8mmol/L would be misclassified as above the threshold with biases up to 1mmol/L, which would result in a large proportion of fetuses receiving unnecessary intervention. Though lower than the rates for positive bias, with negative biases of up to 1mmol/L, 5-20% of fetuses with scalp lactates of greater than 4.8mmol/L would be misclassified as less than 4.8mmol/L, and as such would not receive needed intervention. This study calls into question the ability of POCT with the Lactate Pro meter for fetal scalp lactate to provide meaningful information to guide obstetric management.

## B-248

### Performance Evaluation of the ADVIA Chemistry Total Bilirubin_2 (TBIL_2) Assay in Neonatal Samples

P. Datta, S. Patel, J. Snyder, J. Dai. *Siemens Healthcare Diagnostics, Newark, DE*

**Background:** The ADVIA® Chemistry Total Bilirubin_2 (TBIL_2) Reagents, available on ADVIA Chemistry Systems from Siemens Healthcare, measures total bilirubin in human serum and plasma (lithium heparin) samples. Bilirubin is the end-decomposition product of the heme part of red blood cells. It is extracted and biotransformed in liver and excreted in bile and urine. Bilirubin when exposed to light is transformed into several isomers, the double bonds isomerizing in their usual Z-Z configuration to E-E, E-Z, and Z-E configurations. Total bilirubin measurements are used in the diagnosis and treatment of hemolytic, biliary, and liver disorders,

including hepatitis and cirrhosis. The assay was originally validated for adult bilirubin samples only and now has been tested with neonatal samples.*

**Methods:** The ADVIA Chemistry TBIL_2 Reagents are based on a chemical oxidation method using vanadate as an oxidizing agent. The bilirubin is oxidized by vanadate at about pH 2.9 to produce biliverdin. In the presence of the detergent and the vanadate, both conjugated (direct) and unconjugated bilirubin are oxidized:

Bilirubin + Surfactant + $VO_3^-$ -> Biliverdin

This oxidation reaction causes a decrease in the optical density of the yellow color, which is specific to bilirubin. The decrease in optical density at 451/545 nm is proportional to the total bilirubin concentration in the sample. The Siemens Chemistry Calibrator is used to calibrate the assay. CLSI protocols are used to measure the correlation of the assay with the Roche total bilirubin (BILT3) assay on neonatal samples; limits of blank (LoB), detection (LoD), and quantitation (LoQ); interference of hemoglobin A1 and F, indican, and CYANOKIT; and analytical measuring range. The published total bilirubin assay range was confirmed according to CLSI protocol across neonatal (0-5 days) to other ages (up to >90 years).

**Results:** LoB, LoD, and LoQ of the assay were 0.0, 0.1, and 0.1 mg/dL, respectively. Analytical range was minimum up to 35 mg/dL. When tested with 119 neonatal samples (serum: 13 hours to 39 days) and 5 neonatal samples spiked with bilirubin up to 31.6 mg/dL concentration, the ADVIA Chemistry TBIL_2 assay compared as follows with the Roche BILT3 assay: ADVIA = 1.06(Roche) - 0.24 mg/dL, r = 0.99 (range 0.7-31.6 mg/dL). Interference in the assay (up to) was <10% for hemoglobin A and F (1000 mg/dL), indican (10 mg/dL), and CYANOKIT (40 µg/mL). The literature reference range was confirmed[1]. Earlier the assay was shown to have imprecision <4.7% at bilirubin concentrations of 1-15 mg/dL across all ADVIA Chemistry platforms and <10% interference to ascorbic acid (up to 50 mg/dL) and lipemia (up to 750 mg/dL of triglycerides concentrate).

**Conclusion:** The data obtained in this study show that the ADVIA Chemistry Total Bilirubin_2 assay can be used for quantitative determination of total bilirubin in human serum and plasma on the ADVIA Chemistry Systems.

**References:**

1. Wu AHB. Tietz clinical guide to laboratory tests. 4th ed. St. Louis (MO): Saunders Elsevier; c2006. p. 316.

* For Investigational Use Only. The performance characteristics of this device have not been established. Pending 510(k) clearance. Not available for sale for this indication for use.

## B-249

### Study on Strategic Approach of Prenatal Testing for Trisomy 21 in General Pregnancy Population

S. Lee, E. Lee. *Green Cross Laboratories, Yongin, Korea, Republic of*

**Background:** : Recently, non-invasive prenatal testing (NIPT) by analysis of cell-free DNA (cfDNA) in maternal blood has shown promise for highly accurate detection of common fetal autosomal trisomies. Its clinical studies have primarily included pregnant women identified by prior screening to be at high risk for aneuploidies. It has been uncertain if the results of NIPT in such high-risk pregnancies are applicable to the general pregnancy population. It is known that guideline for application of NIPT is developing. This study is to help establishing guideline for prenatal screening through assessment of the performances of various combination method with NIPT and integrated test.**Methods:** We collected 57,639 integrated test data performed by Green Cross laboratories(Yongin, South Korea). Full integrated test was performed during 1st (PAPP-A, free hCG) and 2nd (AFP, hCG, uE3, inhibin A) trimester. 6 scenarios were supposed. S1 scenario is to perform integrated test (cut off 1:270 for Down syndrome) without NIPT. S2 scenarios are to perform NIPT to high risk pregnancies of integrated test with various integrated cut offs, 1:270 for S2-1, 1:500 for S2-2, 1:1,000 for S2-3, 1:2,000 for S2-4. S3 scenario is to perform NIPT without integrated test. Screening performance such as sensitivity, specificity, positive predictive value, negative predictive value, number of pregnancy needed invasive testing like amniocentesis was calculated to each scenario. Also the comparison of cost between scenarios was performed.**Results:** Among 57,639 data, there were 3.1% pregnancies with below 1:270 cut off, 5.0% for below 1:500 cut off, 8.3% for below 1:1,000 cut off and 13.1% for below 1:2,000 cut off. S1 showed lower screening performance and higher frequency of invasive test. S2-1 showed the possibility of false negative like S1, lower frequency of invasive testing and medical costs compared with S1. S2-2 showed the possibility of false negative, the similar medical costs as S1. S2-3 and S2-4 showed no false negativity and higher medical cost. S3 showed the lowest cost effectiveness.**Conclusion:** We thought that the introduction of NIPT could help improving the screening performance for Down syndrome. Also, considering the cost effectiveness, the practice of integrated test as first line test should be kept. At future, the exploration of the acceptability NIPT to service users and the comparison between NIPT and invasive test should be studied for identify the main barriers and facilitators for implementing NIPT within general pregnancy population

## B-250

### The New Total Bilirubin Assay for the Architect cSystems Instrument

T. M. Alligrant[1], C. A. Kasal[1], K. A. Shaffer[2], R. Acorn[3], A. Gardiner[3], K. B. Sikder[1], J. A. Boomer[1], M. A. Beischer[1]. *[1]Abbott Laboratories, Irving, TX, [2]Abbott Laboratories, Abbott Park, IL, [3]Sekisui Diagnostics, Charlottetown, PE, Canada*

**OBJECTIVE:** To report the performance specifications of the new Abbott ARCHITECT Total Bilirubin assay on the *c*Systems instruments.

**RELEVANCE:** The new Total Bilirubin assay (list number [LN] 07P32) displays a longer calibration stability, but otherwise equivalent performance to the predicate assay (LN 06L45).

**METHODOLOGY:** The new Total Bilirubin assay (LN 07P32) utilizes the same diazonium salt methodology as the predicate assay (LN 06L45). The serum or plasma sample is added to the cuvette followed by reagent 1, which contains hydrochloric acid and surfactants. Later, reagent 2 is added to the reaction mixture. The reaction of 2,4-dichloroaniline and sodium nitrite during manufacture of reagent 2 produces 2,4-dichlorophenyl diazonium salt. Upon addition of reagent 2 to the reaction mixture, the diazonium salt reacts with bilirubin from the sample to create azobilirubin. The production of azobilirubin is proportional to the concentration of bilirubin in the sample and is measured at 548 nm.

**VALIDATION:** The table below displays the performance characteristics of the predicate (LN 06L45) and new Total Bilirubin assays (LN 07P32). All interference data shown, represent the highest acceptable interference levels.

**Table 1.** Comparison of performance characteristics of the predicate (LN 06L45) and new Total Bilirubin assays (LN 07P32).

| **Characteristic** | **LN 06L45** | | | | **LN 07P32** | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Type | Serum and plasma | | | | Serum and plasma | | | |
| **Imprecision** | **N** | **Mean (mg/dL)** | **Total SD (mg/dL)** | **Total %CV** | **N** | **Mean (mg/dL)** | **Total SD (mg/dL)** | **Total %CV** |
| | 80 | 0.75 | 0.01 | 2.0 | 80 | 0.91 | 0.018 | 2.0 |
| | 80 | 15.70 | 0.16 | 1.0 | 80 | 16.88 | 0.234 | 1.4 |
| **Interferents:** | **[Interferent]** | **[Bilirubin] (mg/dL)** | **Difference (mg/dL)** | **% Diff.** | **[Interferent]** | **[Bilirubin] (mg/dL)** | **Difference (mg/dL)** | **% Diff.** |
| Hemoglobin | 2000 mg/dL | 1.07 | 0.02 | 1 | 2000 mg/dL | 1.3 | -0.1 | -9.8 |
| | 2000 mg/dL | 14.08 | 0.01 | 0 | 2000 mg/dL | 13.6 | -0.4 | -3.0 |
| Intralipid | 750 mg/dL | 1.27 | 0.30 | 23 | 1000 mg/dL | 1.0 | 0.0 | -1.0 |
| | 1000 mg/dL | 16.50 | 0.31 | 1 | 1000 mg/dL | 13.7 | -0.1 | -0.5 |
| Indican | 0.175 mmol/L | 1.32 | 0.27 | 20 | 0.063 mmol/L | 1.0 | 0.1 | 12.3 |
| | 1.000 mmol/L | 16.01 | 0.95 | 6 | 0.25 mmol/L | 14.9 | 0.5 | 3.1 |
| Indocyanine Green | 18.8 mg/L | 1.53 | 0.24 | 15 | 18.8 mg/L | 1.5 | 0.2 | 15.6 |
| | 100.0 mg/L | 14.27 | 1.19 | 8 | 100.0 mg/L | 14.4 | 1.2 | 8.5 |
| **Method Comparison:** | **vs. Comparative method** | | | | **vs. 6L45** | | | |
| Adult | N | 138 | | | N | 118 | | |
| | R | 0.999 | | | R | 0.9995 | | |
| | Equation | y = 1.02x + 0.10 | | | Equation | y = 1.02x – 0.07 | | |
| | Range (mg/dL) | 0.1 – 23.5 | | | Range (mg/dL) | 0.3 – 23.7 | | |
| Neonatal | N | 54 | | | N | 58 | | |
| | R | 0.997 | | | R | 0.9976 | | |
| | Equation | Y = 0.99x + 0.32 | | | Equation | y = 1.00x + 0.03 | | |
| | Range (mg/dL) | 1.0 – 19.1 | | | Range (mg/dL) | 0.4 – 23.7 | | |
| Calibration Stability (hours) | 336 (14 days) | | | | 504 (21 days) | | | |

**CONCLUSIONS:** The new Total Bilirubin assay (LN 07P32) has a longer calibration stability while maintaining equivalent performance when compared to the predicate method.

## B-251

### Comparison of Two Automated Immunoassays for Progesterone to Mass Spectrometry to Aid in the In Vitro Fertilization Setting

M. Ali[1], S. M. Roper[1], J. Purviben[2], S. Marilyn[2], J. Mawyer[2], J. Rubin[3], W. E. Gibbons[3], P. W. Zarutskie[3], S. Devaraj[4]. *[1]Department of Pathology & Immunology, Baylor College of Medicine, Division of Clinical Chemistry, Department of Pathology & Immunology, Texas Children's Hospital, Houston, TX, [2]Department of Pathology & Immunology, Texas Children's Hospital, Houston, TX, [3]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [4]Department of Pathology & Immunology, Baylor College of Medicine, Division of Clinical Chemistry, Department of Pathology & Immunology, Texas Children's Hospital., Houston, TX*

***Background:*** Progesterone levels vary during the menstrual cycle, pregnancy and embryogenesis. Progesterone levels are measured to monitor ovarian functions, during infertility treatment, pregnancy or abnormal uterine bleeding. The gold standard method for progesterone measurement is tandem mass spectrometry. However, in the In Vitro Fertilization (IVF) setting, where low progesterone levels are monitored, due to the need for fast turnaround time, decreased cost and sample preparation requirements, automated immunoassays are most often implemented. The main aim of our study was to determine the precision and accuracy of progesterone levels measured on the ADVIA Centaur® CP analyzer and Architect i1000SR (Abbott) analyzer relative to the reference method (LC-MS/MS). Our study will significantly optimize the utility of progesterone assay in patients undergoing IVF**.** The main aim of our study was to compare the analytical performance of two automated immunoassays for progesterone to the reference method, Liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our study will significantly optimize the utility of progesterone assay in patients undergoing IVF.

***Method:*** De-identified serum samples were collected from 23 adult female patients undergoing IVF at the Texas Children's Pavilion for Women. Analytical performance parameters of precision, linearity, accuracy, and correlation were measured on the ADVIA Centaur® CP analyzer and Architect i1000SR (Abbott) analyzer. Results from these immunoassays were compared with an LC-MS/MS method on AB SCIEX 5500 tandem mass spectrometer.

***Results:*** Intra and inter-assay precision for the Architect was <10 %, for Centaur it was <8%, and for LC-MS/MS it was <9 %. Linearity for the Architect was 0.11 to 40.53 ng/ml, for Centaur it was 0.21-60 ng/ml and linearity for LC-MS/MS was 0.17-25.6 ng/ml. For all serum progesterone samples (ranging from 0.7 to 63.9 ng/ml), Deming regression equation for Centaur CP relative to LC-MS/MS was y=0.89x+0.64, r=0.99, (n=23), Centaur CP showed a negative bias of -4.3%. However, for low progesterone samples (ranging from 0.7 to 2.2 ng/ml), Deming regression equation for the Centaur CP relative to LC-MS/MS was y=0.75x+0.57, r=0.90, (n=15), Centaur CP showed a positive bias of 21.9%. On the other hand, for all serum progesterone samples (ranging from 0.4 to 66.1 ng/ml), Deming regression equation for the Abbott Architect relative to LC-MS/MS was y=0.89x+0.04, r=0.99(n=23), the Architect showed a negative bias of -11.1%. For low progesterone samples (ranging from 0.4 to 2.0 ng/ml), Deming regression equation of the Architect relative to LC- MS/MS was y=0.87x+0.26, r=0.92 (n=15), the Architect showed a positive bias of only 6.9%.

***Conclusion:*** Optimization for the progesterone assay can be achieved by comparison across platforms, using LC-MS/MS method. Architect progesterone assay showed better performance and closer correlation than the Centaur assay to the gold standard mass spectrometry, particularly for the testing of low levels of progesterone. Clinically it is very important to report the progesterone levels accurately immediately without delay to initiate IVF treatment. Thus, we aim to use the Architect progesterone assay to test low levels of progesterone and aid in clinical challenges met during IVF treatment.

## B-252

### Acute Infection Etiology of Febrile Children

I. Srugo[1], K. Oved[2], A. Klein[3], M. Stein[3], O. Golan-Shany[1], N. Kerem[1], I. Chistyakov[1], J. Genizi[1], O. Glazer[1], D. Miron[4], Y. Shachor-Meyouhas[5], E. Bamberger[2], M. Shapira[3], T. Gottlieb[2], R. Navon[2], M. Paz[2], L. Etshtein[2], O. Boico[2], G. Kronenfeld[2], E. Eden[2], R. Cohen[6], H. Chappuy[7], F. Angoulvant[8], L. Lacroix[9], A. Gervaix[9]. *[1]Bnai Zion Medical Center, Haifa, Israel, [2]MeMed Diagnostics, Tirat Carmel, Israel, [3]Hillel Yaffe Medical Center, Hadera, Israel, [4]Emek Medical Center, Afula, Israel, [5]Rambam Health Care Campus, Haifa, Israel, [6]Centre Intercommunal de Creteil, Creteil, France, [7]Armand Trousseau Hospital, Pierre et Marie Curie University, Paris, France, [8]APHP, Necker-Enfants Malades Hospital, Paris, France, [9]Geneva University Hospitals and University of Geneva, Geneva, Switzerland*

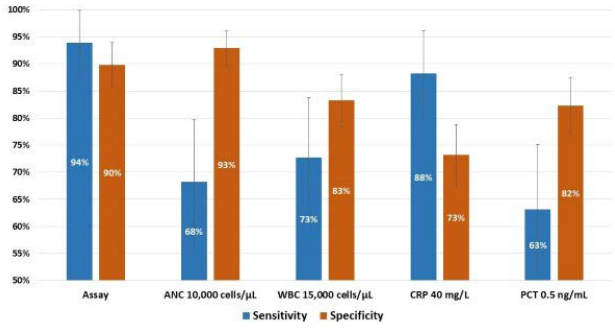
**Background:** A novel assay (ImmunoXpert™) that integrates measurements of three blood-borne host-response proteins (TRAIL, IP-10, and CRP) was recently developed to assist in differentiation between bacterial and viral disease. Here we compare the assay performance with standard laboratory parameters that are routinely used in clinical practice to facilitate diagnosis of infection etiology in febrile children.

**Methods:** We studied serum remnants collected from children aged 3 months to 18 years with suspicion of acute infection presenting at the ED or admitted. Reference standard diagnosis was based on predetermined criteria plus adjudication by an expert panel blinded to assay results. Assay performers were blinded to reference standard. Assay cut-offs were defined before un-blinding.

**Results:** Of 529 potentially eligible patients, 100 did not fulfill infectious inclusion criteria and 68 had insufficient serum. The resulting cohort comprised 361 patients, with 239 viral, 68 bacterial, and 54 indeterminate reference standard diagnoses. The assay distinguished between bacterial and viral infected patients with 93.8% sensitivity (95% CI: 87.8%-99.8%) and 89.8% specificity (85.6%-94.0%); 11.7% had an equivocal assay outcome. Overall the assay outperformed other laboratory parameters, including: (i) white blood count (WBC; cut-off 15,000cells/µl, sensitivity 72.7% (61.7%-83.8%), P<0.002; specificity 83.2% (78.3%-88.1%), P<0.05); (ii) CRP (cutoff 40 mg/L, sensitivity 88.2% (80.4%-96.1%), P<0.37, specificity 73.2% (67.6%-78.9%), P<0.001); (iii) Procalcitonin (PCT; cutoff 0.5 ng/ml, sensitivity 63.1% (51.0%-75.1%), P<0.001), specificity 82.3% (77.1%-87.5%), P<0.03); (iv) absolute neutrophil count (ANC; cut-off 10,000 cells /µl, sensitivity 68.2% (56.6%-79.7%), P<0.001; specificity 92.9% (89.6%-96.3%), P<0.30).

**Conclusion:** The host response-based assay was more accurate than routine laboratory parameters and biomarkers (WBC, ANC, CRP, PCT) in distinguishing bacterial from viral etiologies in febrile children. It has the potential to help clinicians avoid missing bacterial infections or overusing antibiotics.

**Figure 1:** Diagnostic performance of host-signature assay compared to routine laboratory parameters and other biomarkers




## B-253

### Comparison of LeadCare Ultra® to ICP-MS as an initial screen for blood lead levels

L. M. Johnson, M. Lynch, A. B. Karger. *University of Minnesota, Minneapolis, MN*

**Objective**: In 2012, the CDC issued new guidelines for assessing children's blood lead levels (BLLs) based on the National Health and Nutrition Examination Survey (NHANES). The study defined a BLL reference cut-off of 5 µg/dL based on the 97.5th percentile. Prior to these guidelines, children had a BLL "of concern" at or above 10 µg/dL. Given the change of cut-off, it is critical that laboratory methods are accurate

at the lower BLL cut-off of 5 µg/dL. Well-established methods for measuring BLLs include Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) because they are highly accurate and precise methods. However, the instruments are very costly and require expertise to operate and maintain. The LeadCare Ultra® (Magellan Diagnostics) is an FDA-approved instrument that uses anodic stripping voltammetry (ASV) to measure BLLs. The LeadCare Ultra is both cheaper and easier to run and maintain than GFAAS or ICP-MS, and thus is an attractive alternative for clinical labs that are currently sending out lead level testing to reference laboratories. However, there have been no prior studies assessing the performance of the LeadCare Ultra at the BLL cut-off of 5 µg/dL. Therefore, our objective was to perform a method comparison study of the LeadCare Ultra with ICP-MS to determine whether the LeadCare Ultra provides acceptable performance at a BLL cut-off of 5 µg/dL and thus could be used to effectively screen children for elevated BLLs.

**Methods**: 50 µL of heparinized capillary whole blood was transferred into a vial with 250 µL of 0.1M HCl to lyse cells and release lead into the solution for analysis on the LeadCare Ultra. For the method comparison, a blood sample was sent to Medtox (St. Paul, MN) to be analyzed by ICP-MS. Method verification studies included accuracy, precision, reference interval, and reportable range.

**Results**: To assess accuracy, we analyzed 75 samples and statistically compared results of the paired BLLs. We used CLIA proficiency testing criteria of ± 10% or ± 4 µg/dL for allowable total analytical error. Although all measurements passed these criteria, we found that the LeadCare Ultra has a large positive bias of 1.68 µg/dL in the BLL range of ≤ 4 µg/dL (n=38), low negative bias of 0.05 µg/dL in the BLL range of 5-10 µg/dL (n=23), and a negative bias of 0.81 in the BLL range ≥ 10 µg/dL (n=14). The bias across the entire BLL range from 1-45 µg/dL was 0.72 µg/dL (n=75). Deming regression had a slope of 0.91 (95%CI: 0.87-0.94) and intercept of 1.30 (95%CI: 0.94-1.66). Both within-day and between-day precision was less than 10%.

**Conclusion:** The LeadCare Ultra was compared to ICP-MS and found to be acceptable as an initial screen (BLL cut-off of 5 µg/dL) due to the low bias in the BLL range of 5-10 µg/dL. The LeadCare Ultra does have a large positive bias in the BLL range of ≤ 4 µg/dL (1.68 µg/dL), and therefore should not be relied upon to accurately quantify lead levels below the cut-off of 5 µg/dL.

## B-254

### Evaluation of common paediatric creatinine based glomerular filtration rate estimating equations in Black African children in KwaZulu-Natal, South Africa

Y. D. Rampursat[1], R. Bhimma[2], V. Gounden[1]. *[1]National Health Laboratory Services, University of KwaZulu-Natal, Durban, South Africa, [2]Department of Paediatrics, University of KwaZulu-Natal, Durban, South Africa*

**Background**

Accurate determination of glomerular filtration rate (GFR) is important. However use of exogenous substances for measured GFR (mGFR) has limitations. Use of equations for estimating GFR (eGFR) are routinely used due to convenience and widespread availability. We aimed to i) Determine the validity of the revised Schwartz equation in our local Black African paediatric population in KwaZulu-Natal, South Africa and ii) Determine applicability of three other common paediatric GFR estimating equations to the same dataset.

**Methods**

Study performed at the Inkosi Albert Luthuli Central Hospital, Durban South Africa. Results of mGFR determined by intravenous $^{99m}$Tc DTPA clearance, serum creatinine (within 24 hours of mGFR) and anthropometric data were obtained from electronic medical records of African patients aged 2- 18 years old from January 2010 - December 2014. Serum creatinine was performed on Siemens ADVIA 1800. eGFRs calculated using the original and revised Schwartz, Counahan and Leger equations were compared to mGFR. Percentage bias, accuracy within 10 % ($P_{10}$) and 30% ($P_{30}$) of the mGFR and sensitivity and specificity for predicting GFR < 60 ml/min/1.73 m$^2$ was determined.

**Results**

A total of 148 patients were included in analysis. Overall $P_{30}$ and $P_{10}$ values were highest for the original Schwartz equation with 60% and 22% of results respectively. $P_{30}$ was better for the revised Schwartz equation (49%) than for the original Schwartz equation in adolescent boys (24.3 % p=0005). Receiver operating characteristic (ROC) curve analysis revealed similar area under the curve (AUC) for the 4 equations for predicting mGFR < 60 ml/min/1.73 m$^2$. See Table 1.

**Conclusions**

Overall none of the equations met the National Kidney foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines of 90% of eGFR results within 10% of measured GFR.

Table 1

| | Original Schwartz n=111 | Original Schwartz: adolescent boys n = 37 | Counahan n= 148 | Leger n= 148 | Revised Schwartz n= 148 |
|---|---|---|---|---|---|
| Passing and Bablok regression analysis Constant error (95% CI) | -17.23 (-28.6 to -7.89) | -72.57 (-183.57 to -20.74) | -51.94 (-71.43 to -34.42) | -18.59 (-29.52 to -7.62) | -10.48 (-19.38 to -4.42) |
| Passing and Bablok regression analysis Proportional error (95% CI) | 1.29 (1.15 to 1.44) | 2.67 (1.73 to 4.39) | 1.89 (1.66 - 2.19) | 0.95 (0.81 to 1.12) | 0.97 (0.88 to 1.07) |
| Accuracy within 30 % of mGFR | 60 % | 24.3 % | 42% | 34 % | 49% |
| Accuracy within 10% of mGFR | 22 % | 2.7% | 16 % | 10 % | 16% |
| >60ml/min/1.73m2 Sensitivity% (95 %CI) Specificity% (95 %CI) AUC (95 %CI) | 93.55 (78.6-99.2) 95.00 (87.7-98.6) 0.97 (0.92-0.99) | 90.91 (58.7-99.8) 100 (86.8-100) 0.93 (0.80-0.99) | 92.86 (80.5-98.5) 95.28 (89.3-98.5) 0.967 (0.92-0.99) | 90.48 (77.4-97.3) 96.23 (90.6-99) 0.948 (0.90-0.98) | 92.86 (80.5-8.5) 95.28 (89.3-98.5) 0.96 (0.92-0.99) |

## B-255

### Prevalence of sampling errors in umbilical cord blood samples and the effect on determination of reference intervals

F. Leung, M. L. Parker, I. L. Bromberg. *University of Toronto, Toronto, ON, Canada*

**Background:** The measurement of umbilical cord blood gas (UCBG) analytes is a standard procedure intended to document fetal metabolic status at birth. Unfortunately, UCBG analysis is complicated by the anatomy of the cord which consists of a central vein (UV) entwined by two arteries (UA). Due to this architecture, errors from double sampling of the vein or arteries ('same' sampling) or inadvertent swapping of the samples ('swap' sampling) often occur. Such errors have been previously reported to occur in 10-30% of UCBG samples but have not been adequately considered in the establishment of reference intervals (RIs). **Objectives:** To establish a quality indicator for detecting sampling errors in a cohort of deliveries performed at Mount Sinai Hospital (Toronto, Canada) and to determine the effect of such sampling errors on establishing RIs for UCBG analytes. **Methods:** A total of 1301 spontaneous vaginal births were identified from May to October 2015 with at least one associated UCBG sample. Of these, 863 were selected for further analysis using the following inclusion criteria: (1) singleton pregnancy; (2) 5$^{th}$ to 95$^{th}$ centile for birth weight (2640 to 4110 grams); (3) APGAR score >7 at 1 minute; and (4) no resuscitation We developed a scoring system based on differences between paired samples as a sampling error quality indicator, observed about 35% "same" and 5% "swap" errors, and applied the 2.5$^{th}$ and 97.5$^{th}$ centiles as RIs based on the remaining 442 pairs. **Results:**

Table 1 - Reference Intervals of Umbilical Cord Blood Gas Analytes

| Analyte | Difference Calculation | Difference Cut-Offs | | UA Reference Interval | | UV Reference Interval | |
|---|---|---|---|---|---|---|---|
| | | "Same" Within | "Swap" Less Than | Uncorrected | Corrected | Uncorrected | Corrected |
| pH | UV-UA | ± 0.02 | - 0.02 | 7.09 to 7.35 | 7.10 to 7.30 | 7.19 to 7.39 | 7.21 to 7.39 |
| $PCO_2$ (mmHg) | UA-UV | ± 3 | - 3 | 35 to 75 | 44 to 77 | 33 to 56 | 33 to 53 |
| $PO_2$ (mmHg) | UV-UA | ± 5 | - 5 | 10 to 41 | 9 to 28 | 16 to 42 | 19 to 45 |
| $sO_2$ (%) | UV-UA | Not used | Not used | 14 to 85 | 11 to 62 | 29 to 88 | 41 to 89 |
| $BD_{ecf}$ (mmol/L) | UA-UV | Not used | Not used | -0.3 to +11 | -1.4 to +9.8 | +0.4 to +10.2 | +0.3 to +10 |

**Conclusions:** Correction for "same" or "swap" sampling made a conspicuous difference for UA results, which most directly reflect fetal status at birth. Our finding

that UV RIs were only slightly affected by sampling errors suggests that most errors involved collecting UV instead of UA. Nevertheless, UV results are important for confirming UA validity. Detection of common sampling mistakes is crucial for establishing appropriate UCBG RIs.

## B-256

### Rapid Detection of Early Stage *Mycoplasma pneumonie* Infection Using Single-Walled Carbon Nanotube/Colloidal Gold-Based Immunochromatographic Strips

M. Song[1], Y. Zhang[2], S. Li[3], M. Mao[4], C. Zhang[4], M. Tao[4], S. Cai[1], W. Xu[1], Z. Jiang[4], W. Xu[1]. *[1]Shenyang Pharmaceutical University, Shenyang, China, [2]Shenyang Entry-Exit Inspection and Quarantine Bureau Technology Center, Shenyang, China, [3]Liaoning Normal University, Shenyang, China, [4]Benxi TestJet Biotechnology Co. Ltd., Shenyang, China*

**Background:** Current clinical diagnosis of *Mycoplasma pneumonia* infection typically fall under two major categories: immunological assays (ELISA, immunopreciptation, etc.) to detect IgG and/or IgM, and PCR-based assays to test for the nucleic acids of the pathogens. However, a positive result from immunological test usually indicated the existing infection condition of the patient while the PCR test normally requires days of turnaround time. The objective of the current research was to develop quicker and easier way to detect Mycoplasma pneumonia from patient throat swaps.

**Methods:** To achieve a better detection efficiency, the single-walled carbon nanotubes (SWCNT) were coupled with the colloidal gold-monoclonal antibody immunochromatographic strips (CGIC). Two hundred microliters of anti-*M. pneumoniae* (P1) mouse monoclonal antibody (IgG) at 0.5 mg/ml was added into 10 ml colloidal gold solution (pH 8.5) with shaking for 30 minutes. One thousand microliters of 5% BSA was supplemented with shaking for another 30 min. Ten mg of SWCNT (DKnano, China) was dispersed in 100 ml miliQ pure water supplemented with one hundred microliters of Tween 20 by ultrasonicating for 5 min. Five µl of SWCNT solution was mixed with the above colloidal gold/IgG solution with shaking for 30 min. The mixed solution was centrifuged at 9500 rpm for 30 min at 4°C. The pellet was resuspended in 5000 µl of the suspension buffer (0.01mol/L pH 8.5 Tris-HCl, 25% sucrose, 2.5% BSA, 1.5% Tween-20, 0.25% PEG-20000, 0.20% sodium caseinate). Repeating the centrifugation at 9500 rpm for another 30 min at 4°C, and resuspending the pellet in 240µL of suspension buffer. The final SWCNT-colloidal gold-labeled antibody conjugate was jetted onto the glass fiber and dried at 37°C for 4hr. The specific detection antibody (Test line, T) at 1.5 mg/ml and the goat-anti-mouse control antibody (Control line, C) were transferred onto the nitrocellulose membrane and dried at 37°C for 2 hrs. The finalized detection strip was consisted of sample pad, conjugate pad, immobilized nitrocellulose membrane, and absorbent pad. Throat swap was used to collect the samples from the patients and the samples were diluted into 500µl of 1xPBS. Eighty microliters was used for the SWCNT/CGIC strip test for 15 minutes.

**Results:** A sequential cohort of 137 patients aged 3-14 years with fever/cough/fatigue were recruited with custodian-signed informed consent during Dec 1, 2016 to Jan 31, 2017 at the pediatric respiratory department of 3rd Affiliated Hospital of Jilin University. Among them, 97 were diagnosed as positive for *Mycoplasma pneumoniae* based on CRP, WBC, NEUT, LYM, body temperature, pulmonary iconography, immunological passive particle agglutination (PPA, Serodia-Myco II, Fujirebio, Japan), and RT-PCR (Daan gene Co. Ltd. China), and other clinical symptoms. SWCNT/CGIC strips achieved a sensitivity of 72.2% (70/97) and a specificity of 100.0% (40/40). The corresponding positive and negative predictive values were 100.0% (70/70) and 59.7% (40/67), respectively.

**Conclusion:** The SWCNT/CGIC strips demonstrated its effectiveness as an easy and quick detection for *Mycoplasma pneumoniae* infection.

## B-257

### A Creative Approach to Establishing Pediatric Reference Intervals for Ionized Calcium

C. Rosemark, D. Block, S. Bryant, N. A. Baumann. *Mayo Clinic, Rochester, MN*

**Background**: Reference intervals (RIs) represent the range of laboratory test results derived from a group of healthy people. It is important for laboratories to provide accurate RIs, however challenges exist. In practice, pediatric RIs are difficult to establish or verify. Establishing RIs for analytes such as serum ionized calcium (iCal) is especially challenging due to analyte instability and because analyzer manufacturers typically provide only adult RIs for whole blood specimens. The objectives of this study were to (i) establish pediatric and adult iCal RIs in serum using screened, retrospective patient data (ii) verify established adult RIs using healthy donors and (iii) assess bias and RI transferability between serum and whole blood iCal values.

**Methods**: This study was exempt by the Mayo Clinic Institutional Review Board. Serum iCal results from 7/1/2008-7/1/2016 were included (263,724 results from 42,109 outpatients) using GEM3000 Premier analyzers (Instrumentation Laboratories, Bedford, MA). ICD-codes were used to exclude patients with heart failure, bone/renal disease, hypertension, and malignancy. Only the first result per patient was included, yielding n=506 pediatric and n=944 adult values. Age and sex partitioning was assessed. Central 95%RIs and 95% confidence intervals (CIs) were calculated using quantile regression. Age breaks were applied when the calculated range differed by >0.15mg/dL. Healthy adult donors were used to verify adult RIs and assess bias between serum and electrolyte-balanced lithium-heparin whole blood.

**Results:** No significant difference between males and females was observed. The table summarizes the established RIs and CIs. Healthy adult donors verified the RIs in serum and whole blood. Serum demonstrated a 2%(0.1mg/dL) bias compared to whole blood.

Table 1. Reference Intervals for Serum iCal

| Age | N | Central 95% RI | 95% CI on lower bound | 95% CI on upper bound | Verified with Healthy Donors? |
|---|---|---|---|---|---|
| 14 days-<1 yr | 36 | (5.21, 6.01) | (4.88, 5.54) | (5.94, 6.56) | n/a |
| 1-<2 yrs | 35 | (5.04, 5.84) | (4.82, 5.26) | (5.74, 6.11) | n/a |
| 2-<3 yrs | 42 | (4.87, 5.67) | (4.46, 5.28) | (5.39, 5.83) | n/a |
| 3-<24 yrs | 573 | (4.83, 5.52) | (4.80, 4.85) | (5.46, 5.58) | Yes (n=8, age >17) |
| 24-<98 yrs | 767 | (4.57, 5.43) | (4.49, 4.64) | (5.36, 5.49) | Yes (n=20) |

**Conclusion**: Pediatric and adult RIs for iCal were established using screened, retrospective patient data and verified in serum and whole blood. Despite a 0.1mg/dL positive bias in serum, the established RIs are transferable to either matrix.

## B-258

### Transference and Verification of CALIPER Pediatric Reference Intervals to Ortho VITROS 5600 Chemistry Assays

V. Higgins[1], D. Truong[2], A. Woroch[3], M. Chan[3], H. Tahmasebi[1], K. Adeli[1]. *[1]SickKids Hospital, University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]SickKids Hospital, Toronto, ON, Canada*

**Background:** Reference intervals (RIs), defined as the central 95% of laboratory test results obtained from a healthy reference population, are essential to accurately interpret pediatric test results. Although the concept and utility of pediatric RIs is straightforward, developing them can be quite complex, particularly in the pediatric population, As a result, pediatric RIs have been severely lacking, with several laboratories using adult RIs to inappropriately fill the gap. CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) developed an age- and sex-specific pediatric RI database based on thousands of healthy children and adolescents aged 0-<19 years of age. Originally established for Abbott assays, CALIPER has performed a series of transference studies to make CALIPER RIs applicable to additional analytical platforms. This study transfers reference intervals for 34 biochemical assays to the Ortho VITROS 5600 Chemistry System.

**Methods:** In accordance with CLSI guidelines, a method comparison was performed between Abbott and Ortho assays by measuring 200 patient serum samples. If the comparison data yielded an $r^2 \geq 0.95$, simple linear regression using the least squares approach was used to estimate the line of best fit. For data yielding $r^2 < 0.95$, Deming regression is used. If data yields $r^2 < 0.70$, the reference intervals were not transferred due to poor correlation. The appropriateness of the linear model was assessed using Q-Q, standardized residual, and Bland-Altman plots. If the linear model was deemed appropriate, the equation of the line of best fit was calculated and used to transfer RIs and corresponding confidence intervals from Abbott to Ortho assays. Transferred RIs were verified using 86 healthy pediatric serum samples from the CALIPER cohort. RIs were considered verified if >90% of reference samples fell within the transferred confidence limits.

**Results:** Reference limits for most biochemical assays successfully transferred from Abbott to Ortho assays, with the exception of calcium and $CO_2$, which failed to transfer due to poor correlation between Abbott and Ortho assays, with $r^2$ values of 0.611 and 0.305, respectively. Of the 32 successfully transferred analytes, 27 successfully verified with >90% of reference samples falling within transferred confidence limits.

For five of the assays, transferred reference intervals could not be verified using healthy children samples, including total bilirubin, magnesium, LDH, ASO, and C4.

**Conclusion:** This transference study broadens the utility of the CALIPER pediatric RI database to laboratories using the Ortho VITROS 5600 analyzer. These evidence-based RIs will improve the accuracy of pediatric test result interpretation in healthcare institutions using this clinical chemistry analyzer. Prior to implementing these RIs, all laboratories should verify them for use on their individual analytical platform and local population as recommended by CLSI.

## B-259

### Reference intervals for oxysterols as biomarkers in Niemann-Pick C disease with LC-MS/MS method in Turkey

I. Lay[1], A. Samadi[2]. *[1]Hacettepe University Faculty of Medicine, Department of Medical Biochemistry, Clinical Pathology Laboratories, Ankara, Turkey, [2]Hacettepe University Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey*

**Background:** The LC-MS/MS is a sensitive method to quantify colestane-3β,5α,6β-triol (C-triol) and 7-ketocholesterol (7-KC) in plasma to screen for Niemann-Pick type C disease (NP-C). NP-C is a rare progressive autosomal recessively inherited lipid storage disorder with heterogeneous clinical presentation and challenging diagnostic procedures. Definitive diagnosis is based on genetic investigations. Recently, oxysterols that result from non-enzymatic oxidation of cholesterol have been reported to be precise and specific biomarkers for NP-C but knowledge on reference intervals are lacking.

**Methods:** Measurements of plasma C-triol and 7-KC were performed on a triple quadrupole mass spectrometer (Shimadzu 8040 LC-MS/MS, Japan) following derivatization with dimethylglycine esters. The reported method is linear ($r > 0.99$), sensitive, detection limit of 0.03 ng/mL for C-triol, and 0.54 ng/mL for 7-KC, and precise, with an intra-day imprecision of 4.1 - 4.8% and an inter-day imprecision of 7.0 - 11.0% for C-triol and 7-KC, respectively. Reference interval study was performed according to CLSI C28 guideline from a total number of 205 healthy volunteers in both gender. We divided our study group into two categories, under 40 years and over 40 years.

**Results:** Reference intervals for oxysterols were shown in table. A total number of 28 NP-C patients were diagnosed using our reference intervals.

**Conclusion:** Essential reference values were determined with a sensitive LC-MS/MS method for screening test of NP-C in Turkish population. Plasma oxysterols are rapid and reliable biomarkers for NP-C.

Reference intervals for plasma oxysterols by LC-MS/MS

| | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|
| | | n | Mean ± SD | Reference intervals | n | Mean ± SD | Reference intervals |
| 7-KC | <40 years | 53 | 19.66 ±1.70 | 16.32 - 23.00 | 55 | 19.94 ±1.71 | 16.59 - 23.29 |
| | >40 years | 50 | 20.38 ±1.79 | 16.87 - 23.89 | 47 | 20.07 ±1.52 | 16.53 - 22.55 |
| | Total | 103 | 20.05 ±1.78 | 16.57 - 23.53 | 102 | 20.00 ±1.62 | 16.38 - 22.52 |
| C-triol | <40 years | 53 | 8.67 ±3.23 | 3.36 - 15.03 | 55 | 10.08 ±2.98 | 4.25 - 15.91 |
| | >40 years | 50 | 11.10 ±2.60 | 6.00 - 16.20 | 47 | 10.11 ±2.88 | 4.47 - 15.76 |
| | Total | 103 | 9.85 ±3.17 | 3.57 - 15.16 | 102 | 10.10 ±2.92 | 4.38 - 15.81 |

## B-260

### Principle Component and Correlation Analysis of Biochemical and Endocrine Markers in the CALIPER Cohort of Healthy Children and Adolescents

V. Higgins[1], S. Hooshmand[2], M. Chan[2], K. Adeli[1]. *[1]SickKids Hospital, University of Toronto, Toronto, ON, Canada, [2]SickKids Hospital, Toronto, ON, Canada*

**Background:** Reference intervals provide a baseline for which laboratory test results are compared in clinical practice. Reference values of several biomarkers are known to be affected by age and sex. However, an important additional covariate which is often overlooked is the concentration of other biomarkers. Biomarker concentrations are often not independent of each other, but may correlate due to various physiological processes. Identifying significantly correlated biomarkers in healthy individuals can be valuable when interpreting patients' laboratory test results. Here, we report a comprehensive biomarker correlation study in the healthy CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) cohort using principle component and heat map analyses.

**Methods:** CALIPER collected blood samples from healthy pediatric subjects (0-<19 years). Here, we used data from children aged 1-<19 years, as those <1 year only had a small number of blood tests examined, limiting the ability to examine correlations. In total, we examined 35 routine chemistry markers and 20 fertility and endocrine markers measured using Abbott Architect assays. Outliers were detected and removed using a principle component analysis (PCA) score plot and Mahalanobis distance. PCA was performed by finding an alternate set of linearly uncorrelated variables called principal components. Loading plots were created, displaying main patterns of biomarker concentration and dependencies between them. Spearman's rank correlation coefficients were computed and displayed with a heatmap. Analysis was performed on all subjects, including children (<10 years), and adolescent males and females (≥10 years).

**Results:** A high level of agreement was observed between Spearman's heatmaps and PCA loading plots. Eighteen of 595 chemistry marker correlations examined had moderately strong Spearman's coefficients ($r \geq 0.50$) across all subjects, with fewer and unique correlations found when children and adolescents were analyzed separately. Seventeen of 190 fertility and endocrine marker correlations examined had moderately strong Spearman's correlations across all subjects. Again, fewer and unique correlations were found when children and adolescents were analyzed separately. Comparing loading plots and heatmaps between the age and sex separated groups, interesting correlation patterns were apparent. Thyroid hormones were a good example of this, where FT3 is positively correlated (heatmaps) and clustered with (loading plots) TT4 and FT4 in all subjects, children, and adolescent males. However, in adolescent females this is not apparent. Additionally, a high dependency between most fertility and endocrine markers with age is present in adolescent females, but is much less apparent in children and adolescent males.

**Conclusion:** This study provides, for the first time, a robust PCA and Spearman's correlation analysis for 35 chemistry and 20 fertility and endocrine markers in a healthy pediatric population. Identifying biomarker correlations will enable clinical laboratorians and physicians to consider the potential influence of other biomarkers on their biomarker of interest when establishing reference intervals, ordering laboratory tests, and interpreting laboratory test results.

## B-261

### Urinary metabolome in autistic children and in their unaffected siblings: preliminary data on the role of oxidative stress and gut dysbiosis

A. Noto[1], M. Mussap[2], M. Lussu[3], R. Francavilla[4], V. Fanos[1], L. Atzori[3]. *[1]Department of Surgical Sciences, University of Cagliari, Cagliari, Italy, [2]Laboratory Medicine Service, IRCCS AOU San Martino-IST, University-Hospital, Genoa, Italy, [3]Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy, [4]Pediatric Division of University of Bari, Bari, Italy*

Background: Autism Spectrum Disorder or Disease (ASD) is a nonspecific term encompassing a wide range of complex neurodevelopmental disorders, characterized by repetitive and distinctive patterns of behavior and difficulties with social communication and interaction. The development and expression of ASD depend on multiple interactions between genetic, epigenetic, and environmental factors. Behind the plethora of potential risk factors, recent evidences suggest that most of environmental factors may arise from alterations in the gut microbial ecosystem. Indeed, perturbation of the gut microbiota can influence brain development and affect social, emotional, and anxiety-like behaviors by immune-mediated, neural, or humoral mechanisms. Current knowledge points out the role of metabolites derived from the gut microbiota in autism: they can modulate the behavioral phenotype of the host, greatly influencing host metabolic pathways and the immune system and determining the individual susceptibility to disease. The aim of this study was to identify the urine metabolic fingerprint in 21 autistic patients aged 4-16 years (18 males) and to compare it with that obtained in 21 healthy siblings aged 4-17 years (10 males). In addition, we investigated the gut microbiota composition in ASD patients. Methods: Samples analysis was done by Proton Nuclear Magnetic Resonance ($^1$H-NMR) spectroscopy. For each sample, 630 µL were added to 70 µL of a 1.5 M phosphate buffer solution pH 7.4 with a final concentration of 0,5 mM TSP (trimethylsilyl propanoic acid). Each sample was stirred for 60 seconds and transferred into a 5 mm NMR tube for

analysis. Spectra were acquired at 499,839 MHz using a Varian Unity Inova 500 MHz spectrometer (Bruker s.r.l., Milan, Italy). Gut microbiota in ASD patients was characterized by targeted sequencing of bacterial 16S rRNA genes in stool samples. Multivariate statistical analysis was performed by using the software SIMCA-P+13.0 (Umetrics, Umeå, Sweden). The final dataset was scaled using unit variance (UV) scaling to minimize the effect of spectral noise or high variability of the variables. Partial least Square Discriminant Analysis (PLS-DA) identified the set of important variables on the projection (VIPs); they were considered the most important metabolites responsible for differences in urine metabolome between groups. Results: Compared with controls, the urine metabolome in ASD children showed increased levels of hippurate, glycine, creatine, tryptophan and D-threitol as well as decreased levels of glutamate, creatinine, lactate, valine, betaine and taurine. These results suggest that children with ASD may show an imbalance in amino acid metabolism: the increase in tryptophan urine levels indicates alterations of the serotonergic pathway as well as the increase of hippurate, a mammalian-microbial co-metabolite, is closely related with gut dysbiosis. Concomitantly, the decrease in glutamate urine level suggests impairment in excitatory neurotransmission. Changes in urine levels of creatine and taurine are associated with an increasing oxidative stress, which in turn has been previously found in several studies on ASD. Gut dysbiosis in autistic children was characterized by a significant increase in *Clostridium spp*. Conclusion: In conclusion, oxidative stress and gut dysbiosis are involved in metabolic perturbations associated with ASD.

## B-262

### Neonatal Bilirubin Testing in a Community Laboratory: An Opportunity for Physician Education

A. Ptolemy[1], H. Li[2], D. Bailey[1], G. Waite[1]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

**Background:** Significant hyperbilirubinemia must be diagnosed in newborn infants and neonates without delay to prevent the potential development of neurological impairment. If hyperbilirubinemia is suspected in a patient receiving care outside of a hospital setting, community laboratories in Ontario, Canada can provide urgent testing for neonatal bilirubin if requested by the ordering physician. **Objective:** Quantify the turnaround time (specimen collection to result reporting) for urgent and routine neonatal bilirubin testing in our community laboratory. Identify the prevalence of severe and critical hyperbilirubinemia results within our neonatal patient population, as defined by the Canadian Paediatric Society (CPS) and Ontario Association of Medical Laboratories (OAML). **Methods:** The turnaround time (TAT) and patient results from all neonatal bilirubin testing between Jan 2016 and Dec 2016 (*N*=1132) were retrospectively reviewed. Urgent testing was requested for 30.4% (*N*=264) of specimens. The average and median TAT time for each testing priority was tabulated as well as the frequency for which it exceeded 24 hr. Neonatal bilirubin (total serum bilirubin) results were evaluated against: our laboratories age-specific reference intervals (<24 hr, 34-103 µmol/L; 1-2 d, 103-120 µmol/L; 3-5 d, 68-205 µmol/L; 6 d-1 m, <70 µmol/L); and the age-specific concentrations for identifying severe (0-28 d, >340 µmol/L) and critical (0-28 d, >425 µmol/L) hyperbilirubinemia from the CPS and critical hyperbilirubinemia from the OAML (24-48 hr, >260 µmol/L; 49-72 hr, >310 µmol/L; >72 hr, >340 µmol/L). **Results:** Specimens requested for urgent neonatal bilirubin testing had a relatively shorter TAT (mean±SD, 8.3±4.5 hr; median, 8.0 hr) than those processed routinely (mean±SD, 12.3±14.0 hr; median, 10.0 hr). TAT exceeded 24 hr for 3.0% of urgent neonatal bilirubin tests and 9.7% of routine neonatal bilirubin tests, respectively. 90.0% and 81.3% of 1-2 day old neonates respectively receiving urgent and routine neonatal bilirubin testing had results above the upper limit of their reference interval. Regardless of age, the majority (>53%) of neonates receiving urgent testing had total serum bilirubin concentrations above the upper limit of their respective reference interval. The incidence of severe or critical hyperbilirubinemia, as defined by the CPS and OAML, within the examined population was relatively rare. For all urgently (*N*=264) tested neonates, one >72 hr old patient had a bilirubin concentration >340 µmol/L. The CPS defined concentration for critical hyperbilirubinemia (>425 µmol/L) was only exceeded by one neonate who received routine testing. Neonates receiving routine testing had a bilirubin concentration exceeding the OAML critical level hyberbilirubinemia for *N*=1 24-48 hr old (>260 µmol/L) and *N*=5 >72 hr old (>340 µmol/L) patients. **Conclusion:** Severe or critical hyperbilirubinemia is uncommon within the examined population. Urgent neonatal bilirubin testing provided by our community laboratory has a relatively shorter TAT than routine testing. However, it likely does not serve physician needs when the detected level of bilirubin within the neonate necessitates immediate therapeutic intervention. It is recommended that testing for suspected neonatal hyperbilirubinemia should be performed in an acute care setting, not a community laboratory. This study can be used by other community or reference laboratories to educate physicians on the associated risk of this diagnostic practice.

## B-263

### Biochemical sample testing in new MiniCollect® Blood Collection Tubes

S. Griebenow, M. Holzer. *Greiner Bio-One GmbH, Kremsmünster, Austria*

**Background:** Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows highest flexibility and accuracy by collecting blood in unprecedented simplicity. The MiniCollect Serum Separator and Lithium Heparin Separator tubes are intended to collect, transport, separate and process capillary blood for testing serum and plasma, respectively in the clinical laboratory.

**Methods:** Studies were done at Steyr Hospital (Austria) and Laboratory Rainbach (Austria) using MiniCollect tubes with old design vs. new design. Altogether, 80 hospitalized and 70 healthy subjects were recruited. Informed consent was given by all donors and the studies were approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. After centrifugation for 10 min at 3000g, 28 common biochemical analytes (venous) and 10 analytes (capillary) were tested using an AU680 and DxI800 (Beckman Coulter, precision within-run < 3%, total < 3%). Comparison testing to Microtainer (BD) was included. Analysis was done with the instrument's accompanying reagents. Statistical evaluation was done by STATISTICA 13.

**Results:** Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design for performance testing did not reveal any clinically nor statistically significant deviations ($p<0.05$). The values in both serum tubes of venous collection resulted in an initial highest deviation of 3.2%, and in plasma tubes of 4.4%. Comparable highest deviations for initial values in relation to 48h values were obtained for serum (5.3%) and plasma (6.4%). Capillary collection led to a highest deviation for LDH of 6.9% in serum, and in plasma tubes of 8.7%.

**Conclusion:** From a clinical perspective, the MiniCollect Serum Separator and Plasma Separator tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

## B-264

### Evaluation of glucose concentration as a reliable indicator of blood gas sample contamination with total parenteral nutrition, lipid emulsion, and concentrated dextrose solutions

J. C. Jara Aguirre, S. W. Smeets, A. M. Wockenfus, B. S. Karon. *Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*

**Background:** Blood gas samples from the neonatal intensive care unit (NICU) can be contaminated with intravenous solutions high in lipid and glucose such as total parenteral nutrition (TPN), lipid emulsion, or 50% dextrose in water (D50). We currently use glucose concentration >250 mg/dL as a sign of potential contamination. We performed *in vitro* spiking of blood gas samples with either a 1:1 mixture of TPN and lipid emulsion (mimicking administration in the NICU), or D50, to determine whether glucose concentration was a reliable indicator of blood gas sample contamination with these solutions; and determine whether a glucose threshold could be established to predict whether contamination produced blood gas and electrolyte results with greater than allowable error.

**Methods**: Residual lithium heparin arterial blood gas samples were pooled, transferred into tubes and spiked with a mixture (1:1) of TPN solution and 20% lipid emulsion (Intralipid, Germany), to final concentrations of 0, 1, 3, 5, 10 and 15%. Similarly D50 was added to pooled samples to final concentrations of 0, 1, 1.5, 2 and 2.5%. Samples were transferred to blood gas syringes and analyzed for pH, pO2, pCO2, hemoglobin, sodium, potassium, and ionized calcium on a Radiometer ABL 90 Analyzer (Radiometer, Denmark). After centrifugation plasma glucose was measured using a Roche Cobas c501 (Roche Diagnostics, IN). We determined whether a glucose threshold could be established to detect contamination with TPN/lipid emulsion or D50, and to predict when blood gas or electrolyte values exceeded laboratory-defined allowable error for each analyte.

**Results**: Addition of 3% or more of the TPN/lipid emulsion solution resulted in glucose concentrations >300 mg/dL, while addition of even 0.5% D50 resulted in

glucose >400 mg/dL. Any amount of added TPN/lipid emulsion or D50 caused both pH and pO2 to exceed allowable error (±0.02 for pH and ±7.5 mm Hg for pO2). Addition of TPN/lipid emulsion up to 15% of sample volume had minimal impact on ionized calcium. For all other parameters addition of TPN/lipid emulsion resulted in greater than allowable error only when glucose concentrations >500 mg/dL, which occurred with 5% sample dilution. D50 contamination up to 1.5% (producing glucose results >900 mg/dL) did not cause greater than allowable error for any parameters other than pH, pO2 and pCO2.

**Conclusion**: Glucose concentration can be used as an indicator of significant sample contamination with either TPN/lipid emulsion or D50 solution. Sample contamination with TPN/lipid emulsion or D50 produces unreliable blood gas (pH, pCO2 and pO2) results. Contamination with concentrated D50 solution does not impact hemoglobin or electrolyte parameters even when the measured glucose concentration exceeds 900 mg/dL. In contrast with TPN/lipid emulsion contamination, greater than allowable error occurs in many measured blood gas parameters when glucose exceeds 500 mg/dL. The findings suggest that NICU samples with glucose >500 mg/dL should be rejected as potentially contaminated to prevent inaccurate results from being reported.

## B-265

### Verification of CALIPER pediatric Reference Intervals using a large community based pediatric population

N. M. A. White-Al Habeeb[1], H. Li[2], A. Ptolemy[2], D. Bailey[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]Dynacare Medical Laboratories, London, ON, Canada*

Background: The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) program used CLSI guidelines to establish pediatric reference intervals and transfer RIs to other platforms. The applicability of these RIs to other pediatric cohorts has not yet been assessed. The aim of this study was to verify CALIPER-established pediatric reference intervals (RIs) for calcium, iron, ALT, AST, total bilirubin, and alkaline phosphatase (ALKP).

Methods: Test results from a community laboratory obtained from September 2015-August 2016 were extracted from the information system for children <19 years of age. Individuals with abnormal results for clinically-related analytes were excluded. The remaining dataset was age- and sex-stratified according to CALIPER-recommended partitions, pared of outliers via log-transformation and Tukey's test, and used to verify the CALIPER RIs. If CALIPER RI did not pass with a 10% allowance, RIs were adjusted based on population data and verified with a second dataset obtained from September 2016-January 2017.

Results: CALIPER RIs for all age and gender partitions for calcium (n=140) and iron (n=3727) were verified. All partitions for total bilirubin (n=16,802) and ALT (n=6421), two partitions for AST (n=6504), and two partitions for ALKP (n=3609) failed verification, requiring the establishment of population-based RIs. In these cases, the population-based RI more closely matched the limits of the 95% confidence interval (CIs) for each CALIPER RI.

Conclusions: Our data demonstrate the applicability of CALIPER RIs to a filtered, community-based cohort and highlights the importance of verifying proposed RIs for specific populations. CALIPER RIs were adopted for some analytes and partitions, but for others, population-based RIs were required. This was particularly the case for analytes with non-Gaussian distributions, where a larger sample size is required to obtain narrow 95% CIs about the RI limits. With the exception of total bilirubin, the adopted RI was equivalent to the CALIPER RI when the CI was taken into consideration.

Verified age- and sex-specific pediatric reference intervals for biochemical markers

| Analyte | RI Origin | Age | Female Lower Limit (CI) | Female Upper Limit (CI) | Male Lower Limit (CI) | Male Upper Limit (CI) |
|---|---|---|---|---|---|---|
| Total Bilirubin | CALIPER | 0 - 14 days | < | 250 (249-252) | < | 250 (249-252) |
| (mol/L) | CALIPER | 15 days - <1 yr | < | 10 (8- 11) | < | 10 (8- 11) |
| | Population | 1 - < 9 yrs | 2 (2- 2) | 11 (11- 12) | 2 (2- 2) | 11 (11- 12) |
| | Population | 9 - <12 yrs | 2 (2- 2) | 12 (12- 13) | 2 (2- 2) | 12 (12- 13) |
| | Population | 12 - <15 yrs | 2 (2- 2) | 22 (21- 24) | 2 (2- 2) | 22 (21- 24) |
| | Population | 15 - < 19 yrs | 3 (2- 3) | 22 (22- 23) | 3 (2- 3) | 22 (22- 23) |
| ALT | CALIPER | 0 - <1 yrs | < | 25 (17-33) | < | 25 (17-33) |
| (U/L) | Population | 1 - <13 yrs | 9 (8- 9) | 30 (29- 30) | 9 (8- 9) | 30 (29- 30) |
| | Population | 13 - <19 yrs | 7 (7- 8) | 28 (28- 29) | 9 (9- 9) | 38 (37 - 38) |
| AST | CALIPER | 0 - 14 days | < | 155 (140-169) | < | 155 (140-169) |
| (U/L) | CALIPER | 15 days - <1 yr | < | 63 (48 - 78) | < | 63 (48 - 78) |
| | CALIPER | 1 - <7 yrs | < | 41 (26- 55) | < | 41 (26- 55) |
| | Population | 7 - <12 yrs | 20 (20- 20) | 38 (38- 41) | 20 (20- 20) | 38 (38- 41) |
| | Population (Female) CALIPER (Male) | 12 - <19 yrs | 14 (13- 14) | 28 (28- 29) | < | 32 (17- 47) |

## B-266

### Evaluation of the BD Vacutainer® UltraTouch Push™ Button Blood Collection Set in a tertiary pediatric hospital

N. M. A. White-Al Habeeb[1], D. Colantonio[2], M. J. Knauer[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada*

**Introduction**: Blood collection in pediatrics can be stressful for the child and challenging for the phlebotomist. Smaller gauge needles are preferred as they lessen patient discomfort and improve difficult venous access during venipuncture. However, smaller gauged needles are associated with increased incidence of sample hemolysis, which can interfere with many laboratory tests and carries the risk of a re-draw. BD introduced the BD Vacutainer® UltraTouch™ Push Button Collection Set (BD UltraTouch™) with ultra-thin wall technology which provides a needle with a larger inner diameter without increasing the outer diameter (gauge size). The aim of this study was to evaluate the performance of the BD UltraTouch™ in a tertiary pediatric hospital and examine the effect of a smaller gauge needle on sample hemolysis rates.

**Methods**: Blood was collected from patients, aged 7-18 years old, in the phlebotomy clinic at The Hospital for Sick Children, Toronto, Canada, during an evaluation period of four days. We compared hemolytic index (HI) and potassium results in patients with blood drawn using the BD UltraTouch™ versus the current Terumo Surshield Safety Winged Infusion Set (Terumo Surshield™). Patients were age–matched when possible during the evaluation period. 61 specimens collected with the BD UltraTouch™ and 41 specimens collected with Terumo Surshield™ were included in the study. The acceptance criterion for potassium was ±4% between the needle sets. Results from the evaluation period were validated on a larger cohort of patients following the implementation of a hospital wide switch in needle sets. We examined the HI and the rate of critical potassium results due to sample hemolysis using one month of data when phlebotomy was performed using the Terumo Surshield™ and following the implementation of the BD UltraTouch™ for phlebotomy.

**Results**: Analysis of the evaluation period showed 87% (53) of patients collected with BD UltraTouch™ had a hemolytic index (HI) <15; 13% (8 patients) had HI of 15-50, indicating mild hemolysis. Using the Terumo Surshield™, 71% (30) of

patients had HI <15; 21% (9) had HI 15-50; and 7% (3) had HI >51. There was no significant difference in HI between needle sets. Furthermore, no significant differences in hemolysis rates between the BD UltraTouch™ 21G, 23G and 25G needles were observed. Potassium results showed acceptable differences between samples collected with the two needle sets. Validation using a larger cohort of patients showed no significant difference in the rates of gross hemolysis (HI >300) between the needle sets; 1.2% (n=7645) when using the BD UltraTouch™ vs. 1.3% (n=8852) using the Terumo Surshield™. In addition, there was no significant difference in the rate of potassium critical results due to sample hemolysis between BD UltraTouch™ or Terumo Surshield™ sets, as both needle sets reported approximately 80% samples with critical potassium were due to hemolysis.

**Conclusions**: These data show there were no significant differences in sample hemolysis or the number of critical potassium results due to hemolysis when using the new BD UltraTouch™ vs. the Terumo Surshield™. This allows for the use of smaller gauge needles without increasing the risk of sample hemolysis.

## B-267

### Evaluating pediatric eGFR approximation with growth-curve derived height and creatinine reference intervals

P. McAuley, L. A. Beach. *IWK Women's and Children's Health Centre, Halifax, NS, Canada*

*Background:* Although less prevalent than in adults, Chronic Kidney Disease (CKD) among children has steadily increased over time. Late recognition, precluding early intervention to improve growth and development, remains a concern. The aims of this study were to test, in principle, an approximated calculation of pediatric estimated glomerular filtration rate (eGFR) when empirical height data is not available, and to determine whether children identified by the lower approximated eGFR were also flagged by an elevated creatinine. Comparisons of pediatric creatinine reference ranges generated by the Caliper study versus site specific reference ranges employed at local pediatric and adult health centers were also carried out.

*Methods:* Retrospectively, one year of creatinine results for children and adolescents aged 2-18 years were collected from the local pediatric and adult/community health centers. Data were sorted to select for outpatient, non-nephrology, non-emergency department results. Approximate eGFR was calculated using the updated Bedside Schwartz equation by applying age- and gender-specific height data from WHO growth charts at the third, fiftieth, and ninety-seventh percentiles.

*Results:* When an approximation of eGFR was calculated using growth curve heights, the majority of children identified with values under 75 mL/min/1.73m$^2$ were age 13 and older, representing half of identified children at the pediatric center and 98% at the adult/community center. The majority of these values also fell between 60 and 75 mL/min/1.73m$^2$. Using the 3$^{rd}$ percentile from the growth curves, the percentage of children with an approximated eGFR under 75 mL/min/1.73m$^2$ was 2.9% at the pediatric center (P)and 15% at the adult center (A). At the 50$^{th}$ and 97$^{th}$ percentiles these percentages were 1.26% (P) and 5.3% (A), and 0.65% (P) and 1.4% (A), respectively. Of children identified (approx. eGFR <75 mL/min/1.73m$^2$) using the 3$^{rd}$ percentile of height, all under age 11 years had an elevated creatinine flag by both locally established (IWK) ranges and by Caliper-transferred reference ranges. Between ages 11 to <15 years, creatinine did not flag by IWK ranges, but did flag by Caliper ranges. At 15-years and older, IWK reference ranges did not flag a creatinine elevation, while Caliper ranges flagged creatinine elevations for females, but poorly for males. This trend was also apparent, in a lesser degree, in the subset of children identified by using the 50$^{th}$ percentile of height. Chart review of selected cases indicates there may be value added in providing additional information to physicians, with flagging creatinine values or approximation of eGFR, particularly for older children and adolescents.

*Conclusions:* Approximating eGFR using WHO growth charts for age- and gender-specific heights to apply the Bedside Schwartz equation predictably identified an increasing proportion of children with lowered eGFR as lower height percentiles were applied. Interestingly, even at the 3$^{rd}$ percentile, all children identified under age 11 also had a creatinine reference range flag. Caliper reference ranges flagged more children than the IWK reference ranges, particularly for older children and females. Application of the calculation likely generates false positives, depending on the height percentile used, but may identify subclinical cases that would benefit from closer scrutiny.

## B-268

### Defining the reference range of prealbumin in healthy children and adolescents

N. Slhessarenko[1], C. M. A. Jacob[2], D. Fonseca[1], M. Maciel[1], N. Santos[1], C. Berti[1], M. Slhessarenko[3], N. Curi[4], C. J. F. Fontes[4], R. Azevedo[2], G. Campana[5], A. Andriolo[6]. *[1]Federal University of Mato Grosso and DASA, Cuiabá, Brazil, [2]University of São Paulo, São Paulo, Brazil, [3]Faculty of Healthy Sciences Albert Eisntein, São Paulo, Brazil, [4]Federal University of Mato Grosso, Cuiabá, Brazil, [5]DASA, São Paulo, Brazil, [6]Federal University of São Paulo, São Paulo, Brazil*

**Background:** The definition of reference ranges in laboratory is a great challenge, especially in pediatric population. In Brazil, laboratories release their test result with RR based on population from other countries. Prealbumin is a protein synthesized in the liver and it´s important for nutritional evaluation pediatrics because it transports thyroxine and carries retinol-binding protein. To define the reference range (RR) of prealbumin in healthy children and adolescents aged 1-12 years 11 months and 29 days from Cuiabá, capital of Mato Grosso.

**Methods:** This is a descriptive study, conducted in 1,866 healthy children and adolescents from kindergartens and schools of the capital city, obtained by random sampling. A questionnaire assessing the individual background and their relatives besides demographic and anthropometric data had been also carried out. Were defined as inclusion criteria of the study, children and adolescents in the group 1-12 years 11 months and 29 days without any underlying disease or diagnosed clinical complaints at the time of collection. In addition, participants should not take any regular medication. The samples were collected during fasting period and were determined by nephelometry (BN II Siemens). Regarding statistic methodology, we did analyse the homogeneity of variances by Bartlett´s test for each parameter by age and subsequently by ANOVA or Kruskal-Wallis test to check the differences between age groups. The test "pos hoc" Bonferroni was applied when it was found the difference to regroup similar age groups, thus constituting a new age bracket. After this procedure the Bartllet test was applied, as it result, we did conduct ANOVA or Kruskal-Wallis to check if the groups remained. Then proceeded to the exclusion of extreme values (outliers) taken as those values above or below the mean ± 3 standard deviations. After excluding outliers, obtained the RI as the mean ± 2 standard deviations of the remaining values. The significance level was 5%. The project was approved by the Research Ethics Committees of the institutions involved. **Results:** The subjects were grouped into four different age groups: aged 1 year; 2-7 years; 8-10 years and 11-12 years. The RRs found for each age group were 0.09 g/L to 0.22 g/L for the group aged 1 year; 0.11 g/L to 0.26 g/L for the group aged 2-7 years; 0.13 g/L to 0.28 g/L for the group aged 8-10 years and 0.13 g/L to 0.31 g/L for the group aged 11-12 years. A few studies have defined the RR of prealbumin levels in children and adolescents worldwide. The IFCC studied newborns and elderly people aged up to 60 and grouped them into six age groups, showing that prealbumin increased progressively with age. In the age group 1-2 years, the values (0,11 to 0,26 g/L) were very similar to those found in this study (0,09 to 0,22 g/L) for the 1 year-old age group.

**Conclusion:** The RR of prealbumin levels observed in children and adolescents from Cuiabá may be used as a reference for interpreting results of blood tests in Brazilian children and adolescents. Further studies from other Brazilian states should be done.

## B-269

### Establishment of pediatric reference intervals for alkaline phosphatase in Korean children based on retrospective analysis

K. Lee[1], S. H. Song[2], H. D. Park[3], Y. M. Yun[4], S. Chun[5], J. H. Kim[6], J. Song[1]. *[1]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Korea, Republic of, [2]Department of Laboratory Medicine, Seoul National College of Medicine, Seoul National University Hospital, Seoul, Korea, Republic of, [3]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Konkuk University School of Medicine, Konkuk University Hospital, Seoul, Korea, Republic of, [5]Department of Laboratory Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, Republic of, [6]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of*

**Background:** Hypophosphatasia is an inborn error of metabolism characterized biochemically by deficient activity of the tissue-nonspecific isoenzyme of alkaline

phosphatase (ALP). ALP activity is one of sensitive markers of hypophosphatasia. However, it is particularly important and difficult to establish appropriate reference intervals for ALP due to development and growth of children. No more than three subgroups according to the sex were divided on reported studies of pediatric reference intervals in Korea. We aimed to establish more age- and sex- specific partitioned reference intervals for ALP in Korean children by using reported studies.

**Methods:** Blood test results were collected for 4,290 children aged 0-18 year from Korean National Health & Nutrition Examination Survey data which established from a healthy community children, and reported Severance Hospital data and multicenter study data of which children underwent minor surgeries in seven university hospitals. Medical records were reviewed to exclude patients with underlying diseases or any illnesses other than the diagnosis for the minor surgery. The reference interval settings complied with the Clinical & Laboratory Standards Institute (CLSI) guidelines. After eliminating outliers, we used the 2.5 percentile as the lower limit and the 97.5 percentile as the upper limit in the same subgroup as CALIPER. According to CLSI EP9-A2 guideline, reference intervals were transferred to assays performed on five different clinical chemistry platforms including Roche cobas 8000 and modular DP, Abbott C16000, Toshiba 200FR NEO, and Beckman Coulter AU5800.

**Results**: Currently, provided reference intervals for ALP using Roche cobas 8000 were as follows: female, 151-465 IU/L (15 day-1 year), 153-388 IU/L (1-10 year), 134-428 IU/L (10-13 year), 64-258 IU/L (13-15 year), 54-158 IU/L (15-17 year), 38-96 IU/L (17-19 year); male, 153-511 IU/L (15 day-1 year), 145-364 IU/L (1-10 year), 151-435 IU/L (10-13 year), 102-420 IU/L (13-15 year), 77-305 IU/L (15-17 year), 52-192 IU/L (17-19 year). Compared with the results of the CALIPER study, the reference intervals for ALP were similar. Among five different hospital analyzer platforms, the results of each platform except Beckman Coulter AU5800 were not significantly different.

**Conclusions:** This is the first study to present detailed Korean pediatric reference intervals for ALP using three databases. It should assist laboratorians and pediatricians in interpreting test results more accurately and thereby to improved diagnosis of hypophosphatasia.

## B-270

### reference intervals for serum and urinary creatinine urinary albumin and albumin creatinine ratio in healthy children and adolescents in cuiaba mato grosso brazil

N. Slhessarenko[1], M. Cuerva[2], L. Pulcherio[2], I. Souza[2], K. Isaac[2], M. Slhessarenko[3], C. J. F. Fontes[2], R. Azevedo[4], G. A. Campana[5], A. Andriolo[6]. *[1]Federal University of Mato Grosso and DASA, Cuiabá, Brazil, [2]Federal University of Mato Grosso, Cuiabá, Brazil, [3]Faculty of Healthy Sciences Albert Eisntein, São Paulo, Brazil, [4]University of São Paulo, São Paulo, Brazil, [5]DASA, São Paulo, Brazil, [6]Federal University of São Paulo, São Paulo, Brazil*

**Background: :** Establishment of Reference Intervals (RI) is an arduous task for clinical laboratories however it´s fundamental importance to medical decision making. A few studies on pediatric populations have been carried out to determine creatinine dosage by colorimetric assay and cinetic analysis, as well as to quantify urinary albumin by turbidimetry. The objective of this study was define the reference ranges (RR) for serum and urinary creatinine, urinary albumin and albumin/creatinine ratio in the urine of healthy children and adolescents from Cuiabá, Mato Grosso, Brazil. **Methods:** This cross-sectional study was approved by the Ethics Committee of the University of São Paulo and Julio Muller University Hospital of Federal University of Mato Grosso. In a total of 1,886 healthy children and adolescents from daycare centers and municipal schools in the city of Cuiabá were evaluated . A questionnaire assessing the individual background and their relatives besides demographic and anthropometric data had been also carried out. Were defined as inclusion criteria of the study, children and adolescents in the group 1-12 years 11 months and 29 days without any underlying disease or diagnosed clinical complaints at the time of blood and urine collection. In addition, participants should not take any regular medication. The samples were collected during fasting period and were determined using cobas® 6000 (analyser series - Roche Diagnostics). Regarding statistic methodology, we did analyse the homogeneity of variances by Bartlett´s test for each parameter by age and subsequently by ANOVA or Kruskal-Wallis test to check the differences between age groups. The test "pos hoc" Bonferroni was applied when it was found the difference to regroup similar age groups, thus constituting a new age bracket. After this procedure the Bartllet test was applied, as it result, we did conduct ANOVA or Kruskal-Wallis to check if the groups remained. Then proceeded to the exclusion of extreme values (outliers) taken as those values above or below the mean ± 3 standard deviations. After excluding outliers, obtained the RI as the mean ± 2 standard deviations of the remaining values. **Results:** The serum creatinine was studied in 8 aged group: 1-2 years; 3 years; 4 years; 5 years; 6-7 years, 8 years; 9-11 years and 12 years and the Reference Range (mg/dL) for all these age groups were, respectively: 0,20-0,40; 0,22-0,46; 0,27-0,47; 0,29-0,51; 0,32-0,57; 0,33-0,62; 0,35-0,69 e 0,38-0,72. The Reference Range of urinary creatinine (mg/dL) was proposed into three different age groups: 1-3 years (up to 125); 4-11 years (up to 182) and 12 years (up to 261). The studied showed that urinary albumin level was up to 2,12 mg/dL and albumin/creatinine ratio in an isolated sample was up to 0,03 mg/dL in both cases, the subjects were group into a single age group. **Conclusion:** Defining RR for these analytes in Brazilian population may allow us to better interpret test results in Brazil. Other similar studies in different regions may corroborate these results.

## B-271

### Performance Evaluation of the ADVIA Centaur PlGF and sFlt-1 Assays

J. Frenna, A. Palladino. *Siemens Healthineers, Newark, DE*

**Background:** Preeclampsia (PE), a disease found in pregnant women, is characterized by hypertension and proteinuria. Rapid, accurate diagnosis of PE is essential for best clinical practice and maternal/fetal care. PlGF and sFlt-1, both produced by the placenta, regulate fetal angiogenesis. The sFlt-1/PlGF ratio becomes elevated in PE; therefore, this ratio is valuable in helping diagnose PE. Siemens Healthineers (Tarrytown, NY, U.S.) has developed ADVIA Centaur® PE assays* for detecting PlGF and sFlt-1 in serum and plasma to be used in tandem for a clinically relevant ratio of diagnostic value. This study evaluated performance of the ADVIA Centaur PlGF and sFlt-1 assays.

**Methods:** The assays were evaluated on ADVIA Centaur Immunoassay Systems for precision, method comparison, linearity, limit of detection (LoD), limit of quantitation (LoQ), calibration interval, onboard stability (OBS), hook effect, and endogenous interferences. Clinical agreement with Roche ELECSYS PE assays for >150 clinically relevant samples was also evaluated. Also, sensitivity and specificity of the PE cutoff ratios were evaluated at ≤34 and >34 weeks gestation from normal and physician-diagnosed PE patients.

**Results:** The PlGF and sFlt-1 assays have respective ranges of 10-10,000 and 30-85,000 pg/mL, with acceptable sensitivity and specificity within early and late gestational windows. The precision results were <8% CV, and both were linear within each assay's range, with no observed hook effect. The LoD and LoQ were <10 pg/mL (PlGF) and <30 pg/mL (sFlt-1). The calibration interval and OBS for the ADVIA Centaur PlGF and sFlt-1 assays were >14 and >28 days, respectively. <10% endogenous interference was observed for all tested interferents for both assays. In addition, the ADVIA Centaur and ELECSYS PE assays showed strong positive (98.2%) and negative (98.8%) agreement by clinical outcome.

**Conclusions:** These studies indicate that the ADVIA Centaur PE assays are precise and sensitive for measuring PlGF and sFlt-1 across a wide range of concentrations.

*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Point-of-Care Testing

## B-272

### Analytical and clinical accuracy of blood glucose testing by point of care glucose meters when using ISO 15197 and Thailand's national guidelines

W. Treebuphachatsakul, K. Wittayapornpong. *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Thailand, Phitsanulok, Thailand*

**Background:** Glucose meters play an important role in the management of diabetes mellitus (DM) patients. Analytical and clinical performance of blood glucose testing deserves to be evaluated before using it in hospital settings. The objectives of this study were to evaluate the analytical performance of commonly used glucose meters and to evaluate the clinical performance.

**Methods:** Analytical performance including precision, recovery, common interferences, and comparison were carried out. One hundred capillary and venous blood samples obtained from 60 diabetes patients and 40 healthy adults were used in comparison studies. Whole blood samples were measured for glucose by glucose meters and a YSI reference analyzer, while plasma samples were measured for glucose by a Cobas c111 analyzer. All data obtained from the comparative studies was evaluated by using the international guidelines for blood glucose testing, and also Thailand's national guidelines for point of care testing. The clinical performance of blood glucose testing was evaluated by Park Error Grid analysis.

**Results:** The coefficients of variation of blood glucose testing by glucose meters were less than 8% and recoveries ranged from 95 to 105%. We found that hemoglobin and triglyceride can interfere with one of the glucose meter. However, blood glucose levels obtained from four of the glucose meters were compatible with the reference analyzers published in ISO 15197 and CLSI (> 95%). All five glucose meters were in conformance with Thailand's national guidelines for point of care testing by correlation coefficient > 0.975 ($r^2$> 0.95 ), Bland-Altman plot > 95% and clinical performance with biases felt within zone A and B >99% of error grid plots.

**Conclusion:** Four of the five glucose meters tested showed good analytical accuracy, all five glucose meters showed good clinical accuracy.

## B-273

### Application of a Monte Carlo simulation model to estimate clinical risk associated with the analytic performance of point of care INR devices

M. E. Lyon[1], R. Sinha[1], O. A. S. Lyon[2], A. W. Lyon[1]. *[1]Saskatoon Health Region, Saskatoon, SK, Canada, [2]University Saskatchewan, Saskatoon, SK, Canada*

BACKGROUND: In 2016 FDA proposed performance expectations for POCT INR devices in response to a post market risk analysis of serious clinical and patient self-monitoring adverse events: 95% of all INR results should fall within ±0.4 for INR <2; ± 20% for INR ≥2 to 3.5; ± 20% for INR ≥3.5 to 4.5 and ± 25% for INR ≥4.5. OBJECTIVE: To estimate the clinical risk of warfarin dosing error as a consequence of POCT INR assay inaccuracy and imprecision at FDA performance goals. METHOD: INR values (n= 53, 535) were obtained from community adult patients in the Saskatoon Health Region (SHR). Monte Carlo simulation models were used to assess the influence of analytical bias and imprecision on INR values by evaluating the fraction of warfarin-dose-categories according to the SHR algorithm that were unchanged or changed by ≥1, ≥2 or ≥3 dose categories. RESULTS: Simulations used a bias of ±0.4 to ±0.8 combined with 3% imprecision and predicted that 45% to 75% of results would have ≥1 category warfarin dosing error, and 1% to 18% of results would have ≥2 category errors. If INR imprecision was increased to 10%, then the model predicted that 45% to 75% of results continue with ≥1 category warfarin dose error but the fraction with ≥2 category error would increase to 2% to 24%. CONCLUSIONS: Simulation models demonstrated the extent of one category and two category treatment errors for POCT INR assays is highly dependent on method bias and only partially affected by method imprecision ≤10%.

## B-274

### *Point of Care Dipstick Assessment of Ascitic Fluid: Comparison with SAAG in Differentiation of Ascitic Fluid into Exudates and Transudates*

L. EKPE[1], E. Eyam[1], E. Ekanem[2]. *[1]university of calabar teaching hospital, calabar, Nigeria, [2]university of lagos, lagos, Nigeria*

**Background:**

Ascitic fluid accumulation in the peritoneal cavity is seen in all parts of the world by many doctors. This study attempts to differentiate exudates and transudate by use of point of care dipstick.

**Materials/Method:**

A total of 67 patients with ascetic fluid were recruited for this study. Using serum ascitic albumin gradient (SAAG) as standard way of classifying ascitic fluid into exudates and transudate, dipstick protein levels were assessed to classify the ascitic fluid into exudates or transudate. Ascitic fluid was obtained by abdominal paracentesis at the same time of collecting of venous blood from the patients. SAAG was calculated based on albumin value of venous blood and ascitic fluid.{(SAAG less than 1.1 g/dL represented exudates, while SAAG greater than 1.1g/dL represented transudate}.

**Results:**

Of the total 67 adult population in the study, the age range was 18-65 years with a mean age of 47.11± 11.21. The mean age of female and male population was 44.29 ± 11.78 and 50.8 ± 9.4 respectively ($p$ = 0.0168). A total of 38 of them (56.7%) were female and 29 (43.29).the

mean body mass index (BMI) was 24.19± 2.9. The $p$ value of BMI for both sexes was $p$ = 0.95). 41 (61.2%) had high ascitic fluid protein content while 26 (38.81%) had moderate protein content. In calculating the dipstick protein value with exudates or transudate gave odd ratio(OR) value of 2.36 (95 CI: 0.87 - 6.46), Risk Ratio(RR) of 1.405 (0.128 - 2.13), risk difference (RD) of 20.27. The sensitivity was 63% while this study had a specifity of 50%.

**CONCLUSION:**

Dipstick as a point of care, with reference to protein levels may be able to differentiate ascitic fluid into transudate or exudates.

**KEYWORDS:**

Dipstick, point of care, ascites, exudates, transudate, serum-ascites albumin gradient.

## B-275

### Utility and accuracy of transcutaneous bilirubin as a screening test to identify the need for serum bilirubin assessment in neonates in Nepal

S. Timalsina[1], S. Shrestha[2]. *[1]Chitwan Medical College, Bharatpur, Nepal, [2]College of Medical Sciences, Bharatpur, Nepal*

**Background:** Neonatal hyperbilirubinemia is very common and most of the times just a benign problem in neonates. But with high levels of serum bilirubin, there remains a possibility of development of kernicterus which could be devastating. Severity and decision for management are usually based on serum bilirubin (TsB) which requires blood sampling. However, transcutaneous bilirubin (TcB) measurement is a noninvasive point-of-care alternative to TsB, which can be very helpful in the resource constrained setting such as Nepal. This study aimed at evaluating the accuracy of the TcB measurement when compared to TsB and developing a cut-off point of TcB level with desirable sensitivity and specificity values for various clinically relevant TSB levels. **Methods:**

This was a hospital based prospective study done in neonates admitted in NICU of College of Medical Sciences Teaching Hospital, Bharatpur, Nepal. A total of 127 neonates were enrolled in the study from October 2015 to April 2016. TcB was measured using Drager Jaundice Meter JM-103 in the neonates in whom jaundice was suspected by visual assessment following the guidelines mentioned in the information leaflet. A TsB was also sent within 30 minutes of measuring the TcB. The agreement between the two measurement methods was analyzed by Pearson's correlation followed by Bland-Altman analysis. Receiver Operating Characteristic (ROC) curves were plotted for TcB at various clinically relevant cut-off points of TsB that were set at 13, 15 and 17 mg/dl. A p value of less than 0.05 was considered statistically significant. **Results:** Out of the total cases, 81 were male (63.8%) and the rest 46 were female (36.2%). The median post natal age was 56 hours, ranging from 4- 480 hours. The median birth weight was 2560 g (1570 g- 4300 g). The two measurements had high degree of correlation (Pearson's r= 0.872, P <0.01). The Bland Altman error distribution plot shows a tendency of TcB to underestimate TsB throughout the range of measurement with the mean difference of 2.03 ± 1.66 mg/dl, with an uncertainty

of 3.31 mg/dl. The cut off value of TcB at 10.15 mg/dl suggested that the TsB is >13 mg/dl with the sensitivity of 96.1% and specificity of 80%. The cut off value of TcB at 11.75 mg/dl suggested that the TSB is >15 with sensitivity of 93.9 % and specificity of 68.9 %. Similarly, the cut off value of 13.65 mg/dl suggested the TSB value of >17mg/dl with the sensitivity of 93.9% and 73.1% specificity. **Conclusion:**

The accuracy of the TcB measurement is excellent overall and offers a sensitive screening alternative to identify the need for blood sampling for serum bilirubin level. The JM-103 jaundice meter offers painless, accurate point-of-care bilirubin determinations, with advantages such as high level of parent and staff acceptance, easy portability and cost-effectiveness. Furthermore, it can be very helpful in monitoring of the bilirubin level once the treatment has been initiated, to avoid frequent needle-pricks to the neonates.

## B-276

### Simulation Models to Rule-Out Acute Myocardial Infarction with Two Point of Care Testing Devices and a High-Sensitivity Cardiac Troponin T Method

M. E. Lyon[1], P. A. Kavsak[2], A. Worster[2], A. W. Lyon[3]. *[1]Royal University Hospital, Saskatoon, SK, Canada, [2]McMaster University, Hamilton, ON, Canada, [3]St. Paul's Hospital, Saskatoon, SK, Canada*

**Background:** Few studies have assessed the utility of Point of care (POC) cardiac troponin methods to rule-in or rule-out acute myocardial infarction (MI). These studies are difficult to conduct due to lack of method standardization, variation among patients, low incidence of non-STEMI AMI and high cost per test. To overcome study limitations, simulations were used to evaluate the diagnostic performance of the Radiometer AQT90 and Roche h232 cardiac troponin T (cTnT) POC methods and the Roche Elecsys high-sensitivity cardiac troponin T method (hs-cTnT) using an emergency department (ED) patient database expanded to n=10,000 in a finite mixture model. **Objective**: To estimate and compare clinical sensitivity and specificity, positive and negative predictive values (PPV, NPV) for the Radiometer AQT90 and Roche h232 cTnT POC methods with hs-cTnT using simulated data at diagnostic thresholds. **Methods:** Finite mixture analysis of the 0 hr data obtained from the ROMI trial (n=1137 Optimal Troponin Cut Offs for acute coronary syndrome (ACS) by Roche hs-cTnT) enabled derivation of a simulation data set (n=10,000) troponin test results. Regression equations were used to convert hs-cTnT test results into simulated AQT90 and h232 cTnT results. Clinical sensitivity, specificity, PPV and NPV were calculated using the simulated hs-cTnT, AQT90 and h232 data for MI diagnosis using the limit of detection for the assays. **Results:** For the Radiometer AQT90 cTnT at 10 ng/L: sensitivity 98.4%, specificity 29.2%, PPV 15.6% and NPV 99.3%. For the Roche h232 cTnT at 50 ng/L: sensitivity 55.6%, specificity 91.6%, PPV 46.7% and NPV 94%. For the Roche hs-cTnT at 5 ng/L: sensitivity 99.1%, specificity 20.3%, PPV 14.1% and NPV 99.4%. **Conclusions:** Only the Roche hs-cTnT in this simulated data-set achieved both sensitivity and NPV values above 99% similar to the published study (Clin Chem 2017;63:403-414). The Radiometer AQT90 cTnT assay approaches these estimates, suggesting a prospective study is required to determine if the AQT90 cTnT assay can be used to rule-out MI at presentation in an all comer emergency department chest pain population.

## B-277

### Point of care measurement of blood beta-hydroxybutyrate in children with drug-resistant epilepsy

A. Ivanov, U. Vaher. *Tartu University Hospitals, Tartu, Estonia*

**Background:** Incidence of childhood epilepsy in Estonia is 80:100.000 (0-19 years). Up to 70% of patients have their seizures controlled with anti-epileptic drugs. For some children who continue to have seizures, the ketogenic diet (KD) may help to reduce the number and severity of seizures. The KD is a special high-fat, low-carbohydrate diet that helps to control seizures. It is prescribed by a physician and carefully monitored by a KD team. Clinical guidelines advocate the measurement of ketones for the management of KD. A ketone urine nitroprusside test measures only one of the ketones - acetone while a ketone blood test measures the main ketone- beta-hydroxybutyrate (BHB) which accounts for 75% of ketone bodies in blood. POCT BHB and glucose meter Stat Strip (Nova Biomedical, Waltham, MAS, USA) was used for the measurement. **Aim:** To compare ketone measurement in urine and blood of these patients and to describe the impact of the ketone measurements on patient management with the KD.Methods: Case histories were retrospectively reviewed for all of the patients who are treated with KD in the Pediatric Clinic of Tartu University Hospital during years 2011-2016. Epilepsy course, etiology, and outcome as well as laboratory data (ketone, glucose, pH and BE) were collected and analyzed. During 2016, four children with drug-resistant epilepsy were treated with the KD. In two of them ketones were measured from urine (group1, before POCT BHB) and in two of them from blood (group2). Ketones, glucose, pH and BE measurements was performed regularly 1-2 times per day (and more frequently when the child's condition deteriorated) during one week.**Results:** 1. Mean glucose concentration, pH and BE of the patients' group 1 were significant lower than group 2: 2.76, 7.34, and -8.51 vs. 3.35, 7.38, and -5.71, respectively. 2. We compared the mean, minimum, and maximum values of glucose, pH and BE with reference ranges. 90% of values from group 1 were outside the reference range (except max value of pH 7.39: reference range is 7.35-7.45). From group 2 only 40% of values (mean and min of BE and min of pH) were outside the reference range. **Conclusion:** Measuring blood ketones gives more reliable data about the patient's condition and the level of ketosis thus allowing a better management of patients treated with KD.

## B-278

### Increasing the Limit of Detection and Performance of Rapid Immunological Tests

K. Whiting. *BBI Solutions, Cardiff, United Kingdom*

**Background:** Lateral flow immunoassay (LFI) rapid tests are accessible and low-cost diagnostic solutions for many POC applications, fulfilling many of the World Health Organisation's ASSURED characteristics. BBI have identified a market demand for increased performance of tests in terms of assay limit of detection, sensitivity, specificity and time to result. In direct response, they have developed a proprietary signal enhancement technology, Morffi™ that increases the test signal intensity (compared to traditional methods), improves the limit of assay detection and the time to result. This improvement could facilitate quicker diagnosis times for conditions requiring urgent treatment such as cardio metabolic or infectious diseases. The improved limit of detection can also expand the applicability of LFI to compete with more traditional lab based tests. **Methods:** BBI uses an in-house developed BNP-32 LFI as a model system for the assessment of new technologies. The assay combines BBI's colloidal gold label with commercially available antibodies and test materials. 40nm colloidal gold was passively conjugated to an anti-BNP antibody under predetermined optimal conditions. Following antibody immobilisation on the particle, unconjugated sites were blocked with an excess of either a BSA control or BBI's patent-pending Morffi technology. The conjugates were then normalised to the same optical density in buffer . The assay strips were tested against a range of BNP antigen concentrations (n=5). Test strips were read at 15 minutes using a CAMAG TLC Scanner 3 at 520 nm. The limit of detection (LoD) for the BNP-32 assay was then determined for each blocking technology, based on double the standard error (SE) above and below the mean signal for each concentration.

**Results: Improved sensitivity** The use of the novel technology results in a significant improvement in the LoD compared to the BSA-blocked control. The LoD achieved using Morffi was 0.08 ng/ml, compared to 0.8 ng/ml for the BSA-blocked control, representing a 10-fold increase in the limit of detection. The use of Morffi technology leads to a significant increase in signal intensity at all antigen concentrations compared to the BSA-blocked control. This can be attributed to more efficient binding of the detector conjugate to the antigen-capture antibody complex.

**Conclusion:** The results presented demonstrate the feasibility of the Morffi signal enhancement technology, offering a clear improvement in signal intensity over the BSA blocked control for the BNP-32 assay system. This difference was visually discernible at high antigen concentrations, and provided a 10-fold enhancement in the limit of detection. Due to the improved signal intensity the time to result is also improved with an up to four times faster rate to result. To-date more than 30 conjugates have been evaluated and have shown to be stable and robust. BBI has used this technology to enhance the sensitivity of a number of other model assay systems and are continuing to work on the applicability of the technology with other detector labels as well as with different assay formats.

A published whitepaper: Enhanced performance of a lateral flow assay, supports this abstract and provides further data.

## B-279

### Prevalence and factors associated with diabetes mellitus in patients with tuberculosis

S. Aketch, A. Amayo, M. Maritim, W. Mutai. *University of Nairobi, Nairobi, Kenya*

**Background:** Tuberculosis and diabetes mellitus are both global health burdens. Prevalence of both diseases continues to rise in developing countries. Some studies have shown diabetes increases the risk for tuberculosis. The relationship between the two diseases has not been fully investigated in Kenya.

**Methods:** This was a cross-sectional descriptive study involving adult patients diagnosed with tuberculosis attending outpatient clinics in two referral hospitals in Kenya. Informed consent was obtained from all participants. Demographic information was then recorded from each participant after which HbA1c was determined in venous blood using the CLOVER A1c™ Analyzer System. The main outcome variables were age, gender, HbA1c value, type of diabetes and HIV status.

**Results:** A total of 124 patients with tuberculosis were enrolled in the study. Most study participants were female (55.6%); most were aged 18yrs to 40yrs (72.5%); most had pulmonary tuberculosis (69.4%); most were HIV negative (83.1%). Thirteen patients (10.5%) had diabetes mellitus and 32 (25.8%) had impaired glucose tolerance. Majority of the patients with diabetes had pulmonary tuberculosis (8.1%). The mean HbA1c in patients with pulmonary tuberculosis was significantly higher than in those with extra pulmonary tuberculosis (6.9% vs 6.1%; p-value 0.017).

**Conclusion:** The overall prevalence of diabetes mellitus was 10.5 % and that of impaired glucose tolerance was 25.8%. This is higher than the national estimated diabetes prevalence of 3.3% and is suggestive of an association between diabetes mellitus and tuberculosis. Routine screening of patients with tuberculosis for diabetes mellitus is recommended to increase early detection and management.

## B-280

### A Novel Multiplexed Lab-on-Cartridge based POC Device for the Measurement of Common Clinical Diagnostic Tests

A. Singh, H. Singh, V. MATHUR. *Diasys Diagnostics India PVT. LTD, Mumbai, India*

Background:

Measurement of routine clinical biochemistry tests is common in labs worldwide for screening of illness or diseases. Most of the clinical tests require multiple instrumentation devices that perform multiple tests using cartridges for treating an illness. The costs required to maintain such cartridge based POC/Lab instrumentation is high and this limits the use of the device to only highly specialized personnel and sophisticated labs. It is not possible for small labs & clinics in developing countries to afford the instrument infrastructure needed to provide healthcare diagnostics. In order to provide Healthcare diagnostics to ALL at affordable costs, there is a need for a universal device/cartridge that is affordable, reliable, easy-to-use, requires very low sample volume and is maintenance-free and can do multiple tests at the same time.

DiaSys India has developed a world's first, patent pending, versatile electrochemical multiplexed biosensor based lab-on-cartridge device that is capable of performing a 1, 4, 8, or 16 tests simultaneously (QDxInstaLab CC). QDxInstaLab CC incorporates an innovative, high performance, inexpensive microfluidic cartridge for rapid quantitative measurement of diagnostic tests in whole blood/plasma/serum & QC samples. Our proposed methodology utilizes a novel, patented nanomaterial based plastic electrochemical biosensor that uses chronoamperometry to provide a sensitive and accurate result in <3 min for ALL the common clinical biochemistry tests. For the first time in a POC cartridge based device, we have built-in an Onboard QC sensor and a Hematocrit correction sensor to ensure the reliability of the results. The QDxInstaLab CC is capable of performing an eight clinical chemistry multiplexed tests from a fingerpick with just 30 microliters of whole blood sample.

Methods:

We have evaluated the multiplexed QDxInstaLab CC cartridge using samples for linearity, precision, interference and cartridge stability for all the clinical biochemistry profiles such as metabolite profile (glucose, lactate), kidney profile (urea, creatinine), haematology profile(Hb), lipid profile (total cholesterol, triglycerides), liver profile (ALT, AST, bilirubin). Method comparison was done against Repsons®910, a mid-size lab based clinical chemistry analyser. Precision study was done using modified CLSI guidelines with N = 10 samples. Interference study was done against haematocrit variation of 30% to 60% and with ascorbic acid at ±3 mg/dL at two different analyte concentrations with samples run in triplicate. Accelerated stability testing was done at 37°C for 4 weeks for the assays during which linearity samples were run on 0, 4, 7, 14, 21, 28 days respectively.

Results:Data analysis indicates that the assays have a CV < 5%, with $R^2$ > 0.95, interference bias of < 10% and the cartridges are stable up to 12 months at 2-8°C storage temperature based on preliminary extrapolated data.

Conclusion:

The developed technology platform for multiplexed cartridge for QDxInstaLab CC is reliable and meets all the performance specifications of the lab. Hence, it can be easily adapted for low cost, sensitive and rapid measurement of common diagnostics tests in urban, semi-urban and rural areas in the developing countries and also can be used as a general POCT system for worldwide applications.

## B-281

### Point of Care Testing (POCT) Five-Years Performance Review Evidenced Connectivity is Essential for the Establishment and Appraisal of an Effective Quality Management (QM) System for the Best of Patient Care

V. Lo. *Hong Kong Sanatorium & Hospital, Apleichau, Hong Kong*

**Backgrounds**: Our hospital adopted Nova glucometer (Nova) (Nova Biomedical Corporation, U.S.A.) for Point of Care Glucose Testing. Two proprietary systems, BioViewer and NovaNet, enabled connectivity. Bioviewer captured daily internal quality control data of 65 operating Nova devices for laboratory monitoring and troubleshooting; blocking Nova for analysis when satisfactory internal quality control performance was NOT available within 24-hours. NovaNet enabled laboratory to activate lot number of reagent and internal quality control, upload clinically relevant internal quality control range after performance verification and in-house establishment respectively. This study evidenced the criticalness of connectivity for the establishment and appraisal of an effective Quality Management system for the best of service quality and patient care.

**Methods**: In 2011 laboratory adopted ISO-22870 standard to establish Quality Management System, of which the effectiveness was appraised in a five-years performance review according to five quantifiable quality indicators. (1) Royal College of Pathologist Australasian Quality Assurance Program Key Performance Indicator (KPI) score, (2) sigma-metric by (TEa-bias)/CVa , TEa was total error analytical, CVa was analytical coefficient of variation of period collected low and high internal quality control imprecision, (3) analytical goal by CVa/CVi, CVi was intra-individual biological variation, (4) daily internal quality control repeat status was interpreted as repeat frequency and reason of repeat, and (5) external quality assurance sample analysis failure rate.

**Results**: Three biannual KPI scores indicated good performance. Sigma-metric mean of low and high internal quality control were 5.9 and 7.8 corresponded to error rate of 0.00050% and <0.00034% respectively. Analytical goal was achieved satisfactorily. External quality assurance sample analysis failure rate declined from 10.0% to 0.9% in January 2015 and kept persistently low at <1.0% till January 2017. Daily internal quality control repeat status identified two deficiencies of (1) sample application technique and (2) sample verification compliance before analysis owning to lacking focus in the training program.

**Conclusion**: Connectivity and data integration enabled laboratory to establish a well-designed Quality Management system. and conduct a periodic effectiveness appraisal scientifically to identify areas for improvement, aimed to achieve excellent service quality for the best of patient care.

## B-282

### Auditing Point of Care Glucose Test Data for Analysis of Utilization Patterns, Glycemic Control in Hospitalized Patients and Operator Compliance

J. L. V. Shaw. *The Ottawa Hospital, The University of Ottawa and EORLA, Ottawa, ON, Canada*

Background: The implementation of centralized point-of-care testing (POCT) data management software allows for audits aimed at assessing POCT utilization and operator compliance with POCT policies and procedures. Capillary blood glucose is the most common POCT performed at The Ottawa Hospital, a large academic healthcare centre in Eastern Ontario Canada. POC glucose testing was audited to determine which clinical areas perform the highest volumes of testing. Glycemic control in patients (defined as blood glucose concentrations 4-10 mmol/L) was examined for the general medicine, surgical and intensive care units. Operator

compliance with policies and procedures related to repeat of critical values and positive patient identification were also assessed.

Methods: Four weeks of POCT glucose data from were extracted from centralized POCT software. The four weeks examined represented different time points throughout the year. The data were studied for utilization patterns and patient glycemic control of in the following clinical areas: medicine, surgery and intensive care. Operator compliance with hospital policies and procedures related to POCT was also evaluated.

Results: The clinical areas performing the highest volumes of POC glucose testing were general medicine, surgery and intensive care. Patients have, on average, 3-4 POC glucose measurements per day. Hyperglycemia was found to be common in these patients, with 35-41% of patient results measuring > 10 mmol/L on medicine units, 27-31% on surgery units and 48% on the intensive care units. Compliance of POCT operators with positive patient identification policies and procedures was quite good with only 3% of results associated with an invalid medical record number. However, compliance of POCT operators with repeat of critical glucose policies and procedures was poor.

Conclusions: The findings presented here reveal that glycemic control is relatively poor in hospitalized patients, despite frequent POCT for blood glucose. We demonstrate the power of centralized data management software for analysis of POCT data aimed at auditing quality assurance. These audits allow for follow-up with clinical areas performing POCT aimed at improving compliance with policies and procedures.

## B-283

***ACCELLIX Automated Flow Cytometry***

A. Reiter, J. Meissonnier, T. Volker. *LeukoDx, Jerusalem, Israel*

**Background:** Medical flow cytometry (FC) provides diagnostic answers by detecting the presence and concentration of cell populations, and/or by measuring concentrations of cell surface markers expressed on cells. Currently, FC is limited to high complexity labs by time consuming pre-analytical steps, requiring highly trained technologists. Inter-instrument and inter-operator variability limit broad acceptance of IVD FC. Finally, interpretation of FC results requires highly trained professionals typically available only during business hours. The Accellix compact table top multicolor flow cytometer automates the 3 step process required for population identification and/or cell surface marker measurement. Sample preparation and reading are performed in a dedicated disposable cartridge. Analytical data processing utilizing proprietary algorithms provides answers directly to the user. **Methods:Accellix Cartridge:** This disposable cartridge-based platform provides 24/7 availability in a moderate complexity lab - ultimately CLIA waved setting - by implementing sample preparation using three reagent blisters. With different reagents in the blisters the same cartridge structure can be used for multiple applications. The 3 Accellix CD64 cartridge blisters contain staining cocktail of conjugated monoclonal antibodies, lysis buffer, and reference beads respectively. Once sample processing is complete, the sample flows through a dedicated reading channel where data is acquired. **Applications** on Accellix**:**

- Sepsis diagnosis and monitoring based on upregulated CD64 expression on neutrophils.
- Measuring sepsis induced immunosuppression via HLA-DR expression on circulating monocytes.
- CD34+ for stem cell numeration for hematopoietic (bone marrow) transplantation
- Measuring activated platelets using CD41 and Annexin V for real time blood coagulation activity
- Cell characterization:
  - T cell subsets: proportion of T helper cells (CD4) to cytotoxic T cells (CD8) compared with total T cells (CD3).
  - Population analysis of cells: differentiating T cells, B cells, NK cells and monocytes based on cell surface marker expression.

**Results and Conclusions:** In a demonstration of cell surface marker quantitation a comparison study of 53 blood samples showed a correlation coefficient of 0.91 for Accellix determined neutrophil CD64 compared to those determined using a FACS. A similar study showed a correlation coefficient of 0.97 for Accellix HLA-DR compared to using a FACS. In a study to identify lymphocyte subsets a comparison study showed a correlation coefficient of 0.99 for Accellix determined T cell differentiation based on CD3/CD45 ratio compared to FACS. These initial studies show that the cartridge-based Accellix system can determine the presence and concentration of cell populations as well as determine the concentration of cell surface markers. Thus, implementation of a wide range of fully automatic IVD assays with results in 30 minutes or less is possible using Accellix.

## B-284

**Pre-clinical performance evaluation of the XCR GAS Direct Test for the rapid and accurate diagnosis of Streptococcal pharyngitis**

J. Purcell, B. E. Caplin, B. Green. *XCR Diagnostics, Inc, Park City, UT*

**Background:** Group A *Streptococcus* (GAS) is a leading cause of pharyngitis and causes various infections, including strep throat, scarlet fever, and impetigo. Prevalence of GAS infection in children is estimated at 20-30% and up to 5-15% in adult populations. The current standard for pharyngitis diagnosis in primary and urgent care settings involves GAS rapid antigen detection tests, which are about 70-80% sensitive. Additionally, negative tests are generally confirmed with microbial culture which takes 24h to 48h to provide diagnostic results. Rapid and accurate GAS diagnosis at the point-of-care is imperative for timely administration of antibiotic therapy, appropriate patient treatment, and proper antibiotic stewardship. With improper or inadequate treatment, GAS infections can advance to rheumatic fever or other invasive infections. **Methods:** Presented herein is the first clinical performance data demonstrating the diagnostic capabilities of the XCR Diagnostics portable diagnostic system coupled with the XCR™ GAS Direct Test. This fully-integrated system is designed to take advantage of a novel, proprietary amplification technology known as Extreme Chain Reaction (XCR™) that was developed by Fluoresentric, Inc (Park City, UT). In this prospective pre-clinical study, the XCR™ GAS Direct Test was directly compared to rapid antigen and standard bacterial culture to evaluate its potential clinical performance. The study utilized excess, deidentified throat swabs specimens (ESwab™ Liquid Amies FLOQSwabs™ (Copan)) that had been collected from symptomatic patients during the winter of 2016-17 and submitted for routine GAS testing by rapid antigen and/or culture methods. Each remnant sample was evaluated with the XCR™ GAS Direct Test and results were compared to the rapid antigen and culture based results as provided by the collection site. **Results:** The results of this pre-clinical evaluation demonstrate the sensitivity and specificity of the XCR™ GAS Direct Test relative to the current standard-of-care testing. **Conclusions:** The XCR™ GAS Direct Test performed on the XCR Diagnostics portable diagnostic system is a fully integrated sample-to-result assay with automated sample extraction, integrated reaction controls, amplification of nucleic acid targets, and fluorescence-based detection of *Streptococcus pyogenes* from throat swabs. The system provides rapid test results in a timeframe that is amenable to patient office visits, which is highly desirable for use in the near-patient test setting.

## B-285

**Point of Care (POC) International Normalized Ratio (INR) Anti-Coagulation Testing: Human Factors, Quality Patient Care and Error Reduction**

D. J. Augustin, L. K. Beyer, S. M. Woolley. *Mayo Clinic, Rochester, MN*

**Background:** Point of Care (POC) International Normalized Ratio (INR) is used to monitor anticoagulation therapy in the outpatient anticoagulation clinic. We used human factor analysis of the testing process to reduce patient identification errors. **Methods:** Human factors analysis revealed multiple potential sources of error when entering patient identification information into the Roche CoaguChek® XS Plus (Roche Diagnostics, Indianapolis IN). These included failure to follow patient identification procedure, manual entry of medical record number (MRN), and instrument limitations including no backlighting on visual display, small font size, and inability to use dashes when entering MRN. After investigation, the manual MRN entry process was determined to be the most error prone part of the identification process. The process was modified from manual MRN entry using a barcode label on the CoaguChek XS Plus, to scanning of a patient identification card using a device with a barcode scanner (CoaguChek XS Pro). We measured patient identification errors for a 6 month period before implementing the CoaguChek XS Pro, and two six month periods after implementation of the patient identification card and barcode scanning.

**Results:**Sixty-seven errors were observed in a 6 month period in 2014 using the CoaguChek XS Plus. Of those 67, 33 errors were incorrect entries, 32 errors were incomplete entry and 2 were due to transposed numbers. With the introduction of the barcode scanner on the CoaguChek XS Pro, patient identification errors were dramatically reduced to 3 errors during a six month period in 2015, and 2 errors during a six month period in 2016. This represents a 97% error reduction rate.**Conclusion:** Introduction of the barcode scanner on the CoaguChek XS Pro dramatically reduced patient identification errors in the anticoagulation clinic. The additional XS Plus and Pro limitations: no backlighting on visual display, small font size, and inability to use dashes when entering the MRN (e. g. 1-234-567 versus 1234567) were passed onto the manufacturer for consideration in future versions.

## B-286

### Low $pO_2$ Contributes to Potential Error in Oxygen Saturation Calculations Using a Point of Care Assay

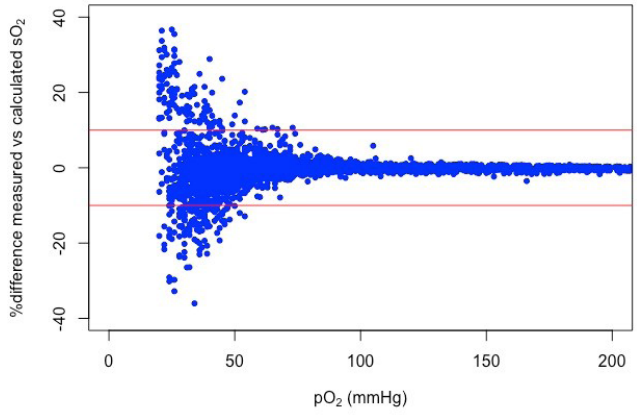
I. L. Gunsolus, S. A. Love, L. P. Kohl, M. Schmidt, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

**Background**: Oxygen saturation ($sO_2$) is an indicator of respiratory status, monitored during in-patient and out-patient clinical settings and surgical procedures. The present study addressed the accuracy of calculated $sO_2$ using point of care (POC) testing compared to measured values on a blood gas analyzer.

**Methods**: 3323 $sO_2$ values were measured in 1180 patients using a CO-oximeter (ABL 800 Flex blood gas analyzer) and using the measured parameters we subsequently calculated the expected $sO_2$ values using an indirect POC method (Abbott iSTAT). Cases in which calculated $sO_2$ differed from measured $sO_2$ by greater than or equal to 10% were classified as discrepant and analyzed.

**Results**: Approximately 60% of patients were male. Venous, arterial, and cord blood samples comprised 79%, 15%, and 6% of measured samples. Of the 3323 comparisons performed, 260 (8%) showed discrepancies (+/- ≥ 10%) between measured and calculated $sO_2$ values. Calculated values that were more than 10% higher or, alternatively, more than 10% lower than measured values occurred with approximately equal frequency. Notably, 94% of discrepant measurements (245 of 260) occurred when $pO_2$ was less than 50 mmHg. The distributions of measured pH and bicarbonate were shifted to lower values in discrepant cases. The frequency of pH ≤ 7.4 and bicarbonate ≤ 25 mEq/L were 16% and 3% higher in discrepant vs. non-discrepant cases.

**Conclusions**: We demonstrate that calculated $sO_2$ values from a POC assay are expected to be clinically discrepant in 8% of cases, the majority of which occur when $pO_2$ is <50 mmHg. In settings where inaccuracies in $sO_2$ calculations may have significant consequences on patient care, direct measurements of $sO_2$ by CO-oximetry should be used.




## B-287

### Clinical Significance of Accurate Total Hemoglobin Measurements in the Perioperative Setting

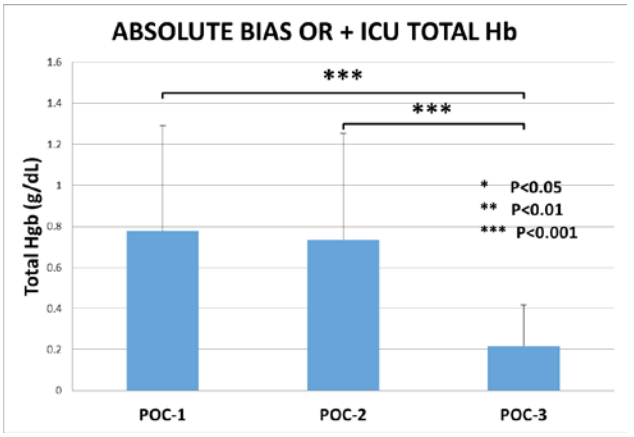
F. Lara[1], R. A. Caynak[1], K. M. Lima[1], N. Lambuth[2], S. Barnhard[1], T. L. Palmieri[1], N. W. Fleming[1], N. J. Klein[1], N. K. Tran[1]. *[1]UC Davis School of Medicine, Sacramento, CA, [2]UC Davis Medical Center, Sacramento, CA*

**Background:** Intensive care unit (ICU) and operating room (OR) patients frequently require transfusions from pathologic and iatrogenic blood loss. Some point-of-care (POC) devices employ conductance-based techniques to determine total hemoglobin (tHb), indirectly calculated through hematocrit measurements, which may be susceptible to hemodilution effects. Conversely, optical sensors are considered more accurate by measuring tHb spectrophotometrically. Inaccurate determination of tHb may result in inappropriate blood product utilization. *The objective of this study is to evaluate the accuracy and clinical significance of POC conductance-based versus optical-based tHb in ICU and OR settings.*

**Methods:** Performance of three POC analyzers (POC-1, -2, and -3) was compared against central laboratory hematology analyzer for burn, surgical ICU, and OR patients. POC-1 and -2 were conductance-based; POC-3 was optical-based. Venous, arterial, or mixed-venous (≥1.0mL) remnant whole-blood specimens obtained from adults (age≥18 yrs.), assayed for tHb, and compared to central laboratory. Administered packed red blood cell (PRBC) units and transfusion events were recorded. Mean (SD) biases (POC minus reference) were compared with paired t-Tests. PRBC units were estimated from institutional recommended thresholds (≤7g/dL non-cardio; ≤8g/dL cardio patients). Cost was calculated by price/PRBC unit alone.

**Results:** Fifty patients (72 samples total) were enrolled. Mean (SD) bias for burn, surgical ICU, and OR populations summarized in Figure-1. POC-1 and -2 exhibited higher mean biases than POC-3 (P<0.001). POC-3 results correlated closer to central laboratory platform; mean (SD) bias=0.2 (0.20) g/dL. Potential bias outcome: 27 unnecessary transfusions (6 missed) with POC-1, 18 (9 missed) with POC-2; 3 missed with POC-3. Point-of-care-1 use would have cost $6,075 and POC-2 $4,050 ($225/PRBC).

**Conclusion:** Conductance-based system bias may result in inappropriate transfusions. Spectrophotometric methods may enable appropriate utilization of blood products. Analytical biases for POC-1 and -2 differ, compared to central laboratory and POC-3. Studies are needed to determine when conductance-based measurements become unreliable, as in cases of hemodilution.




## B-288

### i-STAT Alinity: The Use of Human Factors Engineering and Usability Engineering in the Design of a Next Generation Point of Care Instrument

P. Frank[1], S. Gershtein[2], C. Robertson[2], D. Williams[2], N. Soman[2], J. Tirinato[2], J. Patel[2]. *[1]Abbott Point of Care, Ottawa, ON, Canada, [2]Abbott Point of Care, Princeton, NJ*

**Objective:** To demonstrate that the design of the i-STAT Alinity instrument is safe and effective and meets the needs of end users by using human factors engineering and usability engineering.

**Background:** The next generation i-STAT instrument, the i-STAT Alinity, is a handheld, *in vitro* diagnostic analytical device designed to run i-STAT cartridges. The system is designed for use with-patient care, by trained healthcare professionals and is for prescription use only. Human factors are defined as the application of knowledge about human capabilities and limitations. Usability is defined as the characteristics of the user interface that establishes effectiveness, efficiency, ease of use and user satisfaction. The i-STAT Alinity instrument is certified to the American National Standard ANSI/AAMI/IEC62366-1:2005 *Medical devices - Part 1: Application of usability engineering to medical devices* and has shown to meet the requirements of the Quality System regulation (21 CFR 820.30) where the need for human factors is implied.

**Methods**: To assess the human factors and usability of the i-STAT Alinity instrument, studies were performed that focused on the users, the use environment and the device/user interface. More than eight formative studies were performed with 7 - 12 end users to assess the usability of the instrument. These studies included an evaluation of the shape, weight and balance of the instrument, usability of the display and touch screen as well as the patient test flow, and the customization manager (AlinIQ CWi). With information from the formative studies, the design of the instrument was adapted to meet the needs of the user. A final summative study was performed. This study used 24 healthcare professionals that represented the intended users of the instrument and included the testing of patient blood samples, the reviewing and printing of patient test results, and the running of a quality test of the handheld instrument. Testing consisted of one-on-one sessions in a simulated hospital environment where participants

completed a series of tasks targeted at evaluating the ease of use and overall usability of the instrument.

**Results:** The summative study assessed behavioral and qualitative data. During the running of a patient test no use errors were found 92% to 100% of the time and operational difficulty was noted on 0% to 13% of the tasks. Correct review of results was achieved 96% to 100% of the time with operational difficulty noted on 0% to 21% of the tasks. Proper quality testing of the handheld instrument was achieved 100% of the time with operational difficulties noted on 0% to 21% of the tasks. None of these findings had implications on the effectiveness for user or patient safety and did not require additional risk mitigation. Potential root causes were identified and the residual risk to both patients and users was determined to be minimal.

**Conclusion:** These studies demonstrate that the i-STAT Alinity instrument was designed for usability and meets all of the expectations of a safe and effective device that is easy to use with very high user satisfaction.

These studies were funded by Abbott Laboratories.

## B-289

### Analytical Evaluation of Pleural Fluid pH Measurement on the GEM Premier 5000 Analyzer

K. Manley. *Instrumentation Laboratory, Bedford, MA*

Pleural effusions are an abnormal accumulation of fluid in the pleural space between tissue that lines the lungs and chest cavity. A thoracentesis can be performed to remove the excess exudative fluid and pH is measured for an indication as to the cause of the pleural effusion. Depending on the pH of the pleural fluid, therapeutic decisions can be made.

Pleural fluid with pH levels less than 7.30 are considered to be potentially malignant effusions. Conditions that can result in a low pleural fluid pH are tuberculous pleuritic, esophageal rupture, lupus pleuritic, or purulent pleuritic. The American College of Chest Physicians recommends the use of blood gas analyzers for the measurement of pleural pH. Thus, to meet clinical best practices, the GEM Premier® 5000 (Instrumentation Laboratory, Bedford, MA) was evaluated for performance in measuring pleural pH. Preliminary testing indicate that the GEM Premier® 5000 pH sensor technology has the ability to precisely measure pleural fluid. Samples run under the proposed pleural pH custom sample type denotes that, within the pH range of 7.00-7.50, GEM Premier® 5000 has greater than 95% confidence that pleural fluid pH results are within total allowable error. In-house testing consisted of manipulating pooled pleural fluid with an acid or base, as well as tonometry, in order to span the desired pH range for pleural fluid. Clinical testing was performed at three different hospitals, using clinical samples collected from patients with a variety of pleural effusion presentations. Both in-house and clinical samples were analyzed on the GEM Premier® 5000 and the Radiometer® ABL835 FLEX (Radiometer Medical, Denmark). Method comparison results for samples are shown in Table 1.

**Table 1: GEM Premier® 5000 Pleural Fluid pH Method Comparison Results**

| Pleural pH Decision Level | GEM Premier® 5000 vs. Radiometer® ABL835 FLEX Bias | Total Allowable Error | Pass/Fail |
|---|---|---|---|
| 7.20 | 0.006 | ±0.04 | Pass |
| 7.30 | 0.001 | ±0.04 | Pass |
| 7.45 | -0.007 | ±0.04 | Pass |

A full analytical evaluation and clinical evaluation is planned to establish claims and validate the process by which pleural fluid pH results could be reported.

## B-290

### Clinical Evaluation of the Next Generation i-STAT Alinity Point of Care Instrument using tests for Glucose, and Hematocrit

L. Nguyen[1], B. Wise[2], J. Rogers[2], V. Schaefer[1], D. Uettwiller-Geiger[3], M. Creer[4], S. Meador[4], M. Wayne[5]. *[1]Abbott Point of Care, Princeton, NJ, [2]Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, [3]John T Mather Hospital, Port Jefferson, NY, [4]Pennsylvania State University Hershey Medical Center, Hershey, PA, [5]PeaceHealth-St. Joseph Hospital, Bellingham, WA*

**Objective:** To assess multi-day and whole blood precision of the new i-STAT Alinity instrument, and compare patient results to those from the i-STAT 1 Wireless instrument for glucose and hematocrit. **Background:** The i-STAT Alinity is the latest in Abbott Point of Care's line of *in vitro* diagnostic instruments for with-patient testing. It includes advanced connectivity and quality control features, and measures specific analytes in whole blood using i-STAT cartridge-based technology. A study was conducted at 3 clinical sites using prospectively collected and leftover de-identified venous and arterial whole blood specimens. **Methods:** Multi-day precision was assessed by testing multiple levels of calibration verification materials. Each level was tested once daily for 5 days on 5 Alinity instruments. Whole blood with abnormal low, normal, and abnormal high levels of analyte were tested 3 times each on 7 i-STAT Alinity instruments to assess whole blood precision. Glucose and hematocrit method comparisons were also performed using venous and whole blood specimens tested in duplicate. **Results:** Results are summarized in Table 1. Slope, correlation coefficient, Estimated and Estimated Percent Bias were calculated using Passing-Bablok Regression on the first replicate of the i-STAT Alinity System and i-STAT 1 Wireless System results. For Bias Estimates, the following medical decision levels were used: 45 (L1), 120 (L2), and 180 (L3) mg/dL for glucose, and 33, 53, 56, and 70% packed cell volume (PCV) for hematocrit.

Table 1

| | **Glu (mg/dL)** | **Hct (%PCV)** |
|---|---|---|
| **Multi-day Precision (Total SD, per site)** | **Site 1:** 0.48-4.74 **Site 2:** 0.53-4.2 **Site 3:** 0.44-4.76 | **Site 1:** 0.33-0.52 **Site 2:** 0.35-0.49 **Site 3:** 0.44-0.55 |
| **Whole Blood Precision (Total SD, per site)** | **Site 1:** 0.22-7.46 **Site 2:** 0.51-2.81 **Site 3:** 0.46-3.56 | **Site 1:** 0.48-0.51 **Site 2:** 0.30-0.52 **Site 3:** 0.22-0.50 |
| **Method Comp. Range (Alinity)** | 25-668 | 18-70 |
| **N** | 188 | 229 |
| **Slope (95% CI)** | 1.000 (1.000, 1.000) | 1.000 (1.000, 1.000) |
| **Intercept (95% CI)** | 1.000 (1.000, 1.000) | 0.000 (1.000, 1.000) |
| **Correlation Coefficient r (95% CI)** | 1.000 (1.000, 1.000) | 0.980 (0.975, 0.985) |
| **Estimated Bias** | 1.00, all levels | 0.00, all levels |
| **Estimated Percent Bias** | L1: 2.2% L2: 0.83% L3: 0.56% | 0.00, all levels |

**Conclusions:** Glucose and hematocrit testing on the i-STAT Alinity instrument demonstrated acceptable multi-day and whole blood precision. Method comparisons correlated well with whole blood specimens. Overall, the clinical evaluation of the i-STAT Alinity System with the glucose and hematocrit test demonstrated equivalent performance to the i-STAT 1 Wireless System in a point of care setting.

The study was funded by Abbott Laboratories.

## B-291

### Evaluation of the Next Generation i-STAT Point-of-Care Instrument (i-STAT Alinity) for Chemistry/Electrolyte, Hematology and Blood Gas Tests

L. Thibodeau[1], P. Frank[1], S. Gershtein[2], N. Soman[2], S. Breeze[1], R. Perry[1], I. Cruz[1]. *[1]Abbott Point of Care, Ottawa, ON, Canada, [2]Abbott Point of Care, Princeton, NJ*

**Objective:** The i-STAT® Alinity instrument was designed to provide laboratory quality results in minutes at the patient's bedside. The purpose of these studies was to assess the analytical performance of the i-STAT Alinity system with the following tests: Chemistry/Electrolyte (including sodium, glucose, potassium, blood urea nitrogen, chloride, creatinine, lactate, ionized calcium), Hematology (hematocrit) and Blood Gases (including partial pressure oxygen, partial pressure carbon dioxide, total carbon dioxide and pH).

**Background:** The i-STAT Alinity system represents the latest innovation in with-patient testing. The system uses the same i-STAT cartridges as the i-STAT 1 wireless system while offering enhanced functionality and ergonomics.

**Method:** The analytical performance of the i-STAT Alinity instrument for Chemistry/Electrolyte, Hematology and Blood Gas tests was demonstrated through testing of multi-day precision, linearity, analyte recovery, limit of quantitation (LoQ) and interference from potential substances. Tests were also completed to evaluate total precision in blood and correlation to the currently marketed i-STAT 1 wireless instrument.

**Results:** Acceptance criteria were met for all tests performed. The within-laboratory (total) precision ranged from 0.26% – 10.29% for Chemistry (excluding creatinine level <0.1mg/dL), 0.60% – 4.53% for Hematology (Hematocrit) and 0.03% – 8.52% for Blood Gas tests. Linearity across the reportable range was demonstrated for each test. The % recovery for samples within the reportable range varied from 96.0% to 108.5% for Chemistry tests, 100.1% – 102.8% for the Hematocrit test and 96.6% to 106.9% for Blood Gas tests. Many new potentially interfering substances were tested

and for most tests, there were no new interferences found. All interferences will be labelled accordingly. Correlation between the i-STAT Alinity system and the i-STAT 1 wireless system was demonstrated through a weighted Deming regression slope within the range of 0.98 – 1.02 and a correlation coefficient (r) range of 0.99 – 1.00 for all tests.

**Conclusion:** These studies demonstrate that the next generation i-STAT Alinity instrument provides comparable results to the currently marketed i-STAT 1 wireless instrument.

These studies were funded by Abbott Laboratories.

*Pending review with FDA.

Not all products are available in all regions.

Contact your Abbott representative for availability in specific markets.

## B-292

### Performance Evaluation of Critical Analytes in Capillary Blood Using The epoc Point of Care System

M. Ali[1], C. Jing[1], R. Edwards[2], J. Chairez[2], S. Devaraj[1]. *[1]Department of Pathology & Immunology, Baylor College of Medicine, Division of Clinical Chemistry,Texas Children's Hospital, Houston, TX, [2]Department of Pathology & Immunology,Texas Children's Hospital, Houston, TX*

***Background:*** Close monitoring of blood gases and other critical analytes on point-of-care (POC) devices is crucial during pre-hospital or inter-hospital transfer of patients. Capillary blood is a preferred sample type in transport, due to its better reflection of the arterial oxygen supply status than venous blood, and the convenience of sampling. Limited data are available on the performance of POC devices using capillary blood for critical tests during transport. The objective of this study was to evaluate the performance of capillary blood testing on the epoc Point Of Care Blood Analysis System (Alere Inc).

***Method:*** Institutional Research Board waiver of consent approval was obtained before study initiation at Texas Children's Hospital (TCH). Eleven tests contained in the single-use, self- calibrated epoc BGEM Test Card were evaluated for reference range concordance and correlation with predicate analyzers. The minimum volume requirement for epoc system is 92µl, and results are available in approximately 30 seconds. Capillary blood, obtained from 20 apparently healthy volunteers between 18 and 65 years was tested on the epoc system to assess the concordance to reference ranges used locally at TCH. In addition, 10 whole blood samples were used for method correlation of epoc in the capillary mode. For method correlation of $Na^+$, $K^+$, $Cl^-$, ionized $Ca^{2+}$, glucose, lactate, hematocrit, hemoglobin, $pO_2$, $pCO_2$, and pH, fresh venous blood samples were collected in heparinized vacutainer tubes and were tested on GEM Premier 4000 (Instrumentation Laboratory, a minimum volume of 150µl) and on the epoc system under capillary mode. For method correlation of creatinine, freshly collected venous blood samples were centrifuged within an hour from the collection and were tested on the Vitros 5600 Integrated System (Ortho-Clinical Diagnostics, a minimum volume of 41µl) and the epoc system under capillary mode. Distribution of results from healthy individuals was examined for reference range concordance after excluding outliers based on individual health conditions, fasting states (for fasting glucose) or genders (for gender-specific reference ranges). Correlation of epoc with Vitros 5600 and GEM 4000 was assessed with Deming Regression.

***Results:*** In capillary blood, concordance of greater than 85% to the local TCH reference ranges was obtained for all assays except $pO_2$ and $Cl^-$. The test result distribution of pH, $pCO_2$, hematocrit, hemoglobin, creatinine, $Na^+$, and $K^+$ showed good concordance to the TCH reference ranges (≥90%). $Ca^{2+}$, glucose, and lactate showed between 85% and 90% concordance. Deming regression correlation coefficients for all the comparisons were above 0.65 except for $Ca^{2+}$. For method correlation in the epoc capillary mode, excellent correlation (>0.90) between instruments was observed for $K^+$, glucose, lactate, and creatinine.

***Conclusion:*** Our results, in consensus with previous findings, suggest that majority of the analytes available on the epoc system have comparable results to other chemistry analyzers. In addition, this study has shown an excellent concordance of measured analytes in capillary blood for the following analytes: $Na^+$, $K^+$, glucose, lactate, creatinine, hematocrit, hemoglobin, pH, and $pCO_2$, enabling the use of such critical tests in pediatric transport.

## B-293

### New microscopic counter system ADAMII for enumerating CD3+/ CD4+ T-Cells.

J. Lee, J. Oh, S. Bong, J. Kim, H. Park, J. Yoon, S. Han, C. Chung. *NanoEntek, Seoul, Korea, Republic of*

**Background:** $CD4^+$ T-cell counts are used to monitor the immune status of HIV-infected patients. Laboratory monitoring of HIV-infected patients receiving antiretroviral treatment (ART) is currently done by measuring CD4 cells. World Health Organization (WHO) guidelines recommend that people with HIV to start ART when their CD4 counts fall below 350 CD4 cells/$mm^3$. The CD4 count is very important to know when to start ART and for the evaluation of treatment efficacy. Flow cytometric analysis is used as the standard method for enumerating, but it is difficult to utilize it because of the complicated procedure and the use of expensive equipment in developing countries. In recent years, the flow cytometry analyzers has been developing to miniaturize and to be portable alternative devices, which can save on the cost of reagents consumed in a single test and can be manipulated with minimal training. We have developed a image based cell counter ADAMII™ CD4 system that is cost-effective, easy to use, and maintenance-free. **Methods:** ADAMII™ is a bench top fluorescent cell counting analyzer that can be used in hospitals and research laboratories. The obtained images are processed by an image analysis software integrated in the system. All procedures are automatic once the stained sample is loaded into the disposable assay slide. We have evaluated ADAMII™ and Alere Pima, using the FACSCalibur as a reference method. Since there is limited number of HIV / AIDS patients in Korea, it was practically difficult to receive infected blood periodically. Thus, we have demonstrated the performance of the device by artificially controlling the concentration of leukocyte after collecting the blood samples of the normal person through the recruitment of subjects. We evaluated the new image based cell counter ADAMII™ CD4 system (including the reagents and software) against the predicate system FACSCalibur™ (Tritest™ CD3/CD4/CD45 reagent with Trucount™ tubes) for the determination of absolute count of CD4+ cells to assess the performance characteristics. Statistical analyses, such as the bland-altman, correlation, linear regression and accuracy determination, were performed. **Results:** The numbers of $CD4^+$ cells obtained with the ADAMII™ are highly correlated with the FACSCalibur ($R^2$>0.9837, n=81, bias=-13.5 cell/uL) and PIMA ($R^2$>0.9867, n=81, bias=-14.75 cell/uL). Linearity of $CD4^+$ cell counts was confirmed over a range of dilutions (4-1015.85 cells/uL of sample). Linear regression analysis was performed at a low count range, 4-228.18 cells/µL ($R^2$ = 0.997) and normal range, 4-1015.85 cells/µL ($R^2$ = 0.9968). For the reproducibility test, samples were diluted to an expected concentration. ADAMII™ had a CV of 11.15%, 9.32%, 6.69%, 5.63%, 3.16% at 20 cells/µL, 100 cells/µL, 230 cells/µL, 430 cells/µL, 1100 cells/µL, respectively, and CV's were below 20%. **Conclusion:** The $CD4^+$ counts obtained by ADAMII™ CD4 analyzer showed a good correlation and low bias to the reference method and PIMA CD4 analyzer. The ADAMII™ CD4 analyzer gave CD4+ counts comparable to the reference methods for all CD4 ranges. Furthermore, our data show an excellent correlation with the current standard method. We expect the new image-based cell counter ADAMII™ will be a clinical application by replacing flow cytometry analyzer.

## B-294

### Analytical verification of RAPIDPoint® 500 and multicentric comparison with reference blood gas analyzers

M. Parra-Robert[1], P. Oliver[2], O. Rodriguez-Fraga[2], J. Noval-Padillo[3], J. Liron[4], M. Munoz-Perez[5], B. González de la Presa[1], J. Bedini[1]. *[1]Core Laboratory. Biomedical Diagnosis Centre. Hospital Clínic, Barcelona, Spain, [2]Department of Laboratory Medicine. La Paz University Hospital, Madrid, Spain, [3]Department of Laboratory Medicine. Virgen del Rocio University Hospital, Sevilla, Spain, [4]Department of Laboratory Medicine. La Ribera University Hospital, Alzira, Valencia, Spain, [5]Department of Biochemistry and Emergency Laboratory. Severo Ochoa University Hospital, Leganes, Madrid, Spain*

**Background:** Assessment of blood gases, electrolytes, hemoglobin and other magnitudes is particularly important to manage critically ill patients. RAPIDPoint® 500 (Siemens Healthineers) is a new blood gas analyzer designed to meet the challenges of point-of-care settings. This study evaluates the analytical verification of RAPIDPoint® 500 and compares it with other analyzers located in different clinical laboratories.

**Methods:** Analytical verification of RAPIDPoint® 500 consisted of imprecision evaluation using two levels of quality control in duplicate, with two analytical series

per day, during 20 days. Multicentric comparison was performed in five clinical laboratories in Spain using four blood gas analyzers:

•ABL90 FLEX (Radiometer): Hospital 1

•Cobas b 123 (Roche): Hospital 2

•RapidPoint 405 (Siemens): Hospital 3

•RapidLab 1265 (Siemens): Hospital 3, 4 and 5

Each laboratory included 100 blood samples collected in heparinized syringes and measured the following magnitudes: pH, $pCO_2$, $pO_2$, sodium, potassium, calcium and chloride in all analyzers. Hemoglobin was only measured in three systems. Statistical analysis was performed using Passing Bablok non parametric regression method.

**Results:** The estimated imprecision for intra- and inter-assay was lower than allowable imprecision based on desirable biological variation (Westgard QC). The best correlation between RAPIDPoint® 500 and reference blood gas analyzers was with RapidPoint 405. The comparison with RapidLab 1265 shows a systematic bias, mostly constant, whereas with Cobas b 123 and ABL90 FLEX a systematic bias mainly proportional is observed. A major constant systematic bias is observed with chloride in all comparisons. The table summarizes those arterial blood gases magnitudes, with the corresponding gas analyzer, which have a systematic bias.

| Magnitude | Blood gas analyzer | Regression line | Intercept (95% CI) | Slope (95% CI) |
|---|---|---|---|---|
| pH | Cobas b 123 | Y = -1.144 + 1.157 X | **-1.144 (-1.806 – -0.529)** | **1.157 (1.073 – 1.249)** |
| $pCO_2$ | RL 1265 (Hospital 5) | Y = 2.130 + 0.965 X | **2.130 (0.510 – 3.670)** | 0.965 (0.925 – 1.003) |
| $pCO_2$ | RL 1265 (Hospital 4) | Y = 2.860 + 0.894 X | **2.860 (0.480 – 5.070)** | **0.894 (0.843 – 0.950)** |
| $pO_2$ | ABL90 Flex | Y = -0.820 + 1.038 X | -0.820 (-1.860 – 0.130) | **1.038 (1.014 – 1.063)** |
| $pO_2$ | Cobas b 123 | Y = 2.620 + 0.943 X | 2.620 (-0.110 – 5.170) | **0.943 (0.895 – 0.991)** |
| $pO_2$ | RL 1265 (Hospital 3) | Y = -5.373 + 1.048 X | **-5.373 (-5.878 – -4.675)** | **1.048 (1.034 – 1.061)** |
| $pO_2$ | RL 1265 (Hospital 5) | Y = 8.340 + 0.936 X | **8.340 (7.830 – 9.020)** | **0.936 (0.922 – 0.949)** |
| $pO_2$ | RL 1265 (Hospital 4) | Y = -2.130 + 1.019 X | **-2.130 (-2.610 – -1.620)** | **1.019 (1.009 – 1.028)** |
| $pO_2$ | RP 405 | Y = -0.217 + 0.984 X | -0.217 (-0.659 – 0.326) | **0.984 (0.970 – 0.995)** |
| Hb | ABL90 Flex | Y = 1.080 + 0.947 X | **1.080 (0.760 – 1.380)** | **0.947 (0.925 – 0.973)** |
| Hb | RL 1265 (Hospital 3) | Y = 0.900 + 0.964 X | **0.900 (0.400 – 1.210)** | 0.964 (0.941 – 1.000) |
| Hb | RL 1265 (Hospital 5) | Y = 0.550 + 0.981 X | **0.550 (0.300 – 0.800)** | 0.981 (0.961 – 1.000) |
| Hb | RL 1265 (Hospital 4) | Y = 0.570 + 0.969 X | **0.570 (0.200 – 0.780)** | 0.969 (0.950 – 1.000) |

CI: Confidence Interval

**Conclusion:** The imprecision assessment fulfils the specifications based on biological variation. The bias observed in some magnitudes with other analyzers could be relevant for taking into account when different analyzers are used in the same healthcare center or a change of technology is performed.

## B-295

### Accuracy evaluation of five blood glucose monitoring systems (BGMS) and its influence on insulin dose errors

E. Fernandez[1], B. Morales[1], R. Fernandez[1], J. Bedini[1], T. Petruschke[2]. *[1]Hospital Clinic., Barcelona, Spain, [2]1Global Medical, Clinical & Scientific Affairs EU/EMEA/LATAM/APAC. Ascensia Diabetes Care Deutschland GmbH., Leverkusen, Germany*

**Background:** On July 2016, the Spanish Society of Laboratory Medicine (SEQC) and the Spanish Society of Diabetes (SED) jointly created an official consensus document on "Recommendations for the evaluation of BGMS technical performances". Despite the fact that POCT is becoming more and more relevant; especially in the diabetes field, it's not regulated by law in Spain. That's why; this document is an important and unprecedented initiative. The objective of this study is to evaluate the technical performance, accordingly the specifications defined in consensus document; of 5 BGMS widely used is Spain: Contour TS, Glucomen LX, TRUEresult, OKmeter and Lisubel ChekPlus. As a second objective we have evaluated the influence of the performance results in an insulin dosing error analyses.

**Methods:** 100 venous blood samples, collected with lithium heparin, where analyzed with the 5 BGMS and, after centrifugation, in the laboratory reference chemistry analyzer (Dimension EXL, Siemens Healthineers) using the hexokinase reference method. Time from sample reception to measurement in the Dimension EXL was always below 15 minutes. The study was performed with two different strip lots per BGMS. Accuracy was evaluated according the accuracy criteria defined in the specific ISO 15197:2013 for BGMS: ≥95% of results must be within either ±15 mg/dL of the analyzer result, for glucose concentrations <100 mg/dL, or ±15% for glucose ≥100 mg/dL.

**Results**: Contour TS was the only BGMS fulfilling the accuracy criteria for both strip lots, with 98% and 97%, while the other BGMS presented the following results: Glucomen LX 66% snd 66%, TRUEresult 84% and 80%, OKmeter 80% and 73%, Lisubel ChekPlus 68% and 64%. For each BGMS, the 99% range dose error (measured in insulin units) was the following (the broader the worst): Contour TS: -3.2 to 2.4 ; Glucomen LX: -5.5 to 5.0; TRUEresult: -9.1 to 4.8; OKMeter: -10.3 to 7.1; Lisubel ChekPlus: -13.3 to 9.2. **Conclusion:** Only Contour TS meets, for both lots, the accuracy criteria defined on ISO 15197:2013. Contour TS also presented the narrowest range for insulin dose errors, since 99% of the errors would be included within -3.2 and 2.4 insulin units. Differences in the insulin dose errors are statistically significant (p=0.0001) between Contour TS and the other BGMS. The results of this study showed that accuracy can vary from BGMS to BGMS, with a clinical relevance on insulin dosing. It is important to evaluate BGMS before use in the hospital under routine conditions, in particular for patients under insulin therapy depending on high accurate BGMS for their insulin dosing.

## B-296

### Use of HemoLink™ for blood collection and analysis: Linking patients to clinical testing with accuracy and ease.

K. Sobhani[1], E. Berthier[2], B. Casavant[2]. *[1]Cedars-Sinai Medical Center, Los Angeles, CA, [2]Tasso, Inc., Seattle, WA*

**Background:**

Current blood-draw methods range from unpleasant to difficult for patients and are logistically burdensome for healthcare facilities due to the necessity of performing phlebotomy in most cases. These challenges are heightened for patients with limited mobility, chronic illnesses requiring routine monitoring, and poor venous access. While increased availability of clinical testing (whether at home or the doctor's office) is becoming increasingly relevant, there remains a lack of reliable methods for blood collection that (1) are self-performed and convenient (2) limit pre-analytical variation (e.g., hemolysis), and (3) allow utilization by automated or POC analyzers without modifying tests. Fingerstick blood draws are painful and, more importantly, are often inadequate in terms of analytical quality and blood volume.

**Development and Objectives:**

The Tasso HemoLink™ is a self-administered, disposable capillary blood collection device, which can be used at home or in clinic, and collects adequate volumes of blood for use with both automated and POC instruments. The HemoLink is applied to the deltoid area of the arm, activated by pressing a button to generate a vacuum across four specialized lancets, and uses simple fluidic principles to deliver blood to a detachable tube (with appropriate anticoagulant and/or gel barrier, etc.). The HemoLink addresses three main blood collection issues: (1) simplifying blood draws by removing technical expertise to prevent pre-analytical error, (2) a minimalist design to keep manufacturing costs low, and (3) simple integration of collected samples into existing laboratory workflows. Herein, we compare the HemoLink to fingerstick-lancet and venipuncture for lipid profile measurement (i.e., total cholesterol, HDL, and triglycerides) to assess blood volume yield, pain (standard FACES scale), and linear-regression correlation on 41 male/female adult patients. To further demonstrate HemoLink adaptability, venipuncture and HemoLink blood were shipped to core laboratories for lipid profile correlation.

**Results:**

Blood volumes >=100µL were collected on 88% of subjects. An average pain score of 1.1 was observed for HemoLink (vs. 2.2 for venipuncture and 3.6 for fingerstick), and user preference of over 70% was observed compared to either alternative.

Linear-Regression Through Origin (X-method taken as "Gold Standard"):

**Roche Cobas 6000:**

**TC**: HemoLink=0.992(Venous),

**HDL**: HemoLink=0.99(Venous),

**Triglycerides**: HemoLink=1.03(Venous).

**Piccolo Xpress POC analyzer:**

**TC**: HemoLink=0.998(Fingerstick),

**HDL**: HemoLink=0.996(Fingerstick),

**Triglycerides**: HemoLink=0.998(Fingerstick);

$r^2$>0.98 (all correlations).

The total error observed for all analytes was within allowable error established by the National Cholesterol Education Program (NCEP). For mainframe automated analyzers 200 µL of whole blood was sufficient for a lipid panel and achievable with the HemoLink.

**Conclusion:**

The HemoLink enables blood collection in environments where phlebotomy is not available, suitable, or desired. Blood is collected in easily detached tubes with appropriate anticoagulants enabling excellent correlation to venous plasma for common tests (i.e., lipid panel) on accurate large-scale analyzers or fingerstick blood on POC analyzers. Additionally, preliminary data shows that HemoLink is demonstrating good correlations for other common tests like the comprehensive metabolic panel (CMP). With such studies establishing clinical validity, the HemoLink has the potential to transform monitoring of chronic disease, as well as outpatient, emergency, and pediatric care by offering a versatile blood collection method that addresses individualized patient needs.

## B-297

### Analytical validation of CRP and SAA combined detection (M-(CRP+SAA)) by Fluorescence Lateral Flow Immunoassay

M. GU, C. Zhu, L. Du, X. Yuan, F. Hou, X. Shi, L. Wang. *Upper Biotech Pharma Co.,Ltd, SHANGHAI, China*

Background:

C-reactive protein (CRP) is one of the major acute phase proteins (APPs). It is extremely sensitive and, at the same time, a non-specific systemic marker of inflammation and tissue damage. Another important APP for inflammation is serum amyloid A (SAA) that is synthesized in large quantities in the liver. SAA has a high sensitivity to inflammatory events and it can increase up to 1,000-fold. Like CRP, changes in plasma concentrations occur early after the onset of inflammation and then rapid decline is observed in the attenuation of the process. SAA may be a better biomarker of rheumatoid arthritis (RA) disease activity than CRP, especially during treatment with tumor necrosis factor (TNF) antagonists.

In this study, we evaluated the clinical performance of M-(CRP+SAA) by Fluorescence Lateral Flow Immunoassay (Shanghai Upper Bio-Tech, PRC) which provides results in three minutes. MAYA was constructed to quantify the distribution of fluorescence intensity along the strip. The excitation and emission wavelengths of MAYA are respectively 365nm and 615nm which give a long Stokes shift and ensure a reduced fluorescence background.

Method:

Concentrations of CRP/SAA in the serum samples (n=66) from the same individuals were measured in a side-by-side study using M-(CRP+SAA) and Siemens BNII analyzer as a reference method.

Results:

A. Serum samples comparison

66 samples had CRP and SAA levels within the reportable ranges of M-(CRP+SAA) (0.5-200mg/L for CRP; 5-200mg/L for SAA). A linear regression analysis for CRP showed y (M-(CRP+SAA)) =0.9869x(BNII)+0.2272; $r^2$=0.972;A linear regression analysis for SAA showed y (M-(CRP+SAA)) =1.0674x(BNII)-0.7902; $r^2$=0.982.

B. Analytical Concordance:

The quantitative cutoff value of CRP is set at 10 mg/L, equal to the BNII CRP assay, and the values above which are considered positive. The sensitivity and specificity is 100.0% and 90.3%. The positive predictive value (PPV) and negative predictive value (NPV) is 92.1% and 100.0% respectively. The concordance between two systems is 95.5%.

The quantitative cutoff value of SAA is set at 6.4 mg/L, equal to the BNII SAA assay, and values above which are considered positive. The sensitivity and specificity is 98.4% and 100.0% respectively. The positive predictive value (PPV) and negative predictive value (NPV) are 100.0% and 75.0% respectively. The concordance between the two systems is 98.5%.

Excellent analytical agreement concordance analysis of CRP and SAA showed excellent clinical agreement between M-(CRP+SAA) and BNII.

Conclusions

The results obtained on the M-(CRP+SAA) were comparable to those obtained on the BNII laboratory analyzer. The M-(CRP+SAA) assay is an accurate clinical indicator that can be used to assess inflammation. With both results available in 3 minutes, M-(CRP+SAA) allows for rapid initiation of appropriate patient treatment.

## B-298

### Creatinine and Urea on the ABL90 FLEX PLUS Point-of-care Blood Gas Analyzer

G. Wennecke, L. B. Vang. *Radiometer Medical ApS, Broenshoej, Denmark*

**Background**: To verify the performance of the two new parameters, creatinine and urea (BUN), on the Radiometer ABL90 FLEX PLUS blood gas analyzer studies were conducted using spiked whole blood at Radiometer Medical's laboratories in Copenhagen. The goal is to meet the analytical performance goals based on biological variation as recommended by NKDEP[i]. The goals are based upon intra- and inter-individual biological variation[ii] resulting in a minimum acceptable total error of 13.3 % for creatinine and 23.3 % for Urea[iii].

**Conclusion:** The above requirements for the creatinine and urea measurements were fully met for all the tested levels, covering the reportable range of the two parameters.

**Method**: To verify the analytical performance of creatinine and urea, the evaluation comprised an imprecision profiling according to CLSI EP05-A3.

A bias estimation was conducted by comparing spiked whole blood samples to the corresponding plasma results measured on the reference methods, for creatinine an IDMS calibrated HPLC, and for Urea by enzymatic spectrophotometry. With the precision and bias estimations a total analytical error for each parameter is calculated by: (1.96 x $CV_T$ %) ± I Bias % I

Both parameters were also compared to two other whole blood methods according to CLSI EP09-A3; ABL837 FLEX for Creatinine and i-STAT for Urea.

**Results:**

| Parameter | Mean value | Repeatability $CV_R$ % | Reproducibility $CV_T$ % | Bias % | Total error % | n | Slope | $r^2$ |
|---|---|---|---|---|---|---|---|---|
| Creatinine (µmol/L) | 40 | 0.9 | 5.9 | -0.2 | 11.6 | 101 | 0.97 | 0.998 |
| | 210 | 0.4 | 2.7 | -3.6 | 8.9 | | | |
| | 489 | 0.4 | 3.0 | -3.6 | 9.6 | | | |
| Urea (mmol/L) | 2.6 | 1.3 | 5.7 | -0.7 | 11.9 | 109 | 1.03 | 0.993 |
| | 9.5 | 0.8 | 3.0 | -1.0 | 6.9 | | | |
| | 27.5 | 0.8 | 4.1 | -7.3 | 15.3 | | | |

**References**

i Myers GL, Miller G, Coresh J *et al.* Recommendations for improving serum creatinine measurement: A report from the LWG of the NKDEP. Clin Chem 2006; 52,1: 5-18.

ii Westgard JO. Biologic Variation Database, the 2014 Update. https://www.westgard.com/biodatabase-2014-update.htm. Accessed February 2017.

iii Fraser CG, Petersen PH, Libeer JC, Ricos C. Proposals for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997; 34:8-12.

## B-299

### Analytic Evaluation of the b101 Hemoglobin A1c Point of Care Testing Method from Roche Diagnostics

M. E. Lyon, A. W. Lyon. *Royal University Hospital, Saskatoon, SK, Canada*

**Background:** Hemoglobin A1c (HbA1c) methods are used to screen and diagnose diabetes mellitus. The quality of HbA1c methods has been improved through method certification by the National Glycohemoglobin Standardization Program (NGSP), quality control and proficiency testing. Concern has been expressed that many point of care (POC) methods for HbA1c are not sufficiently accurate or precise enough for diabetes diagnosis, so new POC methods require thorough analytic evaluation. **Objective**: To evaluate the precision and accuracy of the Roche point of care b101 HbA1c method compared with the Cobas Tina-quant Gen. 3 NGSP certified method. **Methods:** b101 method precision (n=15) was assessed with three EDTA whole blood specimens at HbA1c levels at 5.3%, 7.0% and 13.4%. Patient specimens (n=47) with HbA1c levels ranging from (4.5% to 13.2%) were analyzed using the b101 method and compared with the Cobas Tina-quant Gen. 3 NGSP certified HbA1c method on a c501 analyzer. Results were evaluated using Passing Bablok regression analysis and Bland Altman method comparison. **Results:** For the b101, coefficients of variation (precision) was determined to be 1.6% at HbA1c of 5.3%, 1.0% at HbA1c of 7.0% and

1.7% at HbA1c of 13.4%. A patient correlation study yielded the following regression equation with the 95% confidence intervals: b101-HbA1c = 0.9284 (Tina-quant-HbA1c) + 0.6741; slope 95% confidence interval (0.8995 to 0.9574); Y intercept 95% confidence interval (0.43 to 0.92). The average bias over the range of HbA1c levels examined according to Bland Altman analysis was 0.10% HbA1c with 95% confidence interval (0.012 to 0.179). **Conclusions:** The Roche Diagnostics point of care b101 HbA1c method provides excellent precision and accuracy relative to the Cobas Tina-quant Gen. 3 NGSP certified HbA1c method.

## B-300

### GEM Premier 5000 Clinical Evaluation at CHR Citadelle (Belgium)

J. Cervera[1], J. De Marchin[2], A. Randazzo[2]. *[1]Instrumentation Laboratory, Bedford, MA, [2]CHR de Liège, Liège, Belgium*

**Background:** The GEM Premier 5000 is a new critical care analyzer for providing rapid analysis of whole blood samples at the point of care or in a central laboratory. This analyzer contains a single multi-use cartridge PAK to provide quantitative measurements of pH, pCO2, pO2, sodium, potassium, chloride, ionized calcium, glucose, lactate, hematocrit, total bilirubin and CO-Oximetry (tHb, O2Hb, COHb, MetHb, HHb , sO2) parameters. These measurements (and derived parameters) aid in the diagnosis of a patient's acid/base status, electrolyte and metabolite balance and oxygen delivery capacity. Clinical performance of the GEM Premier 5000 was evaluated at the CHR Citadelle Hospital. The analytical performance of the GEM Premier 5000 was compared, under clinical environment, against three Critical Care analyzers from different manufacturers: the GEM Premier 4000 (Instrumentation Laboratory), ABL 90 (Radiometer) and the RapidPoint 405 (Siemens).

**Whole Blood Method Comparison:** De-identified whole blood samples from different clinical locations at CHR Citadelle Hospital were analyzed for method comparison on the GEM Premier 5000 analyzer where the GEM Premier 4000, the Radiometer ABL 90 and the Siemens RapidPoint 405 were used as reference. Method comparison and regression analysis was performed for each reference analyzer according to Clinical Laboratory Standards Institute (CLSI) EP09-A3.

**Results:** Regression results (Slope, intercept and regression coefficients) were summarized in table 1 below. For the analytes where a regression evaluation was not possible due to the limited sample range acquired during the study, the mean bias and 95% confident interval was calculated.

Table 1: GEM Premier 5000 Method Comparison regression results versus listed reference analyzers

| Analyte | GEM Premier 4000 | | | Radiometer ABL 90 | | | Siemens RapidPoint 405 | | |
|---|---|---|---|---|---|---|---|---|---|
| Slope | Slope | Intercept | R | Slope | Intercept | R | Slope | Intercept | R |
| pH | 0.979 | 0.159 | 0.979 | 1.060 | -0.445 | 0.983 | 1.039 | -0.273 | 0.965 |
| PCO2 (mmHg) | 1.000 | 2.000 | 0.978 | 1.053 | 0.000 | 0.977 | 0.909 | 5.545 | 0.951 |
| PO2 (mmHg) | 0.991 | 5.319 | 0.999 | 0.998 | 4.233 | 0.998 | 0.932 | 2.737 | 0.994 |
| Sodium (mmol/L) | 1.014 | -1.506 | 0.982 | 0.911 | 9.938 | 0.977 | 0.964 | 4.678 | 0.943 |
| Potassium (mmol/L) | 1.000 | 0.100 | 0.995 | 1.125 | -0.362 | 0.994 | 1.111 | -0.367 | 0.995 |
| Calcium (mmol/L) | 1.059 | -0.058 | 0.979 | 1.000 | 0.010 | 0.986 | Results not provided | | |
| Chloride (mmol/L) | 1.000 | 0.000 | 0.990 | 0.909 | 11.000 | 0.973 | 1.000 | 2.000 | 0.982 |
| Glucose (mg/dL) | 1.036 | 1.643 | 0.997 | 1.060 | -3.280 | 0.994 | 1.021 | 0.297 | 0.995 |
| Lactate (mmol/L) | 1.000 | 0.000 | 0.995 | 1.000 | -0.100 | 0.991 | Results not provided | | |
| tHb, g/dL | 1.035 | -0.096 | 0.998 | 1.013 | 0.022 | 0.993 | | | |
| O2Hb (%) | 1.001 | 0.617 | 0.999 | 0.951 | 5.896 | 0.998 | | | |
| HHb (%) | 1.003 | -0.907 | 0.999 | 0.964 | -1.8 | 0.998 | | | |
| sO2 (%) | 1.003 | 0.626 | 0.999 | 0.962 | 5.559 | 0.998 | | | |

**Conclusion:** The GEM Premier 5000 systems demonstrate good performance against other Critical Care analyzers selected in this evaluation. Methodology differences between analyzers is attributed to the subtle differences observed between analyzers for some analytes.

## B-301

### Evaluation of a point of care for measurement of plasma free hemoglobin in patients receiving ECMO

L. B. PORTO[1], G. A. CAMPANA[1], L. B. FARO[1], M. D. FREIRE[1], A. F. DUTRA[2]. *[1]DASA, RIO DE JANEIRO, Brazil, [2]HOSPITAL PROCARDÍACO, RIO DE JANEIRO, Brazil*

**Background:**

Hemolysis is common in extracorporeal circuits membrane oxygenation (ECMO) as evident by the elevated PFHb level. Values of PFHb greater than 50 mg/dL checked 24-hour post ECMO implantation are considered a useful tool to predict mortality[1]. There are many methods available for the determination of plasma free hemoglobin (PFHb) in the diagnosis of various hemolytic disorders, like *Cyanomethemoglobin* using *Drabkin's* reagent or *Benzidine*[2], both extremely laborious for routine use in hospital fast response laboratories and with restrictions related to environmental and labor legislation because of its carcinogenicity. This current study evaluated the usefulness of a Point of Care (POC) photometer for the quantitative screening of low levels PFHb (microhemolysis).

**Methods:**

20 consecutive samples of blood were collected of patients with different ages whom Received ECMO at an Intensive Care Unit, using collection materials and tubes from Vacuette Greiner Bio-One®. According to the manufacturer's recommendations the splitted serum samples and quality controls were analyzed with the HemoCue® *Plasma/Low Hb System* (Sweden), which principle is based on a modified azidemethemoglobin reaction.

**Results:**

The results obtained were statistically evaluated for correlation Pearson (p up to 0.05) and by the t-test Student (t of 0.67) paired using Microsoft Excel spreadsheet.

**Conclusion:**

The spectrophotometric microcuvette based technology method is a simple and rapid technique for determination of PFHb. It is linear from 0 to 3000 mg/dl with a measuring range that was shown to be adequate according to the proposal of routine checking of PFHb 24-hours after ECMO initiation is sensitive for early identification ant treatment of the cause of hemolysis[1].

## B-302

### Evaluation of Whole Blood BUN, Creatinine and $tCO_2$ Assays *vs.* Roche Cobas Chemistry Analyzer with Clinical Samples

C. Xu, P. Pamidi, R. Malhotra, M. Curley, D. Cross, Z. Bellio, D. Raimondi, M. Narovlyansky, V. Dobromyslin. *Instrumentation Laboratory, Bedford, MA*

**Background:** Blood urea nitrogen (BUN), creatinine and $tCO_2$ are part of the basic metabolic panel, one of the most commonly ordered blood tests, which provides physicians with a quick assessment of patient electrolyte and fluid balance, blood glucose level and kidney function. A new cartridge that enables fast, accurate and reliable measurements of BUN, creatinine and $tCO_2$ using electrochemical sensing technologies for the GEM Premier whole blood (WB) analyzer (Instrumentation Laboratory/IL) is currently in development. This is an addition to the blood gases, electrolytes, and metabolites currently offered on the GEM analyzer. The goal of this study is to compare the analytical performance of the three new GEM WB assays to an established reference method.

**Methods:** A total of 695 random patient samples were obtained daily from Lahey Hospital and Medical Center (Burlington, MA). Each heparinized WB sample was assayed on up to four GEM analyzers (IL) based on the available sample volume. The plasma portion was assayed on a reference Roche Cobas c311 analyzer (Roche Diagnostics). The study was conducted over the course of four months to cover a wide range of samples and patient populations. The GEM test cartridges (N=47) were replaced on a weekly basis with minimum of 50 samples tested per cartridge.

**Results:** The WB BUN, creatinine, and tCO2 results from GEM analyzer compared well with those obtained from plasma on the reference analyzer across the wide ranges of the unaltered clinical samples. The cartridge-to-cartridge performance consistency is also demonstrated in this study. The results of the GEM WB assays vs. the reference method are summarized in Table 1.

**Conclusion:** Strong correlations were observed between the GEM WB assays and the reference method in clinical samples. These assays can provide reliable WB BUN, Creatinine and tCO2 information with quick turnaround time in Point of Care environments.

Table 1. Method Correlation Statistics for the GEM WB Assays vs. Roche Cobas Assays (N = 2522)

| Ana-lyte | Slope | 95% CI | Inter-cept | 95% CI | R | Sample Range | MDL1 (Bias) | MDL2 (Bias) | MDL3 (Bias) |
|---|---|---|---|---|---|---|---|---|---|
| BUN | 0.9750 | 0.971 - 0.979 | 0.760 | 0.691 - 0.824 | 0.997 | 5.1 - 126 mg/ dL | 6.0 (0.61) | 26.0 (0.4%) | 50.0 (-1.0%) |
| Crea | 0.9678 | 0.960 - 0.976 | 0.045 | 0.037 - 0.053 | 0.994 | 0.2 - 13.2 mg/dL | 0.6 (0.026) | 1.0 (-0.007) | 6.0 (-2.5%) |
| $tCO_2$ | 1.005 | 0.994 - 1.02 | 0.203 | -0.024 - 0.438 | 0.962 | 6.6 - 34.0 mmol/L | 6.0 (0.24) | 20.0 (1.6%) | 33.0 (1.2%) |

## B-303

### Performance Evaluation of A Novel Onsite Screening Test for Synthetic Cathinones

Q. Zheng, W. JIN. *Shanghai Kehua Bio-engineering Co., Ltd., Shanghai, China*

Performance Evaluation of A Novel On-site Screening Test for Synthetic Cathinones

**Abstract**

**Background:**

Recent years, synthetic cathinones like mephedrone, 3,4-Methylenedioxy-α-Pyrrolidinovalerophenone (3,4-MDPV) and methcathinone (MCAT) emerged rapidly as design drugs, also "bath salts". Cathinone is derived from the khat plant (*Catha edulis*), while MCAT was synthetized firstly in 1928 [1] and used as an anti-depression drug for medical therapy in former Soviet Union. Similar to methamphetamine, MCAT has addictive potential as a nervous stimulant. Long-term use of MCAT can cause severe physical injury and psychological toxicity, even death. MCAT has been regulated by many countries, but cases of MCAT abuse are reported frequently [2-4], especially in China. Gas chromatography with mass spectrometry (GC/MS) and liquid chromatography with mass spectrometry (LC/MS) are the main detection methods for synthetic cathinones[5-7], whereas they are not suitable for on-site screening test because of its requirement for sophisticated instruments and professional operators, which leads to high expense and time-consuming. It is imperative to develop a simple, convenient and rapid test device for on-site screening of emerging synthetic cathinones.

**Objective:**

The purpose of this study is to evaluate the performance characteristics of a newly developed lateral flow urine test for MCAT, and the agreement between this screening test and lab method (LC/MS/MS) is also studied.

**Methods:**

Cut-off value of MCAT was set and validated to 1000ng/ml, referred to the cut-off concentration of methamphetamine. 23 interfering substances such as methadone, ranitidine, caffeine etc and 11 structure related analogues including 3,4-MDPV, mephedrone, ephedrine, pseudoephedrine and methamphetamine etc were examined to determine the specificity. The stability of the device at 45ºC was evaluated to test MCAT standards at 0, -50%, +50% and +200% cut-off.

For method comparison, 54 clinical urine specimen were collected on-site and tested by the lateral flow test, the remaining were sent to a certified lab for LC/MS/MS quantification. The screening test results were scored in 5 min based on the color intensity of test line, which was compared with visual score card. The sensitivity, specificity, overall agreement were calculated by IBM SPSS V20.0. Statistical analysis was performed with kappa test.

**Results:**

Performance results showed that all 23 interfering substances did not interfere with MCAT at 100ug/ml, except Ranitidine at 50ug/ml. For other synthetic cathinones, like mephedrone and 4-Methylethcathinone could also be detected by the test at 1000ng/ml. No cross-reaction with common abused drug analogues at 50ug/ml (ephedrine and methamphetamine) or 100ug/ml (pseudoephedrine, amphetamine and (±)-3,4-Methylenedioxymethamphetamine (MDMA)). The device was stable when put into 45ºC for at least one month.

The screening test results showed 20 specimen were positive and 34 negative, which was fully agreed with the quantitative results. The sensitivity, specificity and overall agreement were all 100% with 95% CI 83.89-100%, 89.85-100% and 93.36-100%, respectively. Kappa = 1.

**Conclusion:**

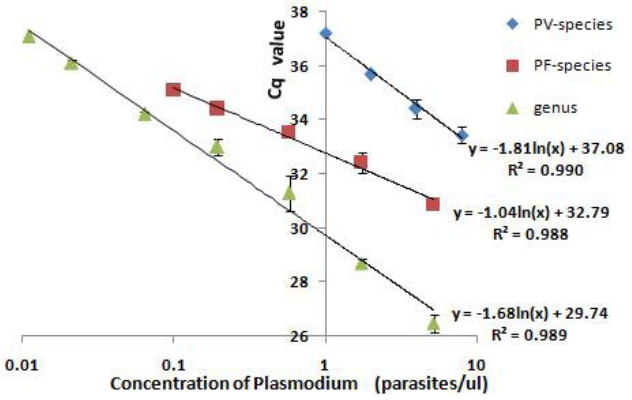
The excellent sensitivity, specificity and stability shows the newly developed lateral flow device is suitable for on-site screening of MCAT. The good agreement with LC/MS/MS results indicates the test is reliable and can be a good complement to the lab method.

## B-304

### A sensitive multiplex assay for high-throughput screening of malaria without nucleic acid extraction

Y. Zhao, Y. Zhao, Z. Zheng. *Institute of Basic Medical Sciences, Peking Union Medical College, Beijing, China*

**Background:** Accurate diagnosis is essential for successful elimination of malaria, new diagnostic tools with simplified procedure and improved throughput are still needed. Capture and Ligation Probe-PCR (CLIP- PCR) has been demonstrated as a high throughput RNA quantification technology for sensitive identification of malaria, but its role in multiplexassay remains to be demonstrated. In this study, we adopt CLIP- PCR to detect both genus Plasmodium 18SrRNA and species Plasmodium (P.falciparum and P.vivax) 18srRNA from whole blood and dry blood samples(DBS) in 3 hours. **Methods:** Target RNA from samples is released by lysis, captured directly to 96-well plate by sandwich hybridization using genus-specific and species-specific probes with different tail sequences. After enzymatic ligation of the probes, the single-stranded DNAs, whose quantities are proportional to target RNAs, are separately PCR-amplified using different primers targeting the corresponding tail sequences and quantified with SYBR green chemistry. **Results:** P.falciparum blood culture or P.vivax whole blood was serially diluted and tested by our multiplex assay. The detection limits (analytical sensitivity) were 0.01, 0.1, and 1.0 parasites/ µl for genus Plasmodium, P. falciparum and P.vivax respectively. We tested our multiplex assay with sixteen DBSs from endemic region. One microscopy-negative sample was shown to be P.falciparum; two P.falciparum infections diagnosed by microscopy were shown to be mixed infections of P.falciparum and P.vivax. Our multiplex assay cost about $1.0 for each sample. All results were confirmed by standard quantitative PCR. **Conclusion:**Our multiplex assay can identify the genus and the species of Plasmodium in 3 hours without RNA purification and reverse transcription. The assay can be used as a highly-sensitive and high-throughput approach for malaria screening with low cost and labor.




## B-305

### qPCR genotyping in crude serum separated by ultralow-cost, portable and hand-powered paper centrifuge: simplification of the pre-analytic and extraction steps for a future molecular point-of-care diagnostics

D. P. Jardim, T. H. Santa Rita, P. G. Mesquita, L. A. Soares, R. H. Jácomo, L. F. A. Nery, G. B. Barra. *Laboratório Sabin, Brasília, Brazil*

**Background**:

An ultralow-cost, portable and hand-powered paper centrifuge inspired by historic whirligig (or buzzer) toy was described recently. The paperfuge achieves high speeds and can be used for serum/plasma separation using only human power. Moreover, as blood coagulation releases leukocyte's genomic DNA (gDNA) to serum and blood clot continuous leakages gDNA to serum *ex-vivo*, this specimen can be used directly as template in qPCR, excepting the DNA extraction. The limitation is that incubation

for 96h at room temperature is necessary for a 200 base-pairs sequence be readily detectable. This turnaround time is impractical. However, just after the coagulation, small DNA molecules are more prevalent than larger ones (1.25 ug/mL for a 65 base-pairs molecule *versus* 0.1 ug/mL for a 200 base-pairs molecule) meaning that small' sizes amplicon could be amplified earlier. Thus, the objectives of this study were: a) test if paperfuge can produce serum from capillary blood for direct use in qPCR and b) reduce the amplicon size of a molecular target and check if a reliable genotyping could be performed in crude serum separated few hours after the blood collection.

**Methods**:

Capillary blood was drawn from 50 volunteers (32 females), ~100ul was transferred for a 1.5mL tube and for a 200ul tube by multiple fill ups and drain out of a 50ul glass capillary. The 1.5 mL tube contained 1mL water to induce hemolysis and its content was submitted to a validated DNA extraction (Chelex method). We adapted paperfuge for 200ul tubes (instead of the original described plastic capillaries). Serum separation occurred between 4-7h after the collection and 4 minutes of spinning was necessary. The variant rs4988235 (associated with lactose tolerance in adulthood) was genotyped by ARMS-qPCR using serum or extracted DNA as template. Two reactions (executed with validated primers pairs), one specific for C allele and another specific for the T allele, were performed simultaneously by using the Maxima SYBR Green/ROX qPCR master mix (Thermo Fisher) in the StepOne qPCR System (Thermo Fisher). Amplicon (90 base-pairs) specificity was checked by melting curve analysis. The genotypes were attributed by the ΔCq between the reactions C and T: CC (ΔCq<-1.5), CT (-1.5<ΔCq<1.5), TT (ΔCq>1.5). The qPCR reactions were performed 2h after the serum separation. The agreement between the tested conditions was verified by kappa statistics.

**Results**:

The validated workflow reveled that 26, 21 and 3 volunteers were CC, CT and TT for the rs4988235, respectively. Identical results were observed for the qPCR performed in crude serum separated by the paperfuge (kappa = 1, perfect agreement).

**Conclusion**:

The paperfuge, adapted for 200ul tubes, can be use for serum separation from capillary blood and this specimen can be used as template for qPCR. Moreover, productive amplification can be obtained from serum separated 4-7h after the blood drawn if molecular target had reduced amplicon size. This study simplified the pre-analytic and extraction steps for a future molecular point-of-care diagnostics in resource-poor settings and open up opportunities for applications in science education and field ecology.

## B-306

### A DNA guided ultrasensitive Cardiac Troponin T testing system compared with hs-cTnT

Y. H. Lin, Y. Li, Y. T. Liu, Z. F. Wang, J. X. Yu, J. S. Kang, Z. Zhou. *Fuwai Hospital, Beijing Key Laboratory for Molecular Diagnostics of Cardiovascular Diseases, Beijing, China*

**Background:** Cardiac troponin T (cTnT) assays have been evolved to show analytical, diagnostic and prognostic improvements over the last decade. The accurate quantification of cTnT levels is crucial in the diagnosis and prognosis of acute myocardial infarction. However, the endogenous anti-streptavidin antibodies, autoantibodies, and biotin might interfere the accuracy of cTnT assays. A novel ultra-sensitive Cardiac Troponin T (*us*-cTnT) testing system, bases on the recently reported 9G technology and DNA guided detection (DAGON) method, employs DNA-DNA interactions between the immobilized oligonucleotide probes and the DNA in the DNA-capture antibody conjugate. In this study, the *us*-cTnT testing system was compared with high-sensitivity cardiac troponin T (*hs*-cTnT) using patient samples.

**Methods:** 200 plasma samples from individuals including men and women of different age groups were collected for this study. Analytical verification included comparison of *us*-cTnT test and the high sensitivity cardiac troponin T (Roche Elecsys TnT *hs* STAT) assay. Assessment of critical outlier data for *us*-cTnT test as compared to *hs*-cTnT assay was verified by a serial dilution test which is one of the best ways to check the interference in the immunoassay.

**Results:** *us*-cTnT test showed linearity in the quantification of serially diluted samples. The CV values for the detection of cTnT at the LoD and the reported 99th percentile were <10%, respectively. The concordance rate between the results of *us*-cTnT test and *hs*-cTnT test was 74.7% in the samples with the cTnT concentrations bellow 15pg/mL. The serial dilution test was performed for 17 clinical samples with the concentrations in the range of 0 - 120pg/mL. Samples at <15pg/mL were detected as 5.0, 5.0, 7.0, 9.0, 164.0, 5.0, 7.0, and 7.0pg/mL in *hs*-cTnT assay and as 9.1, 11.2, 15.0, 14.7, 8.4, 8.4, 13.9, and 7.3pg/mL in *us*-cTnT test, respectively. Samples at 15-30pg/ml were detected to contain cTnT level of 3.0pg/mL in *hs*-cTnT assay and as 15.4, 25.2, and 27.3pg/mL in *us*-cTnT test, respectively. In samples containing cTnT levels >30 pg/mL, *hs*-cTnT assay detected 3.0, 6.0, and 71.0pg/mL. However, in *us*-cTnT test the samples were found to contain 35.0, 44.1, and 40.6pg/mL of cTnT, respectively. It is important to note that *us*-cTnT test showed high degree of agreement with observed and calculated values of cTnT with the correlation coefficient of 0.9952.

**Conclusion:** Our results indicate the satisfactory performance of *us*-cTnT test for the detection of cTnT. The serial dilution test in this study indicate that *us*-cTnT test is a sensitive method that offers a more accurate detection of cTnT values than the conventional cTnT method, because the *us*-cTnT test employing the highly specific DNA-DNA interactions avoids the interference from the endogeno*us*

anti-streptavidin antibodies, autoantibodies, and biotin etc. Therefore, *us*-cTnT test has a very high applicability for the detection of cTnT levels in the clinical settings for regular health check-up. *us*-cTnT test can be a good asset for physicians to track cTnT level and to prevent the incidence like AMI.

**Keywords**: cardiac troponin, accuracy, antibodies interference

## B-307

### Differences in blood gas patient results between POCT and Emergency Laboratory in three intensive care units

L. Bautista Balbás, P. Oliver Sáez, M. Alcaide Martín, P. Fernández-Calle, A. Buño Soto. *Hospital La Paz, Madrid, Spain*

**Background**

Before the installation of a new POCT device in a clinical setting, it is important to evaluate its analytical performance and results interchangeability with laboratory. It is advisable to monitor whether this interchangeability is maintained in the long-term.

Our POCT network includes 27 blood gas analyzers. Six of them are located in intensive care units (1 in Adults Intensive Care Unit (ICU), 3 in the Neonatal Intensive Care Unit (NICU) and 1 in Pediatric Intensive Care Unit (PICU)). All showed results interchangeability (CLSI EP-9) with Emergency laboratory (EL) before installation. The aim was to assess the long-term interchangeability of patient results.

**Material and methods**

POCT and EL results from January 2015 to June 2016 were collected from laboratory information system. Paired data were established by patient and time, considering 30 minutes as the maximum difference between POCT measurement and sample reception at EL. We studied the differences in sodium, potassium, chloride, haemoglobin and glucose measured in POCT (ABL90 Flex, Radiometer) and EL (Dimension Vista 1500, Siemens; CellDyn Sapphire, Abbott). Internal and external quality control was within allowable limits during the study period. The comparison of each measurand was performed using ANOVA, Passing-Bablok regression and Bland-Altman analysis. Results ±2SD were considered as outliers and excluded. The allowable difference criteria were based on biological variation (total allowable error; TAE).

**Results:**

| Magnitude | ICU (n) | r | Intercept (CI95%) Slope (CI95%) | Bland Altman (Mean%; CI95%) | TAE (%) | Outside TAE (%) |
|---|---|---|---|---|---|---|
| Sodium (mEq/L) | ICU (1797) | 0.958 | -15.66 (-18.54 to -11.80)* 1.10 (1.07 to 1.12)* | 0.90* (-2.52 to 4.31) | 1.1 | 56.5 |
| | NICU (791) | 0.918 | -23.42 (-28.56 to -17.89)* 1.15 (1.11 to 1.19)* | 1.87* (-1.91 to 5.65) | | 69.5 |
| | PICU (2141) | 0.963 | -8.79 (-12.22 to -5.54)* 1.05 (1.03 to 1.08)* | 1.04* (-2.75 to 4.83) | | 59.6 |
| Potassium (mEq/L) | ICU (1766) | 0.960 | 0.0 (0.0 to 0.0) 1.0 (1.0 to 1.0) | 0.45* (-9.21 to 10.10) | 8.4 | 4.8 |
| | NICU (755) | 0.931 | -0.1 (-0.1 to -0.1)* 1.0 (1.0 to 1.0) | 2.43* (-9.94 to 14.80) | | 16.2 |
| | PICU (2153) | 0.986 | -0.1(-0.1 to -0.1)* 1.0 (1.0 to 1.0) | 1.68* (-7.16 to 10.51) | | 6.9 |
| Chloride (mEq/L) | ICU (1163) | 0.977 | 0.0 (0.0 to 0.0) 1.0 (1.0 to 1.0) | 0.28* (-3.07 to 3.63) | 2.2 | 14.8 |
| | NICU (702) | 0.970 | -5.8 (-8.86 to -2.00)* 1.04 (1.0 to 1.07) | 1.50* (-2.34 to 5.34) | | 33.5 |
| | PICU (2110) | 0.972 | -2.0 (-2.0 to -2.0)* 1.0 (1.0 to 1.0) | 1.59* (-2.05 to 5.23) | | 33.6 |
| Haemoglobin (mg/dL) | ICU (1820) | 0.990 | -0.33 (-0.3 to -0.26)* 1.02 (1.02 to 1.03)* | 0.69* (-4.96 to 6.33) | 6.36 | 3.3 |
| | NICU (1836) | 0.988 | -0.46 (-0.55 to -0.36)* 1.03 (1.02 to 1.04)* | 0.61* (-8.24 to 9.46) | | 9.4 |
| | PICU (2198) | 0.941 | -0.35 (-0.43 to -0.25)* 1.03 (1.02 to 1.04)* | -0.18 (5.90 to 11.38) | | 8.7 |
| Glucose (mg/dL) | ICU (1734) | 0.987 | 3.47 (2.00 to 4.50)* 0.99 (0.98 to 1.0) | -1.48*(-15.59 to 12.63) | 5.55 | 24.3 |
| | NICU (672) | 0.984 | 4.76 (3.57 to 6.01)* 0.95 (0.93 to 0.96)* | -0.45(-18.77 to 17.87) | | 40.8 |
| | PICU (2131) | 0.984 | 1.45 (0.0 to 2.61) 0.98 (0.97 to 1.00) | 0.09 (-17.60 to 17.78) | | 31.3 |

* Statistically significant differences

Sodium showed the maximum differences between POCT and EL. NICU was the clinical setting with more discrepancies observed, followed by PICU and ICU (ANOVA; $p<0.001$).

**Conclusions**

The assessment of interchangeability in the long-term is relevant. Strict analytical performance specifications might explain the large differences in sodium. Patient type and preanalytical factors could contribute to differences between ICUs. It is important to evaluate this information with POCT settings staff in order to reinforce good practices in POCT use.

## B-308

### Implementing Total Quality Assurance in Point-of-care Testing

S. Mansouri, J. Cervera, E. Kovalchick. *Instrumentation Laboratory, Bedford, MA*

**INTRODUCTION**

Point-of-care testing (POCT) allows healthcare professionals to perform critical tests close to the patient, as opposed to sending samples to laboratory and waiting for the results. Since primary users of POCT are from non-laboratory backgrounds, POCT devices should be designed for ease of use, low maintenance and with comprehensive quality control for rapid detection and correction of errors. Suggested methodologies rely on understanding sources of error during each phase of the testing process. The objective of this study is to evaluate performance of a comprehensive Quality Management program called "Intelligent Quality Management 2" (iQM®2) to replace the use of traditional quality control.

**METHODS**

GEM® Premier 5000 analyzer (Instrumentation Laboratory, Bedford, MA, USA) performs whole-blood testing of pH, $pCO_2$, $pO_2$, $Na^+$, $K^+$, $Cl^-$, $Ca^{++}$, hematocrit, total bilirubin, total hemoglobin and fractions. The analyzer is composed of the instrument and a single, multi-use, disposable cartridge (PAK) containing all the analytical components for sample measurement and quality assurance. iQM2 is the method of control in the analyzer, based on continuous monitoring of sensor drifts by internal Process Control Solutions and analyzing response patterns during the measurement process of every sample with IntraSpect technology. Sources of error as a result of blood micro-clots, interfering substances, micro-bubbles or abnormalities in sample measurement are determined by identifying specific known patterns before, during and after every sample, followed by automatic corrective actions, as well as documentation and notification of all actions.

Data from a large number of PAKs in high-volume clinical use in several European countries were analyzed to identify errors and the outcome of corrective actions conducted by iQM2.

**RESULTS**

A total of 228,677 samples from 721 PAKs collected from 95 analyzers were queried with the following results:

**1. Microclots**

**a.** Affecting one or more electrochemical parameters in 0.3% of samples

**b.** Affecting CO-Oximetry (optical) in 0.1% of samples

**c.** Automated corrective action to remove clots was successful in > 95% of occurrences

**d.** Duration of detection, correction and verification varied from 4 to 11 minutes

**2. Interference**

**a.** Affecting electrochemical parameters in 0.1% of samples, with majority on $Ca^{++}$ and $Na^+$, identified as benzalkonium contamination

**b.** Affecting CO-Oximetry in 1.0% of samples from excess turbidity or other sources of optical interference or sample-integrity-related interference

**c.** Recovery time was immediate with no lasting effect on the analytical system

**3. Sample response**

**a.** Abnormality in sensor response pattern was detected by IntraSpect in 0.6% of samples

**b.** Transient and sample-specific error affecting one analyte was found in the majority of cases

**c.** In-house studies indicate 70% of IntraSpect-flagged analytes had error exceeding CLIA analytical quality requirements

**CONCLUSION**

Data analysis of the GEM PAKs in actual clinical use confirmed the effectiveness of iQM2 in rapid detection of transient, sample-specific errors that could affect analytical results. This is in contrast to traditional quality control (manual or automatic) where no such errors could be detected. iQM2 can effectively help hospitals improve patient care while enhancing the efficiency of testing and reducing the total cost of care.

## B-309

### Assessing the adequacy of laboratory test request from POCT clinical settings

L. Bautista Balbas, P. Oliver Sáez, P. Fernández-Calle, M. Alcaide Martín, A. Buño Soto. *Hospital La Paz, Madrid, Spain*

**Background**

When a POCT network is installed in a healthcare system, it is relevant to determine when the magnitudes should be measured as POCT or in laboratory to avoid unnecessary sample collections, measurements or duplicate information for patient management. The POCT network developed in our third-level hospital over 18 years currently includes 27 blood gas (BG) analyzers (ABL 90 Flex, Radiometer): 3 in Emergency Laboratory (EL) and 24 in different clinical settings. Since a POCT BG analyzer is installed, all BG samples should be performed as POCT.

The aim of this study was to assess the adequacy of BG, electrolytes and glucose testing request to EL from clinical settings with POCT BG analyzers.

**Material and methods**

From every clinical setting, the number of POCT BG and those requested to EL were extracted from laboratory information system during 2016.

We also evaluated the number of glucose and electrolytes (sodium, potassium and chloride) performed in Dimension Vista (Siemens Heathineers) after a request to EL, having had results of these magnitudes in the POCT analyzers within the previous 45 minutes, considering all as the same sample collection.

**Results**

62,996 BG were performed in EL during 2016. POCT BG was 95,535 (60%).

| POCT Setting | POCT BG (n) | BG request to EL n (ratio EL BG/ POCT BG) | Simultaneous EL-POCT requests Glucose n (% of total POCT BG) | Simultaneous EL-POCT requests Electrolytes n (% of Unit EL electrolytes requests) |
|---|---|---|---|---|
| Adults Intensive Care Unit | 11,522 | 71 (0.006) | 2,109 (18) | 1,742 (15) |
| Burn unit | 5,426 | 99 (0.018) | 881 (16) | 760 (14) |
| Coronary Care Unit | 3,662 | 47 (0.013) | 256 (7) | 205 (6) |
| Post-Anesthesia Care Unit - I | 8,254 | 55 (0.007) | 2,036 (25) | 2,021 (24) |
| Post-Anesthesia Care Unit - II | 14,158 | 52 (0.003) | 4,498 (32) | 4,021 (28) |
| Medical Surgical Unit - I | 1,903 | 0 (0.000) | 1 (0) | 2 (0) |
| Medical Surgical Unit - II | 2,273 | 0 (0.000) | 3 (0) | 3 (0) |
| Medical Surgical Unit - III | 567 | 5 (0.009) | 0 (0) | 3 (1) |
| Emergency Department | 5,873 | 37,336 (6.360)* | 2,393 (41) | 2,301 (39) |
| Pediatric Post-Anesthesia Care Unit | 7,990 | 41 (0.005) | 1,255 (16) | 1,241 (15) |
| Pediatric Intensive Care Unit | 10,632 | 109 (0.010) | 2,097 (20) | 2,169 (20) |
| Neonatal Intensive Care Unit | 15,405 | 18 (0.001) | 601 (4) | 598 (4) |
| Delivery Room | 7,870 | 1 (0.000) | 4 (0) | 4 (0) |
| TOTAL | 95,535 | 37,834 (0.396) | 16,134 (17) | 15,067 (16) |

*The elevated number of BG request to EL was related to the gradual installation of the POCT through this year. Without this data, the total ratio would be 0.006.

**Conclusions**

After a POCT is installed, BG is rarely requested to EL. However, some magnitudes are still requested to laboratory often within an ampler request. Surgical Units and Delivery Room showed the lowest duplicate request, possibly due to the immediate clinical decision making in these areas. The locations with more duplicates were Post-Anesthesia Care Units and Pediatric ICU. These findings are relevant in order to work with POCT settings to improve rational use.

## B-310

### A highly sensitive Point of Care Test for GFAP - a brain biomarker in serum

R. Venkatnarayanan, P. Hansia, C. Ramesha, S. Gunupudi. *Dx-Sys, Inc., Mountain View, CA*

**Background:** Glial Fibrillary Acidic Protein (GFAP) is brain specific protein with a molecular weight of about 50 kDa which is released into blood during traumatic brain injury (TBI) and Stroke. A breakdown product of GFAP (38 kDa, GFAP-BDP) has also been detected in patients with TBI. Several ELISA kits are commercially available to quantitate GFAP in serum and most of them have very low sensitivity and require long incubation times. A highly sensitive and quantitative assay which could be used as a point-of-care diagnostic test for GFAP as a biomarker for TBI is not available.

**Methods:** Here we report the development of a sensitive, quantitative and highly specific lateral flow assay for measuring GFAP in human serum. The assay utilizes a high affinity monoclonal antibody against human GFAP for capture and an europium (Eu, III) labeled polystyrene nanoparticles conjugated to the $F(ab')_2$ fragment of a high affinity second monoclonal antibody against human GFAP for detection. The test strips are assembled in plastic cassettes and the assay requires 100 µL of serum sample. Calibration standards containing 0 pg/ml to 250 pg/ml of GFAP and GFAP-BDP were prepared in control human serum. One hundred microliters of the neat serum alone (0 pg/ml) and human serum containing indicated concentrations of GFAP or GFAP- BDP were applied to sample port of each cassette, followed by 50 µL of a chase buffer and allowed to stand at room temperature for 30 - 60 min. Measuring the fluorescence of Eu signal on the lateral flow membrane was performed using a Lateral Flow Fluorescence reader (Qiagen, ESEQuant LFR) and a Lateral Flow Time Resolved Fluorescence reader (Dx-Sys).

**Results:** The background fluorescence from the matrix and associated material (membrane and plastic) were greatly diminished in the time-resolved mode which significantly boosted the signal window as well as detection limit to below pg/ml concentration of GFAP. The assay generates a linear calibration curve from 0 pg/ml to 125 pg/ml. The assay performs equally well with the break-down product of GFAP (GFAP-BDP) as it is with intact GFAP. The sensitivity of the assay was about 15 fold higher when the signal was measured in the Time Resolved Fluorescence reader compared to the fluorescence reader from Qiagen. The assay is highly reproducible (inter assay cassette CV < 12%), sensitive with an LOQ of 0.125 pg/mL, and fast (sample application to detection <45 min).

**Conclusion:** A Point-of-Care, lateral flow immunodiagnostic test using Europium labeled particles and quantitation by a Time Resolved Fluorescence reader gives a very high sensitivity to measure brain biomarker - GFAP at sub-pg levels in human serum in less than 45 minutes. This rapid quantitative assay can be easily adapted to detect several other biomarkers which require high sensitivity.

## B-311

### Europium Based, Quantitative, Point of Care Immunoassay, Fluoro-Check for Measurement of Procalcitonin Has a High Correlation with the BRAHMS Procalcitonin

S. Chae, J. Chung, H. Huh. *Dongguk Univ Medical Center, Goyang, Korea, Republic of*

INTRODUCTION: Procalcitonin (PCT) is a biomarker of bacterial infections and is increasingly used for early risk stratification with suspected sepsis and pneumonia. Point-of-care (POC) test is performed for early decision making, and POC for PCT is useful to diagnose bacterial infection. However, most POC devices for PCT measurement take semi-quantitative methods, and have limitations in monitoring PCT levels for evaluating the response to antibiotics. The Fluoro-Check™ PCT (Nano-Ditech Corp, NJ) is a Europium based immunoassay for the quantitative determination of PCT. In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, within-laboratory precision of Fluoro-Check PCT is less than 15% coefficient of variation at a low, mid-, and high level. We compared Fluoro-Check PCT with Elecsys BRAHMS PCT (Roche Diagnostics, Mannheim, Germany) using clinical specimens. METHODS: The study was approved by the Institutional Review Board of Dongguk University Ilsan Hospital (2016-019) and 125 specimens, whose PCT results by Elecsys BRAHMS PCT ranged from 0.03 to 16.44 ng/mL, were included. After adding 10 uL of serum into sample well, 2 drops of developer solution were applied onto the developer well immediately. The PCT molecules in the sample bond to both biotinylated and Europium particle coupled with anti-PCT antibody at the end of the membrane. Test kits were inserted into Fluoro-Checker TRF reader, and then PCT results were derived by analysis of fluorescence intensity, proportional to the concentration of PCT. Data from the Fluoro-Check PCT were compared with the results using cobas E601 analyzer (Roche Diagnostics) according to CLSI EP 09-A3. RESULTS: The measurable range of Fluoro-Check PCT values was 0.08 - 18.08 ng/mL and distribution was as follows : 21 samples with < 0.5 ng/mL, 33 samples with 0.5-2.0 ng/mL, 56 samples with 2.1 -10.0 ng/mL, and 15 samples with >10.0 ng/mL. Passing-Bablock regression analysis of Fluoro-Check PCT and Elecsys BRAHMS PCT showed a high correlation. Correlation coefficient was 0.921 (95% confidence interval [CI], 0.889 ~ 0.944), slope was 1.236 (95% CI, 1.181 ~ 1.299), and intercept was -0.046 (95% CI, -0.1339 ~ -0.008). CONCLUSION: Fluoro-Check, the europium-based fluorescence immunochromatographic assay, has the advantages of POC. Fluoro-Check PCT showed a high correlation with Elecsys BRAHMS PCT at the measurable range of 0.08-18.08 ng/mL. Quantitative analysis of PCT using Fluoro-Check appears useful for clinical applications to diagnose and monitor patients with bacterial infection.

## B-312

### Optimization of Lactate Measurements for Sepsis Guidelines Using Point of Care Testing

L. M. Evans[1], J. M. Stout[1], B. M. Goldsmith[2]. *[1]Thomas Jefferson University Hospital, Philadelphia, PA, [2]Thomas Jefferson University, Philadelphia, PA*

**Background:** Sepsis is a life-threatening illness caused by an unregulated host response to infection that can lead to septic shock and death. In October 2016, CMS introduced a new core measure based on the Surviving Sepsis Campaign International Guidelines for Management of Sepsis and Septic Shock. This measure requires both a baseline and a 6-hour lactate measurement. Lactate is a marker for cellular hypoxia, and is associated with increased mortality in sepsis. Our hospital, similar to many others, requests baseline and 4-hour measurements to ensure lactate is obtained within 6 hours. The objectives in this study were to evaluate adherence to the hospital's protocol and to evaluate whether Point of Care (POC) lactates improve turnaround time (TAT).

**Methods:** Elevated lactate is defined as >2.0 mmol/L. We evaluated 200 pairs of lactate measurements where the baseline was >2.0 mmol/L and the second specimen was reported at 3-3.5, 3.5-4.5, and up to 6 hours. Lactate was measured by: 1) Epoc Blood Analysis System (Alere, Orlando, FL; whole blood, POC); 2) RapidLab 800 (Radiometer, Brea, CA; whole blood, sent to laboratory); or 3) Cobas 6000 (Roche, Indianapolis, IN; serum, sent to laboratory). Lactates collected <3h or >6h were excluded.

**Results:** 15,701 data points were evaluated from October 2016-January 2017. 200 pairs met the above criteria (Table 1). 54 pairs did not meet the hospital's guideline of lactates reported within 4 hours. Of the 54 pairs, 22 pairs were sent from units where POC lactate was available but not used, indicated by asterisk on the table.

**Conclusion:** Paired lactates performed non-POC fell outside of guidelines (>4.5hr). POC whole blood lactate provides immediate results that could assist in adhering to hospital sepsis guidelines and should be utilized where available.

Table 1

| | ICU POC Lactate (whole blood) | ICU Lactates sent to lab (whole blood and serum) | ED Lactates sent to lab (whole blood and serum) | Other Locations Lactate sent to lab (whole blood and serum) |
|---|---|---|---|---|
| Average Δ Time, Paired results 3-3.5 (h) | 3.14 (n=4) | 3.17 (n=18) | 3.25 (n=3) | 3.27 (n=25) |
| Average Δ Time, Paired results 3.5-4.5 (h) | 3.79 (n=4) | 4.05 (n=29) | 4.00 (n=8) | 3.94 (n=55) |
| Average Δ Time, Paired results 4.5-6.0 (h) | n/a | *4.80 (n=17) | *4.87 (n=5) | 4.89 (n=32) |
| Difference in collection to result time for Non POC lactate (h) | n/a | 0.987 | 0.910 | 0.983 |

## B-313

### Analytical Evaluation of Blood Gas Syringes for Pneumatic Tube Systems

B. Collins, M. Pistorino. *Instrumentation Laboratory, Bedford, MA*

**Background:** There are many options and considerations when choosing arterial blood gas (ABG) syringe to use. It is important to be able to fill the syringe with minimal air contamination, properly mix the sample to suspend cells, as well as the choice of heparin type and concentration. There are many commercially available devices, with different designs to optimize syringe fill, while preventing pre-analytical error. Based on review of data from customer sites, choice of ABG syringe when used in conjunction with a pneumatic tube system (PTS) must be considered.

**Methods:** Several types and brands of ABG syringes were evaluated using the GEM® Premier™ 4000 at Instrumentation Laboratory (Bedford, MA). Each syringe was evaluated by simulated user use to evaluate the impact of the device on blood values after simulated PTS transport. Areas of focus were: venting mechanism (cap, plunger, or both), effectiveness at purging excess air, and syringe volume on blood gas and hematology results. To simulate PTS transport, blood was tonometered to ~30 mmHg $pO_2$, drawn into the syringe, sampled on the GEM Premier 4000, then a slight vacuum was introduced, shaken, and sampled again. Results show that in a negative pressure state with agitation any air contamination through the venting mechanism, or trapped air, causes micro bubbles and a resulting change in patient results. Some venting mechanism combinations are more resistant to the simulated effects of PTS transport.

**Conclusion:** Overall, data demonstrated that vented syringes are best for preventing pre-analytical error during sample draw, where non-vented syringe components are preferred for PTS transportation. Preliminary results are shown in Table 1 for each of the six tested syringes. Each syringe type was tested 5 times. Results are expressed in the fraction averages.

Table 1: Syringe Name and Change to Hemoglobin Fractions

| Syringe | $O_2Hb$ | | | HHb | | | COHb | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Change | Pre | Post | Change | Pre | Post | Change |
| Pro-Vent® - Smiths Medical | 37.7% | 36.3% | -1.4% | 60.8% | 61.8% | 1.0% | 1.1% | 1.4% | 0.3% |
| A-Line® - Westmed | 36.9% | 36.6% | -0.3% | 61.2% | 60.8% | -0.4% | 1.5% | 0.8% | -0.7% |
| Preset™ - BD | 42.7% | 43.0% | 0.3% | 55.1% | 54.1% | -0.9% | 1.5% | 1.0% | -0.4% |
| SafePICO® - Radiometer | 45.5% | 46.4% | 0.8% | 52.1% | 50.1% | -2.0% | 1.7% | 1.1% | -0.6% |
| Pulsator – Smiths Medical | 39.0% | 47.9% | 8.9% | 59.3% | 49.5% | -9.8% | 1.3% | 1.2% | -0.1% |
| Pulset™ - Westmed | 44.0% | 60.2% | 16.2% | 53.8% | 36.2% | -17.6% | 1.6% | 1.7% | 0.1% |

A complete study of venting technique and impact to patient results is ongoing

## B-314

### Comparison of creatinine on the Alere epoc Blood Analysis System against multiple point-of-care and central laboratory assays

A. K. Tsui, G. Cembrowski, A. K. Füzéry. *University of Alberta, Edmonton, AB, Canada*

**Background:** The epoc Blood Analysis System from Alere is a point-of-care (POC) instrument that measures blood gases, electrolytes, and metabolites. In Edmonton, the epoc has been used by emergency medical services since 2015 for patient assessments in continuing care facilities. Recently, Alere added creatinine and chloride to the already existing panel of 9 measured analytes. We evaluated the performance of creatinine and compared it against other assays, including central laboratory and POC, that are used in our health region.

**Methods:** Ethics approval for this study was obtained from our institution's ethics review board. Following informed consent, three tubes of lithium heparin blood were collected from each of 60 healthy volunteers. Creatinine was measured on two POC instruments (Alere epoc, Abbott i-STAT), and two central laboratory analyzers (Beckman Coulter DxC 800, Ortho Clinical Vitros 350). Raw creatinine results, as well as corresponding eGFR values, were compared between the epoc and the other instruments to ascertain any clinically significant differences. eGFR values were calculated using the CKD-EPI formula. Six additional patient samples with abnormally high creatinine values were also analyzed on the epoc, DxC 800 and Vitros 350 and were compared in a similar manner. Within-laboratory precision was assessed for all platforms by assaying two levels of quality control three times daily for 5 days.

**Results:** For healthy volunteers, raw creatinine values from the epoc showed moderate agreement with other methods (R = 0.783 to 0.886). Calculated eGFR values demonstrated 90-100% concordance between the epoc and other methods for all age groups and genders except for females of age 80 years or older. For this latter group, the concordances were 68% (vs DxC 800), 75% (vs Vitros 350) and 82% (vs i-STAT), and mean biases were -7% (vs DxC 800), -4% (vs Vitros 350), -1% (vs iSTAT). Other analyzers demonstrated excellent concordance with each other (90-92%) for this subset of patients. For patients with abnormally high creatinine values, the epoc showed excellent correlation with other analyzers (R = 0.997 to 1.000). Concordance between calculated eGFR values was also excellent (100%). Quality control samples demonstrated coefficients of variation (CV) of 4.3% at a mean creatinine concentration of 57 mmol/L and 11.8% at mean creatinine concentration of 154 mmol/L.

**Conclusion:** The analytical performance of the epoc creatinine assay appears comparable to the assays on the i-STAT, DxC 800 and Vitros 350 platforms. The negative biases shown by the epoc against other analyzers were not clinically significant as they did not exceed total allowable error limits of 26.52 mmol/L or ±15% (CLIA'88) for creatinine. Despite lower concordance between the epoc and other methods for females of 80 years or older, differences in results were still within total allowable error limits. As such, the epoc appears to be a suitable POC alternative or complement to any of the other platforms examined in this study.

## B-315

### ASSESSMENT OF IONIZED CALCIUM AS ANALYZED ON POCT BLOOD GAS SYSTEM: GEM PREMIER 4000 (WERFEN GROUP).

M. Mayor Reyes, C. Cañavate-Solano, J. Santotoribio, J. Cuadros-Muñoz, S. Perez-Ramos. *Hospital Universitario Puerto Real, Cadiz, Spain*

**Background:** Total calcium is part of the basic metabolic panel and is most often used as the primary indicator of calcium status . However, the CaIO (free or ionized calcium) concentration is considered to be a more accurate indicator of calcium status. The gases is a test of laboratory needed for the assessment of the balance acid-base and the study of the oxygenation of the patient. You can carry out in the clinical laboratory, but also in the place of assistance to the patient or Point of Care Testing (POCT). This study assessed the correlation between ionized calcium with in two blood gas Systems. One of cartridges used usually as POCT, GEM PREMIER 4000 (WERFEN GROUP), and other of reference of laboratory clinical, ABL 800 FLEX (RADIOMETER). **Methods:** Included 78 patients with application of study of gases in shows blood venous or arterial. The sample obtained in syringe of gases heparinized was analyzed by potentiometric sensors in two instruments: 1. Blood gas system Y: GEM PREMIER 4000. 2. Blood gas system X: ABL 800 FLEX. The same shows be process first in one and immediately after in the other. study statistical of comparison of methods through analysis of correlation, regression of Passing and Bablok and the test of Bland and Altman, using the program software MEDCALC. **Results:** The medians of ionized calcium from instruments: Y= 1,12 X=1,13; . The following table shows the results of the methods comparison:

| | **Rho de *Spearman*** | ***Passing and Bablok*** | ***Bland and Altman*** |
|---|---|---|---|
| **Calcio iónico** | 0,940 (p<0,0001) | Y=0,1083+1,0833 X | Mean difference of X and Y = 0,01 |

Intercept A = -0,1083; 95% CI: -0,2186 to 0,01000 Slope B= 1,0833; 95% CI: 1,0000 to 1,1818 Analysis of method comparison data revealed good correlation of the ABL 800 FLEX.to the GEM PREMIER 4000. **Conclusion:** The blood gas system GEM PREMIER 4000 and ABL 800 FLEX have a direct linear correlation of high intensity for the determination of ionized calcium, so that both methods can be interchangeable.

## B-316

### ASSESSMENT OF GLUCOSE ON A BLOOD GAS SYSTEM: GEM PREMIER 4000 (WERFEN GROUP).

M. Mayor Reyes, C. Cañavate-Solano, J. Santotoribio, J. Cuadros-Muñoz, S. Perez-Ramos. *Hospital Universitario Puerto Real, Cadiz, Spain*

**Background:** Glucose measurements are used in the diagnosis and treatment of disorders of carbohydrates metabolism such as diabetes melllitus, neonatal hypoglycemia and insulinoma. The blood gas system is a test of laboratory needed for the assessment of the balance acid-base and for the study of the oxygenation of the patient. You can carry out in the clinical laboratory, but also in the place of assistance to the patient o Point of Care Testing (POCT). The aim of this study was to compare the glucose analyzed with a blood gas system, of cartridges used usually as POCT by potentiometry enzyme (glucose oxidase), GEM PREMIER 4000 (WERFEN GROUP), with the glucose analyzed in a Dimension EXL with LM by the method of the hexokinase of Kunst and cols., method of reference. **Methods:** 110 residual samples with results of glucose using Dimension EXL with LM analyzer and blood gas system, were retrospectively selected using data were extracted from the database of the laboratory computer systems. 1 Instrument Y: Blood gas system: GEM PREMIER 4000 2. Instrument x Dimension EXL with LM The same shows is process first in the blood gases system and of form parallel is made the glucose in the Dimension with the tube of biochemistry. Statistical study of comparison of methods using correlation, Passing and Bablok regression and the test of Bland and Altman analysis, was performed using the MEDCALC. **Results:** The glucose medium were: Y= 120 y X= 121. In the following table is show them results obtained from the comparison of methods:

| | **Rho de *Spearman*** | ***Passing and Bablok*** | ***Bland and Altman*** |
|---|---|---|---|
| **Glucose** | 0,9858 (p<0,0001) 95% CI (0,9756 to 0,9917) | Y=-2,000 +1,000 X Intercept A 95% CI: (-7,400 to 3,1387) Slope B 95% CI: (0,9595 to 1,0400) | Mean difference =4,6 |

**Conclusion:** The slope B pending includes to the unit, by what not exists error proportional between both instruments, it ranked in the origin is positive with an interval of confidence that includes to the zero, which excludes an error constant. Therefore, the value of glucose is interchangeable in both methods.

## B-317

### Design and Testing of a Novel Point-of-Care (POC) Device to Convert Whole Blood to Serum at the Bedside for Medical Diagnostics

M. Elias. *University of North Carolina at Greensboro, Greensboro, NC*

Whole blood is used to measure disease-diagnosing biomarkers for a wide range of molecules. Whole blood contains components that can make the measurement of these biomarkers inaccurate, leading to misdiagnosis and increased patient mortality and morbidity. While whole blood is quick and convenient for analyte detection using point-of-care (POC) devices, serum is preferred for most accurate biomarker detection. However, obtaining serum from whole blood requires extensive processing which is not feasible for POC devices and results in delays in diagnosis. What is needed is a method that can quickly coagulate whole blood so that serum can be extracted in sufficient quantities and purities that improve upon whole blood analyte detection. The objective of this study was to design an affordable system that quickly (<5 minutes) and more effectively (resulting in pure serum) converts whole Blood to Serum (B2S System). Several pro-coagulants were analyzed for their ability to induce coagulation without red blood cell (RBC) lysis. It is shown that snake venom in combination with silicon dioxide and protamine sulfate was optimal for coagulation time, firm clot formation, and serum clarity.

Methodology. Nanomaterials (NMs) were tested in the coagulation formula with snake venom, human thrombin, and protamine sulfate. Various conditions were suspended in 1 mL of whole blood and received 10mM $CaCl_2$ to counter the anti-coagulant properties of the citrated tubes used for brief blood storage. 1mL of blood was added to each condition and the blood coagulation time was measured as described below. After coagulation tubes were centrifuged at x 2000g for 10 minutes resulting in two distinct layers (serum and thrombus). Serum was removed and analyzed for RBC lyses as described below.

To quantify coagulation time standard paperclips were sanded to remove buffed layer and provide more surface area to catch the initial fibrin strand. The time at which the initial fibrin strand adhered to the paperclip was recorded as the initial coagulation time.

To investigate RBC hemolysis, serum clarity was assessed using increased absorbance of produced serum. Serum was pipetted off and seeded into a 384-well plate to measure serum purity via absorbance with a spectrophotometer. The addition of NMs facilitates the extraction of purer serum. Comparisons between conditions regarding serum purity and coagulation time using student t-tests have be done resulting in p values < .001.

In conclusion, this coagulation formula was lyophilized within the B2S prototype. The B2S system was able to draw up blood, coagulate the blood, and extrude the serum onto a strip in a time frame suitable for POC devices. Thus, the B2S system represents a novel POC device that could be adapted by existing technologies in clinical settings and improve diagnostic and therapeutic efforts for patients requiring fast and accurate blood biomarker determinations. This will improve patient care, reduce healthcare costs, and bring serum to the bedside for rapid analyte detection.

## B-318

### Giant Magnetoresistive Based Handheld System for Rapid Detection of Human D-dimer and C-reactive Protein

W. Wang[1], T. Klein[2], J. Collins[1]. *[1]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN, [2]Zepto life technology, St. Paul, MN*

**Background:** Many epidemiologic studies have indicated that D-dimer and C-reactive protein (CRP) can be used as reliable biomarkers of most cardiovascular diseases, and some *in vitro* diagnostic kits including the two biomarkers for assessing cardiovascular risk have been successfully commercialized. Biochip-based assay for biomarkers detection using giant magnetoresistive (GMR) sensors and magnetic nanoparticles (MNPs) have been developed by different research groups. However, no portable and handheld GMR biosensor system has been reported yet. In this study, a novel handheld GMR detection system with integrated microfluidics was used to detect human D-dimer and CRP, which revealed advantages of high sensitivity and specificity, multiplexity, and real-time signal readout. The developed assays have great potential for the final development of simple, rapid, automatic and cost-effective point-of-care testing (POCT).

**Methods:** The immunoassay process is set up based on sandwich-type format. D-dimer and CRP capture antibodies (Abs) were printed and immobilized on different sensors on one GMR chip with functional surface. Integrated with microfluidic system, the chip was assembled with plastic substrate and valves to form a test cartridge. After the cartridge was connected with the handheld detection analyzer, TBST buffer (Tris-buffered saline, 0.05% Tween 20) was pumped onto sensor surfaces to wash off unbound Abs. Then sample prepared by mixing D-dimer and CRP analytes to desired concentrations in PBST (1× PBS, 0.05% Tween 20) was loaded into sample entry well which was prefilled with biotin labeled D-dimer and CRP detection Abs. Capture Ab-analyte-detection Ab (biotin) sandwich complex was formed on sensor surface as sample solution flowed along microfluidic channel. At last streptavidin labeled MNPs (SA-MNPs) were introduced and bound onto sensor surfaces via the interaction between SA and biotin. Binding of SA-MNPs to sensor surface can be real-time recorded by the handheld analyzer. Higher detection signal reflected more MNPs binding on sensor surface.

**Results:** *In vitro* multiplex detection of human D-dimer and CRP using a new handheld GMR biosensor platform was well established. The assay can be completed within 20 min, which is much shorter than conventional and widely used enzyme-linked immunosorbent assays (ELISA). The novel assay provides linear analytical ranges of 0-5000 ng/mL for D-dimer and 0-500 ng/mL for CRP, and their detection limits are 8.42 ng/mL and 0.72 ng/mL respectively. D-dimer and CRP with varied concentrations were spiked into human plasma, and recoveries of 85-115% are observed for the two analytes. It is also shown that the assay is not interfered with hemoglobin, fibrinogen, human anti-mouse antibody and rheumatoid factor.

**Conclusion:** The developed technology platform for GMR based immunoassay is able to sensitively and specifically detect human D-dimer and CRP. Not only the assay time has been shorten, but also simple and automatic assay operation has been accomplished. Hence, we believe it can be further integrated and developed for POCT diagnostics.

## B-319

### A Novel One-Step One Blood Drop Beta-hCG Rapid Assay

M. Tsang[1], W. Wang[2], B. Zha[1], Q. Shi[1]. *[1]ZBx Corporation, Toronro, ON, Canada, [2]Now Diagnostics, Inc., Springdale, AR*

**Background:** Human chorionic gonadotropin (hCG) is a common hormone used to detect pregnancy. hCG is a glycoprotein heterodimer hormone composed of an α- and β-subunit. The α-subunit is common to luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH), while the β-subunit is unique to hCG. hCG is synthesized by trophoblastic tissue of the placenta in pregnancy making the hCG β-subunit a good predictor of pregnancy. Early detection of pregnancy is important for caregivers to avoid certain medications and procedures that could harm a fetus. Unfortunately, clinics across the country are experiencing too many false-negative results from point-of-care urine-based tests. In a March 2015 report in Clinical Chemistry, Robert N. Nerenz and Ann M. Gronowski detailed their evaluation of 11 common POC hCG urine-based devices and determined that 9 of the 11 were susceptible to false-negative results. Urine-based hCG tests are not as sensitive as blood-based tests leading to false-negative results in early pregnancy. The objective of this study is to evaluate the performance of a new blood-based hCG rapid test. **Principle:** The ADEXUSDx hCG Rapid Test ("hCG Test") was developed using a direct sampling immunoassay technology for whole blood, plasma, serum, or capillary blood. The test requires a 35ul sample. This could be from a capillary stick or from a venous blood, plasma, or serum sample. The hCG test uses a sandwich format to detect the presence of hCG above an established reference concentration. The appearance of a purplish-red band in the test window indicates that the sample contains hCG above 10 mIU/ml. The hCG test is unique in that the sample moves directly from the patient into the device without any sample preparation, buffer application, or complex machinery required. **Performance:** A one step rapid detection of β-hCG test requires only 35µl of sample with no additional buffer. Test results can be read at 10 minutes. Using WHO hCG standard added to whole blood, the C5 concentration (5% of samples positive) was confirmed as 1.25 mIU/ml hCG and the C95 concentration (95% of samples positive) as 4.5 mIU/ml hCG, which are equivalent to approximately 2.3 mIU/ml hCG and 8.2 mIU/ml hCG, respectively, in plasma. There was no cross reactivity to the α-subunit portion of hCG as demonstrated using three other hormones. Further testing demonstrated no high-dose effect at hCG levels up to 150,000 mIU/ml. A clinical study was conducted using 84 samples above the cutoff and 476 samples below the cutoff. Results were comparable to the predicate device, Unicel® DxI 600® Access® Clinical System, with 97.6% sensitivity, 99.8% specificity, 98.8% PPV, and 99.6% NPV. **Conclusion:** The ADEXUSDx hCG Test is a true one-step rapid test with demonstrated specificity to β-hCG. It has a cut-off at 10 mIU/mL making the ADEXUSDx hCG Rapid Test a useful test for the early detection of pregnancy.

## B-320

### A novel one-step one drop of blood sampling HSV-2 antibody rapid test

J. K. Babra[1], L. Wang[2], M. Fortune[2], V. Thompson[2], Q. Shi[1]. *[1]ZBX Corporation, Toronto, ON, Canada, [2]Now Diagnostics Inc., Springdale, AR*

**Background:** HSV-2 infection is widespread throughout the world and is almost exclusively sexually transmitted, causing genital herpes. Genital herpes can also be caused by herpes simplex virus type 1 (HSV-1). Both HSV-1 (cold sores) and HSV-2 (genital herpes) viruses infect epithelial cells and are ubiquitous and contagious. To some extent genital herpes is attributable to HSV-1 which is not as clinically severe whereas, HSV-2 infection increases the risk of acquiring human immunodeficiency virus (HIV) infection by approximately three-fold. Neonatal herpes can also occur when an infant is exposed to HSV in the genital tract during delivery. The screening of all pregnant women and their sexual partners for HSV-2 antibodies would help to prevent primary maternal infections and neonatal infection. It would also help to identify all women at risk for recurrent HSV-2 infection as well as asymptomatic HSV-2 seropositive mothers. Immunological or molecular based tests are available to determine HSV-2 infection and are complicated to perform. A rapid one-step HSV-2 Antibody Test format is developed which detects only HSV-2 specific antibodies. The objective of the test study is to demonstrate the performance of the new one-step test.

**Principle:** Adexus-Dx HSV-2 Antibody test was developed using a direct sampling immunoassay technology for whole blood, plasma serum or capillary blood. Purified HSV-2 recombinant antigen has been employed for the detection of HSV-2 antibodies. 35 µl of serum/ plasma/venous blood or one drop of blood directly from the fingertip with no addition of extra buffer is required for the test. When sufficient sample volume fills the Receiving Channel, the sample flows into a dry porous test strip composed of a membrane array with colloidal gold conjugated HSV-2 specific antigen. The appearance of visible pinkish-red band at the test window region indicates that the individual sample contains HSV-2 antibody.

**Performance:** A one step rapid detection of HSV-2 antibody test only requires 35 µl of sample with no additional buffer. Test results can be read at 15 minutes. Testing with WHO anti-HSV-2 and anti-HSV-1 quality control serum samples confirmed that HSV-2 antibodies were recognized by the test. A clinical sample study was conducted using 102 HSV-2 positive and 109 negative samples. Results were comparable with the EIA based method for both positive and negative clinical samples. There was no cross reactivity with 30 HSV-1 positive clinical samples. Further testing of characterized 21 member anti-Herpes mixed titer performance panel showed 100% agreement with the Focus Herpes Select 2 IgG ELISA.

**Conclusion:** The ADEXUS-Dx HSV-2 Antibody Test is a true one-step rapid test with excellent sensitivity as well as specificity for the detection of HSV-2. It is the simplest and most suitable test to use in the detection of HSV-2 infection at the point of care or for self-testing.

## B-321

### A Comparison of Four Methods of Urine Sediment Analysis

A. T. Schaeffer, M. Ban, R. Chan. *Quantimetrix Corporation, Redondo Beach, CA*

**Background:** Urine sediment analysis is an important diagnostic tool for of a number of diseases including glomerular disease, tumors of the urinary tract, upper and lower urinary tract infections, and passage of renal stones. Laboratories have a wide variety of choices as to how to analyze patient urine sediment, including both manual and automated methods. While many factors must be considered when a laboratory determines which sediment analysis method to adopt, method precision must be considered. This study was designed to compare the % coefficient of variance for three manual and one automated urine sediment analysis methods

**Methods:** Three human urine pools were created from multiple donors. The pools were preserved using antibiotics to prevent microbial growth. Each of the urine pools were split into two portions. The portions were then spiked with preserved human cells at an abnormal (leukocytes 30±10 cells/µL, erythrocytes 60±10 cells/µL) or normal (leukocytes 5±3 cells/µL, erythrocytes 8±5 cells/µL) concentration. The cell concentration for each of the pools was determined by three manual methods, Kova Glasstic® Slide 10 with grids, Kova Glasstic® Slide 10 without grids, and the CenSlide®2000 urinalysis system. The same pools were analyzed using an IRIS Diagnostics iQ®200 automated sediment analyzer. All four methods were performed as per manufacturer's recommendations.

**Results:** Analysis by Student T-Test (Paired, 2 tailed) showed that the iQ200 had a significantly lower %CV than the Kova without grids and CenSlide methods for both

leukocytes and erythrocytes. The iQ200 was significantly better than the Kova with grids method for quantitation of the leukocytes but not the erythrocytes. The Kova with grids method was significantly better than the Kova without grids and CenSlide methods for erythrocytes.

**Conclusion:** In general the automated iQ200 device offers superior precision when compared with manual methods. The Kova with grids method offers better precision when compared with other manual methods**.**

| Mean % Coefficient of Variance for the Analytical Methods | | | | |
|---|---|---|---|---|
| Method | Normal Pool RBC | Normal Pool WBC | Abnormal Pool RBC | Abnormal Pool WBC |
| Kova with grids | 39.9 | 55.3 | 23.5 | 38.6 |
| Kova without grids | 102.4 | 118.7 | 44.3 | 53.6 |
| CenSlide | 116.5 | 117.0 | 58.5 | 65.7 |
| Iris | 29.1 | 24.2 | 15.5 | 15.7 |

## B-322

### Development of a Multiplexed Immunochromatographic Assay for Detection of Acute Bacterial Sinusitis

O. Hawkins[1], O. Okiya[1], J. Shirajian[2], J. Skraba[1]. *[1]Entvantage Diagnostics, Fort Worth, TX, [2]Kestrel Biosciences, Carlsbad, CA*

**Background:** Annually, 29 million people are diagnosed with sinusitis in the U.S. and 80% receive a prescription for antibiotics. Although less than 10% of sinusitis cases are caused by bacteria, sinusitis accounts for more adult antibiotic prescriptions than any other outpatient diagnosis. Currently, there are no products available to diagnose bacterial sinusitis at the Point of Care (POC). With the looming threat of antibiotic resistance and 1 in 1000 patients suffering serious, sometimes life-threatening adverse effects due to antibiotics, physicians need better tools to diagnose and inform the clinical management of patients with sinusitis. Entvantage Diagnostics is developing the first multiplexed, Point of Care (POC) test for rapid diagnosis of bacterial sinusitis in the primary care and urgent care setting where 90% of sinusitis patients are seen. **Methods:** Mouse monoclonal antibodies were generated or licensed for each of three pathogens responsible for >90% of bacterial sinusitis; *non-typeable H. influenzae* (NTHI), *M. catarrhalis*, and *S. pneumoniae*. Antibody pairs were initially selected for the final assay prototype based on inclusivity, analytical sensitivity, analytical specificity, and interference by enzyme-linked immunosorbent assay (ELISA). Prior to licensing from the Respiratory Diseases Branch of the CDC, Anti-*S. pneumoniae* antibodies had screened positive for recognition of 90 serotypes and demonstrated minimal crossreactivity with 22 genera and 29 species of respiratory pathogens and commensals. Inclusivity: Bacterial lysates were generated using a hyperosmotic lysis buffer containing an anionic detergent, a chaotrope, and protease inhibitor and utilized as antigen by ELISA. Anti-*H. influenzae* OMP-P5 specific antibodies were selected for positive recognition of two reference strains, 5 clinical isolates, and an OMP-P5 knockout. Anti-*M. catarrhalis* CD antibodies were selected for recognition of a reference strain. Analytical Sensitivity: The target limit of detection for the final assay was $1x10^4$ CFU/ml. Antigen was diluted from $1x10^7$- $1x10^2$ CFU/ml and interrogated with the antibody pairs. Antibody pairs that met this specification were selected for additional testing. Analytical Specificity: Down-selected antibody pairs were further screened for reactivity to lysates of 12 bacterial species commonly found in the nasal cavity, including *S. aureus* and *S. epidermidis*. Those demonstrating no crossreactivity were selected for prototyping of individual lateral flow-based immunochromatographic assays. **Results:** These prototyped assays were tested in an IRB approved pilot study composed of 7 symptomatic patients, 15 healthy participants, and 22 contrived samples. Bacterial culture of endoscopically guided swab of the Middle Meatus served as the comparator method. Sensitivity for *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* were 94 %, 87.5% and 90%, respectively. Specificity for *M. catarrhalis H. influenzae*, and *S. pneumoniae* were 92%, Not determined due to no true positives, and 100%, respectively. **Conclusion:** The results of this study support full validation and clinical assessment of this product. This rapid diagnostic will improve patient care by equipping health care providers with a means to quickly and accurately diagnose patients with sinusitis who otherwise would be treated empirically.We acknowledge the contribution of the Respiratory Diseases Branch, Division of Bacterial Diseases, CDC.

## B-323

### Microfluidic-based Point-of-Care Immunoassay for Quantitative Determination in Capillary Whole Blood

A. Srinivasan, Y. Adhikari, B. Cahill, A. Fiore, J. Kassas, D. Okrongly, J. Taylor, D. Wright, V. Linder. *OPKO Diagnostics, LLC, Woburn, MA*

Fast and accurate delivery of diagnostics results contributes to improving quality of healthcare. In those settings, where phlebotomists and trained laboratory staff are not readily available, the design characteristics of an immunoassay diagnostic device must be able to accommodate capillary blood samples, and pass a high bar for both ease of use and accuracy of the test result. The Claros® 1 instrument, associated with its Sangia™ microfluidic device, returns a laboratory-quality, quantitative result in approximately 10 minutes (from sample collection to result). The sample collection interface was optimized for the user to collect precisely 12uL capillary blood samples at the fingertip of the patient, before subsequent attachment to the microfluidic device. After insertion of device in the analyzer, the user may enter patient specific information and then walk away until the test result is reported. The Sangia measures analytes directly from capillary whole blood (WB), and the test is designed and calibrated to return a result matching that of conventional immunoassays performed in serum or plasma. In this contribution we present the analytical performance of the Claros 1 with the Sangia total PSA kit, and a clinical evaluation based method comparison against an established laboratory method (Roche Cobas® and total PSA Elecsys® kit).

The limit of quantification of the PSA assay was found to be 30pg/mL (per CLSI guideline). Precision profile of the assay showed a CV=9-16% across the entire assay range (0.05-16ng/mL). Equimolarity between free-PSA and alpha-1-antichymotrypsin-PSA was demonstrated at three levels of PSA (0.3, 3 and 14 ng/mL). Following an IRB-approved protocol, a cohort of 68 men was enrolled for PSA testing in a two-arm study design: a EDTA blood tube was collected and centrifuged to obtain plasma for testing with PSA Elecsys test in a CLIA-accredited laboratory, and a fingerstick blood sample was collected for testing with PSA Sangia in the point of care. Five lots of PSA Sangia were included in the study, and some men were tested on more than one lot of PSA Sangia, yielding a total of 86 PSA determinations with both methods. The method comparison (Passing Bablok) fingerstick WB on Sangia vs. plasma on Elecsys showed excellent correlation with a slope 0.999, intercept 0.0007 ng/mL, and $R^2$=0.95.

The data presented demonstrates that the determination of PSA from a single drop of capillary blood using a truly point-of-care system matches closely the results from an established laboratory method. Pending completion of regulatory activities, the Claros 1 system appears to be well suited for safe and reliable use in the doctor's office.

## B-324

### Evaluation of a POC INR Program in a Long-Term Care Setting: Comparison of the CoaguChek XS and Time in Therapeutic Range versus Laboratory-Based Monitoring

D. Bailey[1], A. McFarlane[2], R. Elphinestone[2], T. Dunn[2]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

**Background:** Point-of-care (POC) testing of international normalized ratio (INR) enables immediate reporting of results and dose adjustment of oral anticoagulation therapy. Time in therapeutic range (TTR), a measure of the quality of the therapy as TTR is inversely correlated with adverse events, is thought to improve with POC versus laboratory-based INR testing. However, previous studies have demonstrated statistically significant differences between POC and laboratory-based INR testing indicating that laboratory-based INR may be necessary in certain clinical scenarios. The objective of this study was to evaluate the comparability of the CoaguChek XS POC meter against the Siemens CS2100 laboratory-based instrument and to examine the impact of POC testing on TTR in an elderly population.

**Methods:** For the method comparison, paired INR results were obtained for 100 patients covering a range of INR values (0-8). For the TTR evaluation, residents in long-term care facilities were included. Repeat INR values were obtained from a total of 64 patients enrolled in the POC program from March 2016 - February 2017. POC INR results were provided to an attending medical practitioner (nurse or physician) who would adjust the OAT dosage as required. To monitor the impact of the POC INR program to patient care, the change in TTR versus baseline (laboratory-based INR) was calculated.

**Results:** Excellent correlation was observed between the POC and laboratory-based INR methods (R=0.96). Additionally, the following ISO 17593:2007 criteria were met: for INR values within 2.0-4.5, the mean bias was +/-0.3 INR and >90% of the difference between values was within +/-0.3 INR or +/-30%. However, statistically

significant differences did occur between seven paired values (7%). These differences were attributed to preanalytical errors in either the POC or phlebotomy process and to unidentified patient-specific factors. In a long-term care setting, upon introduction of the POC program, the average TTR improved from 53% to 76%.

**Conclusion:** For the majority of patients, POC INR testing offers a reliable alternative to laboratory-based INR testing with the potential to improve TTR and reduce adverse events. However, statistically different results may be obtained for some patients, with potential impact to patient care. These differences may be caused by the increased complexity of the whole blood matrix used in POC INR testing. POC INR programs should include a rigorous quality assurance program to ensure accuracy in reporting and investigation of spurious results.

## B-325

### Comparison of three glucose meters: Accu-chek Performa-Nano, Accu-chek Active and Prestige, commercially available in Peru

S. M. Flores Toledo[1], J. C. Jara-Aguirre[1], M. N. Lucano[1], R. J. Fiestas[1], S. M. Perez[1], A. C. Palacios Ramirez[2], G. C. Ferreyros Brandon[1]. *[1]Escuela de Tecnologia Medica. Facultad de Medicina. Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Departamento de Laboratorio Hospital Nacional Cayetano Heredia., Lima, Peru*

**Background:** The point-of-care (POC) glucose devices are routinely used for monitoring glucose levels and play a key role in the management of hyperglycemia and hypoglycemia at the patient's bedside. In addition glucometers has an important function in diabetic patients as a self-monitoring of blood glucose in a convenient manner. The purpose of this study was to evaluate the accuracy of 03 most common glucose meters: Accu-chek Performa-Nano,Roche, US (Nano);Accu-chek Active, Roche,US (Active); Prestige, Nipro,Mexico (Prestige), commercially available in Peru.

**Methods:** Accu-chek Performa Nano, Roche (glucose dehydrogenase/amperimetric); Accu-chek Active, Roche (glucose dehydrogenase/amperimetric) and Prestige, Nipro (glucose oxidase/amperimetric) were assessed and results compared to central laboratory method (glucose oxidase, dry chemistry), using VITROS 4600 System chemistry analyzer (Ortho Clinical Diagnostics, Inc). 80 whole blood samples from patients were prepared and divided in three groups of glucose concentration were evaluated: 20 blood samples with glucose ≤ 70 mg/dL, 40 with 70–160mg/dL and 20 with 180-500 mg/dL, were measured in duplicated with each glucometer and immediately centrifuged, the glucose plasma concentration by the routine glucose method (glucose oxidase) of the central laboratory using the VITROS 4600 System was performed. The plasma glucose values were adjusted by +/-10%. Analytical performance precision parameter of these 3 glucometers was verified using CLSI EP15-A3, with manufacturer control material (level 1-normal and level 2-high level) and was measured in quintupled during 5 days, according CLSI EP 15-A3 to estimate repeatability and intralaboratory precision. Glucose meter results were compared to reference glucose central laboratory method to estimate the bias, acceptability according ISO15197:2013 (>95% of glucose result <100mg/dL:+/-15mg/dL or >100mg/dL:+/-15%) and the Color–Coded Surveillance Error-Grid plot for each glucose meter was evaluated.

**Results:** The mean bias for Nano, Active and Prestige was -6.8, -7.94 and 8.93 mg/dL with $r^2$ values of 0.995, 0.994 and 0.979, respectively. The Accuracy using Color–Coded Surveillance Error-Grid plot, for Nano and Active, only showed 8.75 and 2.5% of the results were with slight lower degree of risk (light green/region A and B); and for Prestige, 2,5% of the results were slight higher degree of risk (yellow/region C). For Nano and Active, 100% of the glucose results met the ISO 15197:2013 requirements for glucose <100 mg/dL and 95% and 100% for glucose >100 mg/dL, respectively. The Prestige <90% of the glucose results met the ISO 15197:2013 for glucose >100 mg/dL and <100 mg/dL. Nano, had a precision of repeatability (level 1=1,4% and level 2=1,8%) and intralaboratory precision (level 1=2.8% and level 2=2,2%), for Active the precision of repeatability (level 1=2.1% and level 2=1.6%) and intralaboratory precision (level 1=3% and level 2=3%), and for Prestige, the precision of repeatability (level 1=2.6%

and level 2=1.3%), and intralaboratory precision (level 1= 9.5% and level 2=3.7%), the precision was accepted according manufacturer specifications.

**Conclusion:** Glucose meters meet the requirements of analytical performance described by the manufacturer and showed good accuracy, correlation and clinical concordance in relation to the reference central laboratory results, but not all meet the analytical quality specifications proposed by ISO 15197:2013.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Proteins/Enzymes

## B-326

### Diagnostic value of serum Adenosine deaminase and its isoenzymatic activities in type 2 diabetic patients attending tertiary care centre in Nepal.

L. B. Sapkota, S. Thapa. *Chitwan Medical College, Bharatpur, Nepal*

**Background:** Adenosine deaminase (ADA), an enzyme of purine metabolism is suggested to play an important role in cell mediated immunity and modulation of insulin activity, but its clinical and diagnostic significance in Nepalese type 2 diabetes mellitus (type 2 DM) is not yet characterized.

**Methods:** This is a hospital based case control study including 80 type 2 DM patients and same number of age and sex matched healthy controls aged between 30-70 years attending Manipal Teaching Hospital (MTH), Pokhara, Nepal. All the demographic and anthropometric data were collected using a preformed set of questionnaire and biochemical data were obtained from the laboratory analysis of the patients' blood samples. Statistical analysis was done with SPSS version 17.

**Results:** A significantly higher mean values of body mass index (BMI), fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c), and lipid profiles except high density lipoprotein cholesterol (HDL-C) were found in type 2 diabetic cases compared to controls ($p<0.001$). Similarly, the cases were also found to have significantly raised ($p<0.001$) serum total ADA ($35.55 \pm 7.53$ U/L), $ADA_1$ ($14.16 \pm 4.74$ U/L) and $ADA_2$ ($21.48 \pm 4.60$ U/L) activities compared to controls (total ADA- $20.46 \pm 3.27$ U/L, ADA1-$8.3 \pm 2.21$ U/L and $ADA_2$-$12.2 \pm 2.17$ U/L). Highest diagnostic accuracy and agreement with blood glucose test was observed with $ADA_2$ followed by total ADA and $ADA_1$.

**Conclusions:** Based on their higher serum activities, diagnostic sensitivity, specificity and substantial agreement with plasma glucose estimation, serum ADA and its isoenzymes could be utilized as biomarkers for identifying the immunological origin of the uncomplicated type 2 DM.

**Key words:** type 2 Diabetes Mellitus, Adenosine deaminase, Isoenzymes

## B-327

### The effects of protein supplements on liver enzymes activity

N. Serdarevic[1], L. Cano[2]. *[1]Clinical center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]Faculty of health sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

**Background:** Training and diet are closely related, because intensive training causes increased metabolic, physical and mental activity, so the energy requirement of athletes is greater than the requirement of people who does not have sport activity. The input of protein supplements increases the influx of amino acids in the liver tissue and that increases the catabolic conversion of amino acids in terminal nitrogen products which are excreted from the body. This is caused by increasing the activity of liver enzymes involved in these processes. In our study we determine the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LD) in athletes with high intensity and low intensity training. To establish whether there is a significant difference between the activity of the enzymes ALT, AST, GGT and LD in athletes after a seven-day break of using the supplementation.

**Methods:** The research included 180 male athletes. It contained three groups of subjects, athletes with high intensity training, athletes with low intensity training and the control group. In the first group it was professional athletes that take a 4000 and 5000 kcal a day, second group athletes who have low intensive training and daily take from 3,000 to 4,000 kcal. The control group was males who are not involved in sports and do not use protein supplements. The athlets take protein suplements Whey protein, Gainer, Isoactive, BCAA (branched-chain amino acids). The activity of enzymes (ALT, AST, GGT and LD) was analysed using BS-200 Mindray autoanalyzer. The method of determining these parameters is on the autoanalyzer modified and adapted according to the IFCC.

**Results:** The study showed that the mean value of the examined parameters is significantly lower in the serum of athletes who do not use supplements before, during or after training (ALT - 56.68 %, AST - 48.78 %, GGT - 14.17 and LD i 9.71 %) in comparison to athletes who use supplements. In the investigation of the differences between the parameters ALT, AST, GGT and LD between the two groups (athletes who use supplements and athletes who do not use supplements), we concluded that there was a statistically significant differences between the parameters ($p <0.05$) subjects who use supplements and those who do not use supplements. The Man Whitney U test showed that between the two groups (subjects with high intensity training and subjects with low intensity training) there is a statistically significant difference between the examined parameters ALT, AST and GGT ($p <0.05$), while the LD did not show a statistically significance difference ($p > 0.05$).

**Conclusion:** The results confirmed that the increased activity of the enzyme ALT, AST, GGT and LD in athletes decreases in the serum after a seven-day break of using the protein supplements.

## B-328

### The Effect of Shıkonın on $A\beta_{25-35}$ Induced Cell Cytotoxıcıty and Oxıdatıve Injury In Pc12 Cell Lıne

N. Oztas, S. Taysi, M. Tarakcioglu. *Gaziantep University, Gaziantep, Turkey*

**Aim:** Alzheimer's Disease (AD) is a chronic neurodegenerative disorder characterized by progressive loss of memory. Although its etiology has not been fully explained, basic features seen its AD includes the accumulation of extracellular amyloid plaques (AP) and formation of intracellular neurofibrillary tangles. Studies carried out on the human brain and in animals (*In vivo* and *in vitro)* show that oxidative stress plays an important role in the neurodegeneration observed in AD. Shikonin has been shown to have many positive effects such as anti-oxidant, anti-inflammatory, antithrombotic, antimicrobial, anticancer and wound healing properties. In this study, using the AD model induced with $A\beta_{25-35}$ in the PC12 cell line, we aimed to investigate the potential protective effect of Shikonin against Aβ toxicity and its resulting oxidative damage.

**Method:** The effects of Aβ25-35 and shikonin on PC12 cell viability was determined by MTT assay. To examine the effects of Aβ25-35 and Shikonin oxidative stress, we analyzed the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH Px), catalase and malondialdehyde (MDA) levels using Enzyme Linked Immunosorbent Assay (ELISA) method. Nitric oxide ($NO^{\bullet}$) was colorimetrically analyzed by Griess method.

**Results:** On addition of 20μM shikonin, reduced cell viablility noted with 5μM $A\beta_{25-35}$ was significantly increased ($p < 0.01$). Increased concentration of nitrite and MDA was observed with $A\beta_{25-35}$, decreased with addition of shikonin ($p < 0.001$). Reduced SOD and GSH Px activities observed with $A\beta_{25-35}$ increased on addition of shikonin ($p < 0.05$). Similarly, reduced catalase (CAT) activity enzyme noted with $A\beta_{25-35}$ increased on addition of shikonin ($p < 0.001$).

**Conclusion:** Results obtained from this study reveal the positive effects of shikonin on neurotoxicity and oxidative damage in $A\beta_{25-35}$ induced AD model created in vitro in the PC12 cells We therefore are of the opinion that shikonin may be a potential agent in preventing the cytotoxicity and oxidative damage induced by Aβ (an important pathophysiologic mechanism in AD).

**Key Words:** Alzheimer's Disease, Amyloid β, Oxidative stress, Shikonin.

## B-329

### Oxidative stress parameters in accute ischemic stroke patients

M. Begovic[1], J. Kotur Stevuljevic[2]. *[1]Center for Laboratory Diagnostics Biomedica, Belgrade, Serbia, [2]Faculty of Pharmacy, Belgrade, Serbia*

**Background:** Brain stroke is defined as local or global dysfunction of brain activity caused by obstraction of brain circulation to the level which is not sufficient for brain metabolic requirements for oxygen and glucosa. Deficit of antioxidants in brain ischemia leads to increasing of free radicals which could exceed the physiological defense mechanisms.

**Objective:** Oxidative stress parameters: prooxidant-antioxidant balance (PAB), total oxidant status (TOS) and total antioxidant status (TAS) were determined in stroke patients and also in healty control group.

**Methods:** The study group included 53 patients with acute ischemic stroke (37 survivors and 16 with a lethal outcome) and 24 healthy subjects. PAB was determined using the 3,3 '5, 5'- tetramethylbenzidine (TMB) chromogen, TOS by o-dianisidine-spectrophotometric method, and TAS with ABTS as the chromogen.

**Results**: TOS concentration was higher in patients (10.4 µmol/L (6.81-5.93) vs. 6.5 µmol/L (5.29-7.71), p<0.05). Patients were in a state of depleted antioxidant defense, which was confirmed by significantly lower values of TAS comparing to control (253 µmol/L (± 176) vs. 438 µmol/L (± 138), p <0.01). Patients had significantly higher concentrations of PAB, compared to control (73.6 (± 33.92) vs. 46.1 (± 21:59), p <0.05). PAB was significantly higher in patients with lethal outcome (94.9 (± 37.63) vs. 65.8 (± 29.52), p <0.01) compared to survivors. Logistic regression analysis showed that the best predictive ability for survival had PAB compared to other parameters (Odds Ratio (95th confidence interval)): 1,028 (1004-1054), p <0.05). PAB was positively correlated with MDA (ρ = 0,363, p <0.05), hsCRP (ρ = 0.390, p <0.01) and age (ρ = 0.319, p <0.05). This parameter showed significant negative correlation with the concentration of HDL-cholesterol (ρ= -0.390, P>0.05), indicating dyslipidemia and oxidative stress connection in the pathogenesis of stroke.

**Conclusion:** Higher values of PAB and TOS, and lower levels of TAS in ischemic stroke patients compared to healthy subjects confirmed the participation of oxidative stress factors in the pathogenesis of this disease.

## B-330

### Correlation between plasma cholinesterase & liver enzymes among the patient with organophosphorus poisoning

P. Risal[1], R. Karki[2], S. Maharjan[1], B. Adhikari[1], R. Bhatta[1]. *[1]Dhulikhel Hospital-Kathmandu University Hospital, Dhulikhel, Kavre, Nepal, [2]Kathmandu University, School of Medical Sciences, Dhulikhel, Kavre, Nepal*

**Background:** Organophosphorus (OP) poisoning is a major public health problem in developing country like Nepal, leading to high degree of morbidity and mortality. OP compounds act by inhibiting the enzyme Acetyl cholinesterase and the severity of symptoms correlates with the degree of decreased enzyme activity. Evaluation of various serum enzymes is important for the accurate diagnosis of OP poisoning thereby early recognizing the complication and management. Therefore, this study was designed to find out the correlation between hepatic enzymes and plasma cholinesterase level in the OP poisoning.

**Methods:** Cross-sectional study was conducted at Dhulikhel Hospital-Kathmandu University Hospital, Kavre district from 1st January 2015 to 1st December 2016. In present study, 97 cases of OP poisoning were admitted during the study period. Serum cholinesterase level and liver enzymes were estimated at the time of admission in all the patients.

**Results:** Out of 97 cases, 33(34.0%) were males and 64 (66.0%) female. Most of the cases were young people 77% (< 40years). There was wide variation in age ranging from a minimum of 10-70 years with mean age of 33.79 years. Depression of serum cholinesterase level below normal reference range was noticed in 53.6% patients and mild elevation in liver enzymes was noticed in 19.2% patients. Serum cholinesterase level did not correlate with AST, ALT, ALP and GGT and no significant increase in morbidity/mortality was seen in patients with deranged liver function test [P > 0.05].

**Conclusion:**

In conclusion, serum cholinesterase was found to be decreased in many patients at the time of admission and no correlation was seen between serum cholinesterase and liver enzymes.

## B-331

### Role of RNase L in Kidney

N. A. Alghamdi, A. Zhou. *cleveland state university, Cleveland, OH*

**Background:** Renal diseases is continuing to be a prevalent problem. Current data indicate that 1% of patients admitted to hospitals are diagnosed initially with acute kidney injury(AKI), while 2-5% of hospitalized patients develop AKI secondarily. It has been reported that epidermal growth factors(EGF)/EGFR activation contributes to development and progression of renal diseases such as obstructive nephropathy, diabetic nephropathy, hypertensive nephropathy, and glomerulonephritis through mechanisms involved in induction of tubular atrophy, overproduction of inflammatory factors, and/or promotion of glomerular and vascular injury. In this study, we showed that RibonucleaseL(RNaseL), enzyme playing a role in interferon functions, mediated EGF/EGFR activation. Interestingly, we found that kidneys from aged RNaseL deficient mice were significantly smaller than that from wild type mice under the same condition. Histological staining revealed that there were remarkably more vacuoles in the kidney of KO RNaseL mice than WT mice. Proteomic analyses of urine discovered that lack of RNaseL exclusively block EGF excretion to urine. In this study, we will determine the role of RNaseL in the pathogenesis and elucidate how RNase L regulates the level of EGF in the kidney.

**Methods:** Investigate the role of RNaseL in the pathogenesis of AKI: Wild type(WT) &RNaseL null mice(KO) treated w/wo folic acid stained with PAS. Podocyte count and density analysis performed. Frozen kidney slides subjected to Oil Red O. Creatinine level in plasma and urine measured using HPLC MS. Investigate the mechanism by which RNaseL regulates the EGF: Total protein extracted from WT and KO kidneys and other organs. The level of EGF in blood investigated by qRT-PCR and ELIS under. The signaling of EGF/EGFR investigated by immunoblotting assays and the target molecules identified by using HPLC MS/MS. Investigate the corresponding enzyme of EGF cleavage: A Disintegrin and Metalloproteinase10(ADAM10). Renal cells isolated from WT and RNaseL KO mice. The cells treated w/wo PMA, total RNA and cell extracted. The expression of ADAM10 is examined by qPCR and Western blot. The release of ADAM10 to the media measured in the presence/absence of ADAM10 inhibotor Batimastat(BB-94).

**Results:** 1.Ratio of kidney weight/body for WT mice was 1.89-fold higher than KO mice. 2.kidney sections from KO RNaseL mice possess more vacuoles. 3.Two urinary protein bands in WT mice were missing in the urine of mice deficient RNaseL. The protein bands identified using LC-MS/MS &Western blot. Deficiency of RNaseL blocked the excretion of pro-EGF in urine. Determined expression at mRNA level in kidneys from 2-month-old WT &Ko RNaseL male mice. RT-PCR showed RNaseL deficiency doesn not affect the expression of EGF/EGF family at the transcriptional. 4.RNaseL has no protein-protein interaction with pro-EGF as the co-immunoprecipitation results show. 5.Western Blot analysis using p-Tyr and p-Thr antibodies showed that p-Protein profile is dramatically differs between WT &KO RNaseL kidney.

**Conclusion:** we should be able to obtain direct evidence that RNaseL contribute to kidney function. Data from this study will provide a better understanding of involvement of RNaseL in pathogenesis of AKI. The information about how RNaseL impacts ADAM10, subsequently regulating the EGF/EGFR activation, will reveal the molecular mechanism underlying the pathogenesis of AKI.

## B-332

### Screening and Selection of Antibodies for the Detection of MIP-1 alpha and Its Application to the Study of Chronic Kidney Disease

L. Staunton[1], E. Harte[1], M. Tokarska[2], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background**

The identification of the correct antibody pair is the most important step in ensuring the development of a highly specific and sensitive immunoassay. In order to do this, a high number of antibodies have to be screened as quickly as possible in order to find the best antibody pair for the development of an accurate immunoassay. Using Biolayer Interferometry (BLI) and Biochip Array Technology (BAT) the aim of this study was to develop a rapid, cost-effective screening and selection strategy for the establishment of a high sensitivity MIP-1alpha assay and evaluate its application to early detection and progression of Chronic Kidney Disease (CKD). CKD is defined as the progressive and irreversible decline in renal function and is classified into stages 1-5. Patients frequently present with chronic elevation of inflammatory biomarkers which appears to be exacerbated by disease progression. MIP-1alpha is known to have pro-inflammatory effects and has previously been shown to be significantly elevated in stages 1-3 of CKD patients as compared to control subjects. The effective detection of MIP-1 alpha with an optimal antibody pair, allows for the development of efficient immunoassay that can be used as an analytical tool in clinical studies.

**Methods**

A total of 22 purified MIP-1alpha sheep monoclonal antibodies (mABs) were screened using regenerable biosensors on the Octet RED96 instrument (ForteBio, USA). Capture and detector screening was performed by immobilisation of mABs and his-tagged antigen respectively. Top ranking MIP1-alpha pairs were then brought forward for assay development on the biochip platform using the Evidence Investigator. The final MIP-1alpha antibody pair was selected following sensitivity, reproducibility and cross-reactivity assessment. The clinical utility of the assay was then assessed by the analysis of 120 patient serum samples (normal n=60, CKD n=60; CKD stage 1 n=20, CKD stage 2 n=20 and CKD stage 3 n=20).

**Results**

Each MIP-1alpha mAB was ranked according to on- and off-rates using recombinant MIP-1alpha antigen. Top mABs exhibiting fastest on-rates and slowest off-rates were used in a single epitope binning experiment resulting in the screening of 42 possible antibody pairs and the identification of three unique epitope bins. Using BAT six

of the matched antibody pairs were used to successfully generate highly sensitive calibration curves (1pg/ml-500pg/ml). The assessment of serum samples using two sample Wilcoxon rank sum test showed significant separation of normal and stage 1 CKD patient samples (p-value<0.05), normal and stage 2 CKD patient samples (p-value<0.01) and normal and stage 3 CKD patient samples (p-value<0.001).

**Conclusion**

The data demonstrate the successful screening and selection of a suitable antibody pair that can be used for the development of efficient highly sensitive MIP-1alpha immunoassay using BLI and BAT. The strategy used shows how its resulting data can tailor the selection of mABs for their end-point assay platform such as BAT through the attention to antibody-antigen on- and off-rates. The resulting antibody pair was used to demonstrate the potential for MIP-1alpha as an early stage screening tool and disease monitoring biomarker for CKD.

## B-333

### Development of a Biochip Assay for the Detection of Thyroxine-Binding Globulin (TBG) on the New Random Access Fully Automated Evidence Evolution Analyser

E. O'Connor[1], T. Doherty[1], S. Ward[1], M. Foley[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background**

Thyroxine Binding Globulin (TBG) is the main carrier protein for thyroid hormones in the blood. This 54 kDa protein reversibly binds thyroxine and tri-iodothyronine in circulation. Only free levels of these hormones are metabolically active, entering cells to regulate metabolism and body temperature. The normal reference range of TBG is 12-30 µg/ mL. Changes in TBG concentrations do not alter the metabolic state or cause thyroid disease but can produce changes in total thyroid hormone concentrations in serum. This may be mistaken for serious thyroid disorders such as hyper or hypothyroidism and if unrecognized can lead to inappropriate treatment. The aim of this assay was to develop a biochip based immunoassay for the quantification of TBG levels in serum applied to the new high throughput, fully automated random access Evidence Evolution analyser. The application provides a new analytical tool in the assessment of thyroid function.

**Methods**

A competitive chemiluminescent immunoassay was employed. The capture antibody was immobilised and stabilised on the biochip surface at a discrete test region. Calibrator material was prepared from purified human TBG antigen. The conjugate was also made from purified human TBG antigen labelled with HRP. The assay was applied to the Evidence Evolution analyser. The sample is diluted on-board the analyser at 1:10 and requires 8.3 µL of neat sample. Interference was tested for the following; haemaglobin 10 mg/ mL, triglycerides 10 mg/ mL, intralipids 40 mg/ mL and bilirubin 2 mg/ mL. The shelf life of key assay components was investigated. Serum patient samples (n=20) were assessed and the results compared with a commercially available method.

**Results**

A calibration range of 0-100 µg/ mL was established for the assay. Haemoglobin, triglycerides, intralipids and bilirubin were found to have no negative effects on performance. The stability of calibrators and conjugate were assessed and predicted to be stable for up to two years at 4°C. An $r^2$=0.96 and agreement of 69% were obtained based on the assessment of 20 patient serum samples with the biochip assay and another commercially available method.

**Conclusion**

The results indicate applicability of the developed biochip assay on the Evidence Evolution analyser for the detection of TBG. The assay presented a wide calibration range (0-100 µg/ mL) and the assessment of serum samples showed favourable correlation with another method. This new system represents a new analytical tool for a high throughput, assessment of samples and also incorporates random access and STAT sample capabilities. Moreover, the biochip platform offers flexibility to incorporate other tests on the biochip surface thus increasing the information to facilitate clinical understanding.

## B-334

### Development of a New Enzyme-Linked Immunosorbent Assay Kit to Detect NGAL in Human Serum and Its Application to Chronic Kidney Disease

K. Cawley[1], M. Summers[1], E. McCole[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd, Crumlin, United Kingdom*

**Background**

Neutrophil gelatinase-associated lipocalin (NGAL) or Lipocalin-2 (LCN2) is a member of the lipocalin family of proteins which are known for the transportation of small hydrophobic ligands. NGAL was originally discovered in the granules of neutrophils but has since been found in many other human tissues including breast, kidney and liver. NGAL itself has many functions, for instance it's sequestering of iron, prevention of bacterial growth, chemoattraction of neutrophils, reduction of oxidative stress and regulation of cancer cell survival. It has been reported however that NGAL is highly upregulated upon kidney damage where levels can rise by ~ 10 fold in 3 hours depending on the type and severity of injury. The early detection of biomarkers for kidney injury may improve the diagnosis of conditions such as chronic kidney disease (CKD) or acute kidney injury (AKI) and allow timely determination of treatment which may ultimately slow progression. The availability of tests enabling the detection of this protein represents an advantage in clinical research settings. This study aimed to develop a new enzyme-linked immunosorbent assay (ELISA) for the detection of NGAL in human serum.

**Methods**

A colorimetric 2-step sandwich immunoassay was employed. The capture antibody was immobilised and stabilised on a 96-well microtitre plate surface. The analyte, if present in the sample, binds to the capture antibody and then a second antibody labelled with horseradish peroxidase binds to the analyte. Absorbances were read at 450nm. The signal is proportional to the concentration of the analyte in the sample. All assay kit reagents are ready to use. Recognition of NGAL was tested with analysis of 40 CKD samples (10 normal, 10 stage 1, 10 stage 2 and 10 stage 3). Statistical analyses were performed by Mann Witney test (with bon ferroni correction) (Medcalc version 16.4.3).These samples were also measured on another commercially available ELISA and the results compared by linear regression analysis.

**Results**

The assay exhibited a functional sensitivity of 20 ng/mL (measuring range of 0-2000 ng/mL, allowing for a 1 in 100 sample dilution). The intra assay precision value, expressed as %CV, was 8.9% and 6.6% based on 10 measurements at two different concentrations. Concentrations of native NGAL from Stage 3 CKD serum samples (Median 205ng/mL) were significantly elevated when compared to controls (Median 72ng/L), p=0.0030. Stage 1 and 2 samples were not significantly elevated when compared to controls. The correlation study showed, with 40 samples ranging from 32 to 302ng/ml, a correlation coefficient of 0.96 and a slope of 1.1.

**Conclusion**

The results show applicability of the developed ELISA for the sensitive detection of NGAL in serum. NGAL is shown to be a useful biomarker for patients at stage 3 CKD. The assay presents all kit reagents ready to use and 48 samples can be measured in less than 3 hours. This assay is a useful analytical tool for clinical research studies.

## B-335

### Evaluation of Receptor Activator of Nuclear Factor K Ligand and Osteoprotegrin Levels in Thalassemia Intermedia

N. A. Bashir[1], Y. S. Khader[1], M. Barqawi[2], S. M. Alqudah[1]. *[1]Jordan University of Science and Technology, Irbid, Jordan, [2]Jordan Ministry of Health, Irbid, Jordan*

**Background:** The clinical phenotype of thalassemia intermedia is somewhere between beta thalassemia minor and beta thalassemia major, without need for regular blood transfusions.The increase in erythropoietin will stimulate erythropoiesis that will lead to bone fragility.

**Methods:** In this cross-sectional study we are investigating thalassemia intermedia related bone loss by evaluating the levels of OPG(Osteoprotegrin) and RANKL(Receptor Activator of Nuclear factor- Kappa B Ligand) among 45 patients in comparison to a 39 healthy control group, and determining the correlation between RANKL and OPG and bone turnover markers such as carboxy terminal telopeptide of collagen type I (CTXI) and serum procollagen type I N-terminal propeptide (PINP), using Sandwich and competitive ELISA. IBM-SPSS version 23.0 was used in our

descriptive and inferential statistics. Independent T-test was used to compare the means between thalassemic patients and control group. Pearson correlation was used to study the correlation between RNKL/OPG and other parameters (erythropoietin, CTXI, estrogen/testosterone, and procollagen). The two sided null hypothesis were used in our analysis. Finally, all nominal and ordinal data were reported in frequencies and percentages, and numerical data were reported in term of mean (+_SD).

**Results:** The mean erythropoietin concentration for thalassemia intermedia patients and controls is (638.36±272) ng/ml and (137.69±81) ng/ml, respectively. The mean concentration of OPG in thalassemia intermedia patients and control is (3370.02±1142.40) pg/ml and (3931.2±47907) pg/ml. RANKL concentration in thalassemia intermedia patients and controls is (4980.9±1313) pg/ml, (2621±568) pg/ml, respectively. The mean RANKL/ OPG ratio in thalassemia intermedia patients and control is (1.66±0.85), (0.7±0.2), respectively. The mean concentration of bone formation marker PINP in thalassemia intermedia patients and controls was (64.36±51.9) pg/ml, (269.40±127.7), respectively, while mean concentration of bone resorbtion marker CTXI in thalassemia intermedia and control is (459.18±120.1) pg/ml , ((185.87±53.5) pg/ml., respectively.There was no correlation between Osteoprotegrin and RANKL with all tested parameters; Erythropoietin, Estrogen and Testosterone, PINP, and CTXI

**Conclusion:** Our results suggest that thalassemia intermedia patients have an alteration in the RANKL, OPG levels, and a consequence RANKL/OPG ratio.This alteration lead's to an increase in bone resorption, demonstrated by the elevation of CTXI levels, and to a decrease in bone formation, demonstrated by the reduction of PINP levels, eventually bone loss and osteoporosis. The increase in Erythropoietin implies that the erythroid hyperplasia and marrow expansion also plays an important role in bone lost through the mechanical interruption of bone formation, distortion and fragility.

## B-336

### Development of a Highly Specific Monoclonal Antibody Pair for the Detection of Glutathione S-transferase Pi (GST Pi)

M. Tokarska, P. Ratcliffe, W. J. Curry, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd., Crumlin, United Kingdom*

Background Renal pathologies that drive distal tubular damage may cause increased release of GST Pi into the urine. Elevated urinary GST Pi levels are indicative of renal tubule damage in transplant rejection, nephrotoxicity, infection, diabetes and chronic renal injury. Increased plasma concentrations of GST Pi are associated with chronic cholestatic diseases, cholangiocarcinoma and a range of malignancies, including colorectal cancers and lung cancer. Furthermore, the concentration of GST Pi in plasma is significantly elevated following stroke and some reports indicate that measurement of GST Pi in the early stages post-symptoms onset could indicate time of stroke. The aim of this study was to develop a highly specific monoclonal antibody pair (capture antibody and detector antibody), which can be employed for the development of a robust, quantitative immunoassay for the detection of GST Pi. Methods Sheep were immunized with E. coli-derived, recombinant human GST Pi. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies generated were purified and evaluated by direct binding ELISA to determine their specificity for GST Pi and cross-reactivity with other GST family members, including GST A1, GST T1, GST mu, GST M1. Sandwich pairs were evaluated by employing biochip based immunoassays on the Evidence Investigator Analyser and the optimal combination was identified for assay development. The resulting assay was employed for analysis of plasma samples from stroke patients (n=32) on admission (within 6 hours of onset of neurological symptoms) and healthy control samples (n=9). The Mann-Whitney t-Test was applied to determine statistical significance ($p<0.05$) of the median from each group. Results The selected monoclonal antibody pair exhibited specificity for GST Pi (%cross-reactivity to other family members was <1%). It was employed in development of a biochip immunoassay with a calibration range of 0-200ng/mL, sensitivity <1.0ng/mL and within-run precision, expressed as CV (%), <10%. Results from analysis of stroke patients revealed an increased median concentration of GST Pi in ischaemic stroke (12.93ng/mL, p=0.0005) and haemorrhagic stroke (3.94ng/mL; p=ns) when compared to controls (1.17ng/mL). Conclusion Data indicate optimal analytical performance of the monoclonal antibody pair for the specific detection of GST Pi and its suitability for application to the development of robust, quantitative immunoassays. The determination of GST-Pi will contribute to the study of its role in various disease states, including renal pathologies, a range of malignancies and stroke

## B-337

### Development of a Highly Specific Monoclonal Antibody Pair for the Detection of DJ-1

M. Tokarska, A. Riddles, P. Ratcliffe, W. J. Curry, R. I. McConnelll, S. P. FitzGerald. *Randox Laboratories Ltd., Crumlin, United Kingdom*

Background The protein DJ-1 regulates redox signalling kinase pathways and acts as a transcriptional regulator of many anti-oxidative genes, consequently it is an important redox-reactive signalling intermediate controlling oxidative ischemic stress and neuroinflammation during age-related neurodegenerative processes. DJ-1 mutations are associated with rare forms of autosomal recessive early-onset Parkinson's disease, therefore augmenting DJ-1 activity could potentially offer novel approaches to treat such chronic neurodegenerative illnesses. DJ-1 is readily detected in tau inclusions in brain tissue from patients with neurodegenerative diseases such as Alzheimer's disease and other related tauopathies. Analysis of DJ-1 expression in 3 independent cohorts of stroke patients demonstrated significant early elevation following a stroke event, in some patients within 30 minutes of stroke onset. This early detection may offer meaningful thrombolytic therapy intervention. The aim of this study was to develop a highly specific monoclonal antibody pair (capture antibody and detector antibody), which can be employed for the development of a robust, quantitative immunoassay for the detection of DJ-1. Methods Sheep were immunized with E. coli expressed recombinant human DJ-1. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies generated were purified and evaluated by direct binding ELISA to determine their specificity for DJ-1. Sandwich pairs were evaluated by employing biochip based immunoassays on the Evidence Investigator Analyser and the optimal combination was identified for assay development. The resulting assay was employed for analysis of plasma samples from stroke patients (n=32) on admission (within 6 hours of onset of neurological symptoms) and healthy control samples (n=9). The Mann-Whitney t-Test was applied to determine statistical significance ($p<0.05$) of the median from each group. Results The selected monoclonal antibody pair exhibited specificity for DJ-1 (%CR to other family members was <1%). It was employed in development of a biochip immunoassay with a calibration range of 0-100ng/mL, sensitivity <1.0ng/mL and within-run precision, expressed as CV (%), <10%. Results from analysis of stroke patients revealed a significantly increased median concentration of DJ-1 (9.55ng/mL; p=0.0004) in ischaemic stroke when compared to controls (1.5ng/mL). Conclusion Data indicate optimal analytical performance of the monoclonal antibody pair for the specific detection of DJ-1 and its suitability for application to the development of robust, quantitative immunoassays. The determination of

DJ-1 will contribute to the study of its role in various disease states, including neurodegenerative conditions, malignancies and stroke.

## B-338

### Development of a Biochip Array for the Simultaneous Measurement of Distinct Fatty Acid-Binding Proteins (FABPs)

N. Cutliffe[1], R. McGinley[1], E. Harte[1], M. Foley[1], T. Doherty[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd., Crumlin, United Kingdom*

**Background** Fatty Acid Binding Proteins (FABPs) are small 14-15kDa proteins which belong to a family of intracellular transport proteins. They are involved in the transport of long chain fatty acids and other hydrophobic ligands across cell and nuclear membranes. The family share moderate sequence homology of 20-70% and their tertiary structure is virtually superimposable. To date nine different FABPs, each with tissue specific distribution have been identified. Each FABP is named according to its tissue localisation; Liver-FABP, Intestinal-FABP, Heart-FABP, Adipose-FABP, Epidermal-FABP, Ileal-FABP, Brain-FABP, Myelin-FABP and Testis-FABP. These proteins have shown to be valuable biomarkers for a wide variety of conditions, including, but not limited to cancer, metabolic syndrome, irritable bowel disease, diabetes, acute myocardial infarction and renal failure. Biochip Array Technology enables the simultaneous detection of multiple analytes from a single sample. The development of a biochip array for the simultaneous measurement of multiple FABP proteins, will give a holistic profile of various organs and identify anomalies in certain tissues that may warrant further investigation. **Methods** The biochip incorporates multiple discrete test sites on its surface and allows quantification of specific FABPs using a chemiluminescent sandwich technique. Sixteen specific monoclonal antibodies were developed to bind a unique epitope on eight of the nine FABP family

members. This facilitated the multiplexing of FABP proteins applied to the biochip analyser Evidence Investigator and analytical performance was evaluated. A study was conducted to measure the FABP serum levels in diseased cardiac patient samples (n=26) and a healthy patient cohort (n=36). A significant difference of FABP serum levels measured between healthy and diseased patients' samples was evaluated using the Kruskal-Wallis test (P value ≤ 0.05). **Results** The multiplex biochip immunoassay developed for FABP family members were specific for the targets with cross reactivity ≤ 1% observed when cross reactants were tested at excessive concentrations. The FABP multiplex array has shown sensitivity of 1.2ng/mL (Liver-FABP), 1.21ng/mL (Intestinal-FABP), 0.56ng/mL (Heart-FABP), 0.78ng/mL (Adipose-FABP), 1.95ng/mL (Epidermal-FABP), 1.46ng/mL (Ileal-FABP), 0.08ng/mL (Brain-FABP) and 0.72pg/mL (Testis-FABP). A cohort of cardiac disease samples were compared to normal healthy patient samples. Significantly elevated levels of Heart-FABP (P= <0.0001) and Adipose-FABP (P=0.0001) were observed in cardiac samples compared to healthy control patients, whereas other FABP family members such as Brain-FABP did not show significant differences. Average FABP levels measured in healthy individuals for Heart-FABP, Adipose-FABP and Brain-FABP were 3.18ng/mL, 1.92ng/mL and 0.02ng/mL respectively. For cardiac disease samples the average Heart-FABP, Adipose-FABP and Brain-FABP levels measured were 15.56ng/mL, 13.885ng/mL and 0.01ng/mL respectively. **Conclusion** The results indicate the applicability of the developed biochip array for the simultaneous detection of FABP proteins from a single serum sample. The simultaneous immunoassays are specific and sensitive to each FABP protein, and capable of discriminating sample types. This novel multi-analytical test allows for the initial screening of tissue damage which can then be a foundation for further patient investigations.

## B-339

### Evaluation and Performance of a New and Novel Visible Method for the Measurement of ALT on VITROS® Systems

J. L. Fyles, M. Barbero, T. DiMagno, C. Felder, G. Labenski, A. Porter, H. Robords, V. Thai-Paquette. *Ortho Clinical Diagnostics, Rochester, NY*

VITROS Chemistry Products ALTV Slides quantitatively measure alanine aminotransferase (ALT) activity in serum and plasma using VITROS 250/350/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System in the visible spectrum at 670nm. Alanine amino transferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases. The VITROS ALTV Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate in the presence of pyridoxyl-5-phosphate (P-5-P) to produce pyruvate and glutamate. Pyruvate is oxidized to acetylphosphate and hydrogen peroxide by pyruvate oxidase. The final reaction step involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored dye. The rate of oxidation of the leuco dye is monitored by reflectance spectrophotometry. The rate of change in reflectance density is proportional to enzyme activity in the sample. Accuracy was evaluated for 124 patient serum samples (6 - 728 U/L) on the VITROS 350 and VITROS 5600 Systems compared to the IFCC comparative method adapted to a centrifugal analyzer at 37°C. The VITROS ALTV Slides assay showed excellent correlation with the IFCC method. VITROS 350 System = 1.00 * IFCC +2.0; (r) = 0.999. VITROS 5600 System = 1.00 * IFCC +1.9; (r) = 0.999. The 20-day precision studies conducted on the VITROS 350 and 5600 Systems showed excellent precision. Mean ALT concentrations of 33 U/L and 172 U/L resulted in within-laboratory percent coefficient of variation (%CV) of 1.9% and 1.7% respectively and within day %CV of 1.3% and 1.1% respectively on the VITROS 350 System. Mean ALT concentrations of 32 U/L and 171 U/L resulted in within-laboratory %CV of 1.3% and 1.6% respectively and within day %CV of 0.9% and 1.2% respectively on the VITROS 5600 System. The within run precision studies (n=44) also showed excellent precision. Mean ALT concentrations of 70.1 U/L, 324.0 U/L and 634.7 U/L resulted in within run %CV of 0.9%, 0.9% and 1.0% respectively on the VITROS 350 System. Mean ALT concentrations of 68.3 U/L, 318.2 U/L and 611.4 U/L resulted in within run %CV of 0.7%, 0.6% and 0.8% respectively on the VITROS 5600 system. The VITROS ALTV Slides assay exhibits higher sensitivity with a Limit of Detection (LoD) of 1.0 U/L based on 350 determinations with 5 low-level samples. The Limit of Blank (LoB) is 0.7 U/L based on 70 determinations with 5 blank samples. The VITROS ALTV Slides assay has exhibited good correlation with serum samples across a broad measuring range compared to the IFCC comparative method. In addition excellent precision and low end sensitivity has been observed on the VITROS 350 and 5600 Systems.

## B-340

### Results of a time and motion study of special protein analyzers

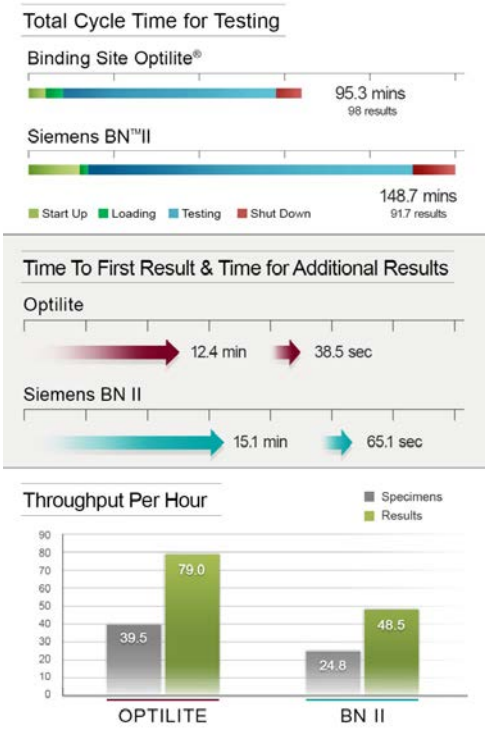
K. Pacella[1], S. Stone[2]. *[1]Health Network Labs, Allentown, PA, [2]Argent Global Services, Oklahoma City, OK*

**Background**: The objective of this independent, observational time and motion study was to compare performance, labor and time requirements of the Binding Site Optilite® system and the Siemens BN™II system for special protein assays.

**Methods**: A before and after study was performed by observing all activities related to system operations. Observations were conducted and data was collected for four days on each system in the same location with the same layout, processes, volumes, schedules and staffing. The test menus were identical and daily average volumes were very similar. Testing was performed once per day with batch loading. The data is presented as daily weighted averages.

**Results**: The study revealed that the Optilite required significantly less time to complete all testing. On average, the Optilite required 95.3 minutes to produce 98 results and the BNII needed 148.7 minutes to complete 91.7 results. The time to first result on the Optilite was 12.4 minutes compared to 15.1 minutes for the BNII; and the time for each additional result was nearly twice as fast at 38.5 seconds for the Optilite compared to 65.1 seconds for the BNII. The Optilite produced higher throughput of both specimens and results at 39.5 specimens and 79.0 results per hour compared to the 24.8 specimens and 48.5 results per hour for the BNII. The total labor and cycle time for daily, weekly and monthly maintenance needed for the Optilite was less than the BNII (see figure).

**Conclusions**: The study design provided an ideal comparison of the two systems. The Optilite demonstrated faster turnaround times, faster time to first result, increased throughput and reduced maintenance requirements when compared to the BNII.



## B-341

### Multisite study of analytical performance reveals disparities in global performance specifications

S. Westgard[1], N. Chararuks[2], J. Jairaman[3], E. Kondrashova[4], Z. Hua[5], J. Litten[6]. *[1]Westgard QC, Madison, WI, [2]Bumrungrad International Hospital, Bangkok, Thailand, [3]Sunway Medical Centre, Selangor, Malaysia, [4]INVITRO Laboratories, Moscow, Russian Federation, [5]Guizhou Provincial Hospital, Guiyang, China, [6]Winchester Medical Center, Winchester, VA*

**Background:**

**Analytical Performance specifications** for AST are not harmonized throughout the world. Allowable total error specifications range from 12% to 21% depending on the country or EQA/PT program. Since the use and interpretation of the AST test is standardized, the goals to judge method acceptability should also be standarized

**Methods:** 30 instruments in 19 laboratories in 8 countries participated in a Sigma Verification program. Imprecision was estimated from routine controls, with 1 to 3 months of data. Bias or inaccuracy was estimated from EQA/PT programs, peer group comparisons, or comparisons vs. the assayed/target values of the controls.

Allowable total errors from Rilibak, "Ricos Goals", CLIA Goals, and the Australian RCPA goals were compared. These range from 12% to 21% allowable total error.

Performance was evaluated using analytical Sigma-metrics. The standard Sigma-metric equation was used (TEa - bias) / CV

The percentage of laboratories able to achieve 5 Sigma (excellent) performance or better based on these goals was determined. A target of achieving 80% or better was considered success.

**Results:**

100% of labs and instruments can achieve CLIA and Rilibak goals, indicating that these performance specifications may be too lenient. These goals may be more like a rubber stamp than a performance standard.

In contrast, only 1/3 of instruments and labs are able to achieve an excellent level of quality using the RCPA goal, and nearly 1 in 6 labs would be considered unacceptable (less than 3 Sigma performance). This is a high level of failure and would represent a true crisis in the laboratory diagnostics market, if the goal was clinically appropriate. However, we do not see such problems, which is further evidence that this goal may not match up with the clinical use of the test.

For the Ricos goal, nearly 80% of the labs can achieve 5 Sigma or higher performance at the critical decision level, with less than 4% receiving unacceptable grades. This is a much better spread of evaluation. It does not show that too many labs are going to fail, nor is it so low a bar than all labs are passing it.

**Conclusion:**

For the AST method, instrumentation quality has improved since the CLIA goals were established in 1992, and tighter goals are now achievable by a significant majority of the methods. This is demonstrated by the ability of multiple instruments and laboratories operating under a wide variety of circumstances reflective of typical routine operation. Data of this type can help inform the global debate on which analytical performance specifications to adopt during the harmonization efforts.

## B-342

### Production of recombinant Streptolysin O from *Streptococcus pyogenes*

Y. Sumida, H. Kitazawa, A. Kawai, T. Kishimoto. *Toyobo co., Ltd., Fukui, Japan*

Background: The ASO test is a method to determine a recent infection with group A *streptococcus*. Steptolysin O (SLO) is an essential raw material for ASO *in vitro* diagnostic (IVD) reagents. Recently several groups are studying the recombinant expression of SLO in *Escherichia coli*. On the other hand, it has been reported that low molecular form of SLO is also produced due to degradation by protease from *E. coli* (Pinkey et al. 1995). Hence, it is difficult to produce highly purified SLO cost-effectively in industrial scale. In addition, SLO has originally hemolytic activity which is an undesirable property from a viewpoint of the safety of production workers.

Methods: We attempted to produce a truncated SLO to avoid the degradation and eliminate hemolytic activity. The SLO gene without C-terminal region was cloned for the construction of a recombinant expression system in *E. coli* based on the fact that C-terminal region is involved with the degradation as well as hemolytic activity (Pinkey et al. 1995 and Yamamoto et al. 2001). The truncated SLO was overexpressed in *E. coli*, and the SLO was purified to homogeneity by general purification systems. Subsequently, we evaluated hemolytic activity, heat-stability and application using the highly purified SLO.

Results: As a result of the hemolytic activity test, it was elucidated that the hemolytic activity of the truncated SLO was completely eliminated. Besides, it was shown that the heat-stability of the truncated SLO was much higher than that of full-length form. In addition, a competitive assay using 50 serum samples was performed showing that no clinically relevant epitopes are lost in this truncated SLO compared with native SLO. Interestingly, ASO-latex IVD reagents were successfully prepared with this new raw material. The details will be discussed at the conference.

Conclusion: This novel truncated SLO will be useful for the manufacturing of ASO IVD reagents.

## B-343

### It's Complex: A Multistep Process to Determine a Clinical Cutoff for Macroenzyme Determination

S. P. Wyness[1], S. L. La'ulu[1], J. J. H. Hunsaker[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT*

**Background:** Macroenzymes are antigen-antibody complexes formed between a specific autoantibody and enzyme. Their formation increases enzyme half-life and therefore inhibits in vivo clearance from circulation. Lack of clearance may cause abnormal elevations in serum enzyme activity, independent of pathology. Detection of macroenzyme forms is therefore crucial to avoid unnecessary clinical intervention. Macroenzyme detection methods range from complex gel filtration chromatography (GFC) and electrophoresis methods to less labor-intensive ultrafiltration and polyethylene glycol (PEG) precipitation techniques. **Objective:** Validate a clinical cutoff for the detection of a macroenzyme complex, macro-aspartate aminotransferase (AST), using PEG precipitation of serum. **Design and Methods:** PEG precipitation was performed by vortexing, incubating, and centrifuging equal amounts of PEG (250g/L) and patient sample. Neat samples and supernatant were analyzed using Roche cobas 602. Monomeric recoveries (measurement of unbound enzyme after complex removal) were determined by dividing the activity of the supernatant (following dilution correction) by the neat activity and converting to percent. To determine a clinical cutoff for macro-AST, central 95% reference intervals (RIs) were first established in well-characterized, healthy adults and children. Serum was collected from 124 adults (age 19-62 years, 58 males, median 33 years) and 74 children (age 6 months-17 years, 36 boys, median 7 years). Second, newly established RIs were verified using 252 residual specimens with elevated AST concentrations (> 31 U/L). AST concentrations ranged from 33-10,923 U/L, median 115 U/L. Third, a group of clinically suspect samples (n=6; 4 adults, 2 children) were analyzed. All suspect cases had elevated AST concentrations and clinical suspicion of macro-AST presence. Last, a subset of the above groups was evaluated using confirmatory GFC (n=8; 2 healthy, 2 verification, 4 suspect samples). **Results:** Nonparametric central 95% RI for the monomeric recovery in healthy adults was 33-83%. Parametric central 95% RI for healthy children was 26-71%. Using this statistically derived RI, 15% (37/252) of the verification samples (AST >31 U/L) would be considered positive for macro-AST. This likely exceeds the prevalence of macro-AST in the general population, thus the lowest monomeric recovery observed in the healthy populations (19% adults, 16% children) was evaluated as alternative positive cutoffs. This reduced positivity in the verification group to 0.8% (2/252). Four of six clinically suspect samples had monomeric recoveries ≤12%, 2 had recoveries ≥33%. Confirmatory testing of healthy (recoveries ≥33%) and verification (recoveries 25%, 29%) samples were negative by GFC. The 4 clinically suspect samples (with recoveries ≤12%) were positive by GFC. Based on these results, samples from adults with calculated monomeric recoveries ≤12% were considered positive for macro-AST, recoveries 13-18% were considered indeterminate, ≥19% was considered negative. In children, samples with calculated monomeric recoveries ≤12% were considered positive for macro-AST, recoveries 13-15% were considered indeterminate, ≥16% was considered negative. **Conclusions:** Macroenzymes are relatively rare, however, identification and diagnosis of these primarily benign anomalies is important. Extensive evaluation of the clinical cutoffs used for these assays is necessary. When empirically-derived RI do not align with clinical expectations, further evaluation using well-characterized samples is essential for proper clinical interpretation.

## B-344

### IFCC Traceable Calibration Factors for Abbott ARCHITECT non activated ALT and non activated AST assays

J. Miao[1], J. Ulloor[2], R. Thillen-Chennault[2], Y. Qian[1], C. Chen[3], K. Sikder[2], J. Reid[4], P. Yin[1]. *[1]Abbott, Shanghai, China, [2]Abbott, Dallas, TX, [3]Abbott, Singapore, Singapore, [4]Abbott, Longford, Ireland*

IFCC Traceable Calibration Factors for Abbott ARCHITECT non-activated ALT and non-activated AST assays

**Objective**: Determine metrological traceability by optimizing calibration factors (k-factors) for Abbott ARCHITECT non-activated ALT (ALT, 7D56) and non-activated AST (AST, 7D81).

**Method:** The study was performed in three phases: 1) Estimation of IFCC traceable k-factors for ALT/AST using IFCC traceable activated ALT (AALT, 8L92) and activated AST (AAST, 8L91) method means and non-activated method means with the current k-factors ; 2) Verification of the new adjusted k-factors for ALT/AST by a method comparison with AALT/AAST; 3) Validation of IFCC traceable k-factor for ALT/AST by comparing the results to AALT/AAST in 31 laboratories across China.

**Results:** Sixty serum samples were used for estimating the IFCC traceable k factors, the comparison between current factor and IFCC traceable factor. Fifty serum samples were used for verification of IFCC traceable k-factors for ALT/AST. Method comparison results between ALT/AST with the optimized k- factor and AALT/AAST. See table below:

| Enzyme | Estimation of IFCC traceable k-factors | | | | Verification of IFCC traceable k-factor | | |
|---|---|---|---|---|---|---|---|
| | ARCHITECT | Current factor | IFCC traceable factor | %diff current vs adjusted factor | R | Slope | Intercept |
| AST | C8000 | 8141 | 9860 | 21.12 | 0.9962 | 1.00 | -5.76 |
| | C16000 | 8492 | 10077 | 18.66 | 0.9959 | 1.00 | -6.41 |
| ALT | C8000 | 8141 | 9810 | 20.50 | 0.9937 | 0.95 | 2.39 |
| | C16000 | 8492 | 10024 | 18.04 | 0.9935 | 0.94 | 2.88 |

The correlations for validation studies using 8 pooled serum samples across 31 laboratories were: ALT (R2: 1.00; Slope: 0.98) and AST (R2: 1.00; Slope: 0.92). The Bland Altman analysis showed a mean % difference of 0.22% for ALT and -7.78% for AST.

**Conclusion**: The study demonstrated comparable performance between AALT/AAST (IFCC traceable methods) and ALT /AST assays with newly optimized k-factors. The use of new optimized k-factor for ALT /AST assays may provide results that are comparable to IFCC traceable methods for patient samples in the clinical setting. However, AALT and AAST reagents contain the coenzyme pyridoxal -5-phosphate (P-5-P) whereas ALT and AST reagents do not, so the use of the IFCC traceable k-factor for ALT and AST may result in either over or underestimation of true values for some patient or external proficiency survey samples.

## B-345

### Unnecessary Repeat Gamma Glutamyl Transferase Requesting

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

**Background:** There is presently much interest in reducing waste in health care. In laboratory medicine, unnecessary repeat testing is such a focus. Various Minimum Retesting Intervals (MRIs) for gamma glutamyl transferase (GGT) have been proposed: 24 hr, 36 hr, 3 days and 1 week. This study examined the pattern of repeat GGT testing in adult patients at a 1400 bed general hospital in Singapore. **Methods:** Anonymised details of all GGT testing (Beckman-Coulter DxC-800; glutamyl-p-nitroaniline and glycylglycine; 410 nm) for Jan-June 2016 were extracted from the laboratory information system for analysis in Excel. Repeat testing was calculated for the above time cut-offs (with a generous 2 hour buffer subtracted for day-to-day variation in ward round timing, phlebotomy rounds etc.). **Results:** There were 30446 requests in 6 months. 9776 (32 %) were repeat samples with up to 50 repeats in a single patient in a single admission. The cumulative distribution of repeat testing was: 1.0% within 10 hr (12 hr minus 2 hr) of the initial test, 4.0% within 22 hr, 10.8% within 34 hr, 13.3% within 46 hr, 19.7% within 70 hr, 27.7% within 166 hr. **Conclusion:** Using conservative MRIs of 36 hr and 72 hr, 11-20% of all GGT requests on inpatients are inappropriate repeats. This represents 19-34 tests per day. Poor understanding by clinicians of the timeframe for GGT change may contribute to this practice - better education and/or introduction of computerized minimum retest interval guidelines could reduce such over-requesting.

## B-346

### Evaluation of the Clinical Utility of Neutrophil Gelatinase Associated Lipocalin as a Biomarker of Kidney Injury in Patients with Monoclonal Gammopathies

P. Park, J. Seo, H. Lee. *Gachon medical school Gil medical center, Incheon-shi, Korea, Republic of*

***Background*** Monoclonal proteins are frequently associated with kidney injury in multiple myeloma (MM) and other plasma cell dyscrasias. We aimed to evaluate the clinical utility of neutrophil gelatinase associated lipocalin (NGAL), a sensitive marker of acute kidney injury, as a biomarker of renal impairment in patients with monoclonal gammopathies. ***Materials and Methods*** We studied 131 samples from 73 patients with monoclonal gammopaties (57 MM, 4 monoclonal gammopathy of undetermined significance [MGUS], 4 solitary plasmacytoma, 2 smoldering MM, 4 POEMS [polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes] syndrome, and 2 malignant lymphoma) and 30 healthy control samples. We measured serum creatinine (Cr), cystatin C, and NGAL (Bioporto Diagnostics, Denmark) on ADVIA Chemistry XPT System (Siemens Healthcare Diagnostics, USA). Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI cystatin C equation. Serum M protein and free light chain concentrations were also measured. ***Results*** There were significant correlation between serum NGAL level and Cr ($r$=0.68, $p$<0.001), cystatin C ($r$=0.73, $p$<0.001), and eGFR based on cystatin C ($r$=-0.43, $p$<0.001). Regarding the disease group, the median level (range) of NGAL were 122.8 ng/ml (29.0-1190.6 ng/ml) for MM, 234.7 ng/ml (137.0-428.9 ng/ml) for MGUS, 135.1 ng/ml (68.2-334.6 ng/ml) for solitary plasmacytoma, 132.1 ng/ml (73.5-190.7 ng/ml) for smoldering MM, 195.2 ng/ml (111.3-528.6 ng/ml) for POEMS syndrome, and 150.6 ng/ml (127.7-173.4 ng/ml) for malignant lymphoma. The median levels (range) of serum NGAL were 112.2 ng/ml (29.0-710.9 ng/ml), 162.7 ng/ml (64.2-563.3 ng/ml) and 380.5 ng/ml (173.4-1190.6 ng/ml) for patients with eGFR ≥60 ml/min, 30-59 ml/min and <30 ml/min, respectively ($p$-ANOVA<0.001). However, serum NGAL level did not correlate with serum M protein burden. Cystatin C levels were in significant relationship with M protein ($r$=0.33, $p$=0.005) and involved free light chain concentration ($r$=0.34, $p$=0.007). ***Conclusions*** Our data showed that serum NGAL levels were correlated with serum Cr and cystatin C, and measurement of serum NGAL could provide supportive information for monitoring of kidney function in patients with monoclonal gammopathy. **Keywords:** Cystatin C, Kidney injury, Monoclonal gammopathy, Neutrophil gelatinase associated lipocalin

## B-347

### CSF Total Protein Reference Intervals Determined from 20 years of Patient Data

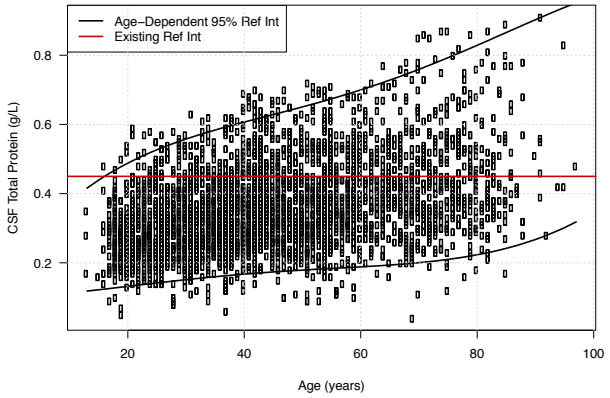
C. McCudden, P. Figurado, J. Brooks, P. Bourque. *The Ottawa Hospital, Ottawa, ON, Canada*

**Background:** Reference intervals are vital for interpretation of laboratory results. Many existing intervals employed for CSF total protein (CSF-TP) are from old literature, yet performing studies for such invasive samples is challenging. The objective of this study was to determine reference intervals for CSF-TP using patient data.

**Methods:** Twenty years of hospital database information was mined for previously reported CSF total protein results. Associated demographic, laboratory, and clinical diagnosis (ICD-9/10 codes) were extracted. CSF-TP results included three different analytical platforms, the Siemens Vista 1500, Beckman Lx20, and Roche Hitachi 917. From an initial data set of 18,119 samples, we removed cases with incomplete data and applied the following laboratory exclusion criteria: WBC>5×$10^9$/L, RBC>50×$10^9$/L, and glucose>2.5 mmol/L. Patient charts were also reviewed in detail to exclude 60 different conditions where elevated CSF-TP would be expected (e.g. Guillan Barre, brain tumors, MS); pediatric patients were also excluded. After exclusions, outliers were removed using Tukey's interquartile range method. A total of 3,186 samples were included (59% female, median age 44 years old). Continuous reference intervals were determined using quantile regression. Age and sex partitioned intervals were established using the quantile regression equation and splitting age groups into 5-year bins.

**Results:** CSF-TP showed a marked age dependence (Figure). Males had a significantly higher CSF-TP than females across all ages. CSF-TP results from the three different instruments and manufacturers showed no differences and values were consistent across two decades. CSF-TP showed weak, but statistically significant correlation with WBC, RBC, and creatinine ($R^2$<0.1), but was completely independent of serum total protein concentration.

**Conclusion:** CSF-TP reference intervals based on available literature and published in manufacturer's package inserts appear much lower than those determined in this study, particular with advancing age. This is the first report of a sex difference for CSF-TP. Improved diagnostic accuracy from age-partitioned reference intervals for CSF-TP may benefit patients.




## B-348

### Development of an Automated Enzymatic Method to Quantify Pyruvate Kinase in Red Blood Cells

J. Lu[1], B. Pulsipher[2], D. G. Grenache[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

**Background:** Pyruvate kinase (PK) deficiency, an autosomal inherited disorder, is the most common cause of nonspherocytic hemolytic anemia due to defective glycolysis. The objective of this study was to develop and validate an automated method to measure PK activity in red blood cells (RBC).

**Methods:** PK catalyzes the reaction of phosphoenolpyruvate with ADP to form pyruvate and ATP. In the presence of lactate dehydrogenase and NADH, pyruvate is reduced to lactate and NADH is oxidized to $NAD^+$. The rate of absorbance decrease at 340 nm is proportional to PK activity. PK and hemoglobin (Hb) measurements were performed on a Roche Cobas c501 analyzer using RBC hemolysates from residual patient samples sent to ARUP Laboratories. Hemolysates were prepared by combining RBCs with hemolyzing solution (5% Triton X-100, 0.27 M EDTA). After establishing a rate constant (k), accuracy, linearity, imprecision, analytical sensitivity, and analyte stability were validated and a PK reference interval was verified.

**Results:** The k-factor was established as -9477 by measuring PK activity in 10 patient samples on the c501. Accuracy was evaluated with replicate measurements of PK in 56 patient samples analyzed by the current laboratory-developed, manual PK assay and the c501 over 10 days. Linear regression produced a slope of 1.0, y-intercept of -0.57, and $R^2$ of 0.93. Linearity was determined by combining a high PK sample with hemolyzing solution in different ratios to create 6 samples that were tested in two replicates. Linear regression analysis produced a slope of 1.02, y-intercept of -2.68, and $R^2$ of 1.0. The assay was linear to 87 U/dL. Precision was evaluated by testing hemolysates stored at -70°C in 3 replicates once each day for 10 days. Within-run imprecision was 1.9 and 2.5% and total imprecision was 4.0 and 5.6% at 14.0 and 8.1 U/g Hb, respectively. The limit of blank (LOB) was 0.0 U/dL as calculated from the mean plus 3 SD of 10 replicates of saline. The limit of detection (LOD) was determined to be 1.0 U/dL calculated as the LOB added to 3 SD of 10 replicates of a hemolysate pool with a low PK activity (2.3 U/dL). Analyte stability was determined in 4 sample types, each at 2 PK activities. Compared to time zero, the changes of the PK activities were less than 7% when 1) whole blood specimens were stored at 4-8°C for 21 days; 2) hemolysates prepared from washed RBCs stored at -70°C for 1 month; 3) hemolysates in hemolyzing solution stored at -70°C for 2 weeks; and 4) hemolysates in hemolyzing solution stored at -20°C for 2 days. The current PK reference interval of 4.6-11.2 U/g Hb was verified by measuring PK activity in 20 healthy individuals (10 males and 10 females; 21-59 years old). The mean PK activity in paired EDTA and heparinized blood samples obtained from the same individuals was not significantly different (7.8 vs. 7.9 U/g Hb, respectively; p=0.94).

**Conclusion:** This automated assay for quantifying PK in RBCs has acceptable performance characteristics and is fit for intended use.

## B-349

### Performance Evaluation of the ADVIA Chemistry System Alanine Aminotransferase P5P (ALTPLc) and Aspartate Aminotransferase P5P (ASTPLc) Assays

J. Thomas[1], S. Janas[1], S. A. Lewisch[1], K. Piper[2], L. Sinclair[2], Z. Burnside[2]. *[1]Siemens Healthineers, Newark, DE, [2]Randox Laboratories Ltd., Crumlin, United Kingdom*

**Background:** The ADVIA® Clinical Chemistry (CC) Alanine Aminotransferase with liquid P5P (ALTPLc)* and Aspartate Aminotransferase with liquid P5P (ASTPLc)* liquid reagent assays are IFCC traceable. Each assay contains liquid, ready to use P5P (pyridoxal-5′-phosphate), an enhancement over the lyophilized P5P used in the Siemens Heathineers ADVIA ASTP_c and ALTP_c assays.

**Methods:** The ALTPLc and ASTPLc assays are adaptations of the IFCC Reference Methods. The reactions are initiated by the addition of α-ketoglutarate. The concentration of reduced nicotinamide adenine dinucleotide (NADH) is measured by its absorbance at 340/410 nm; the rate of absorbance decrease is proportional to the analyte concentration. Both the ADVIA ASTPLc and ALTPLc assays provide IFCC reference assay traceable results on both serum and plasma within the analytical range of 8-1000 U/L (ASTPLc) and 9 - 1000 U/L (ALTPLc) undiluted. Each assay can be extended up to 7800 U/L with dilution. Both assays accomplish this with a 25 µL sample size at a time to first result of 10 minutes.

**Results:** Observed patient sample agreement via method comparison studies for the ALTPLc assay is as follows: ADVIA CC ALTPLc = 0.96 * IFCC Reference Assay - 1.6 U/L (r = 1.00, n = 101, range 12 to 1016 U/L); ADVIA CC ALTPLc = 1.03 * ADVIA CC ALTP_c + 0.2 U/L (r = 1.00, n = 104, range 10 to 950 U/L) ADVIA CC ASTPLc = 1.03 * IFCC Reference Assay - 1.6 U/L (r = 1.00, n = 103, range = 9.9 to 910.9 U/L) ADVIA CC ASTPLc = 0.97 * ADVIA CC ASTP_c - 1.4 U/L (r = 1.00, n = 103, range = 11 to 970 U/L). Precision was evaluated across the assay range per CLSI EP05-A2 using serum/plasma pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 3.6 % CV and ≤ 4.9 % CV for ALTPLc and ≤ 1.1 %CV and ≤ 2.1 %CV for ASTPLc, respectively. Limit of Blank, Limit of Detection and Limit of Quantitation were observed to be 2, 4 and 9 U/L for ALTPLc, and 2, 4 and 8 U/L for ASTPLc respectively. Minimal interference (≤ 10%) was observed for both assays for Bilirubin (20 mg/dL) and Lipemia (500 mg/dL). Agreement of serum and plasma in the ALTPLc assay is represented by: Plasma = 0.99 * Serum - 0.3 U/L (n = 50, range = 15 to 916 U/L) and for the ASTPLc assay by: Plasma = 1.02 * Serum - 1.0 U/L (n = 53, range = 21 to 961 U/L).

**Conclusion:** The ADVIA CC Alanine Aminotransferase (P5P; ALTPLc) and Aspartate Aminotransferase (P5P; ASTPLc) assays exhibit equivalent performance characteristics to the current Siemens ALTP_c / ASTP_c assays respectively and provide greater ease of use by virtue of ready to use liquid P5P. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements. ADVIA and all associated marks are trademarks of Siemens Healthcare Diagnostics, Inc. or its affiliates.

## B-350

### Performance Evaluation of the Atellica CH Creatinine Kinase, Alanine Aminotransferase (with P5P), and Aspartate Aminotransferase (with P5P) Assays

J. Thomas, S. Janas, S. A. Lewisch. *Siemens Healthineers, Newark, DE*

**Background:** This investigation evaluates the performance of clinical chemistry enzyme assays including creatine kinase (CK_L), alanine aminotransferase with P5P (ALTPLc), and aspartate aminotransferase with P5P (ASTPLc) on the Atellica Chemistry (CH) Analyzer.*

**Methods:** Performance testing, evaluated using Clinical and Laboratory Standards Institute (CLSI) guidelines, included precision (CLSI EP05-A3); method comparison (CLSI EP09-A3); limit of blank, detection, and quantitation (CLSI EP17-A2); interference (CLSI EP07-A2); and serum/plasma equivalence (CLSI EP09-A3).

**Results:** Assay range for the CK_L assay is 15 to 1300 U/L, the ASTPLc assay is 8 to 1000 U/L, and the ALTPLc assay is 9 to 1000 U/L. Observed agreement in patient sample method comparison studies using Deming regression: Atellica CH CK_L assay = 0.96 * ADVIA® Clinical Chemistry CK_L assay − 3.1 U/L (r = 1.00, n = 177, range: 17-1289 U/L); Atellica CH ALTPLc assay = 1.02 * ADVIA CC ALTPLc assay − 0 U/L (r = 0.999, n = 103, range: 10-995 U/L); and Atellica CH ASTPLc assay = 1.00 * ADVIA CC ASTPLc assay − 4 U/L (r = 0.999, n = 110, range: 13-984 U/L). Precision was evaluated across the assay range using serum/plasma pools

and commercial quality control materials. Each sample was assayed in duplicate twice a day for 20 days. Creatine kinase assay repeatability and within-lab precision were ≤1.6% CV and ≤2.4% CV, respectively. Aspartate aminotransferase assay repeatability and within-lab precision were ≤1.8% CV and ≤1.8% CV, respectively. Alanine aminotransferase assay repeatability and within-lab precision were ≤1.8% CV and ≤2.7% CV, respectively. Limit of blank, limit of detection, and limit of quantitation were observed to be 1, 6, and 6 U/L for creatine kinase; 0, 1, and 7 U/L for ASTPLc; and 2, 3, and 5 U/L for ALTPLc, respectively. Minimal interference (≤10%) was observed for the creatine kinase assay with hemolysate (125 mg/dL), bilirubin (60 mg/dL), and lipemia (1000 mg/dL). Minimal interference (≤10%) was observed for both the ALTPLc and ASTPLc assays for bilirubin (20 mg/dL) and lipemia (500 mg/dL). Agreement of serum and plasma in the CK_L assay is represented by plasma = 0.99 * serum − 0.3 U/L (r = 1.00, n = 57, range: 57-1062 U/L); in the ASTPLc assay by plasma = 0.99 * serum + 2 U/L (r = 1.00, n = 56, range:10-982 U/L); and in the ALTPLc assay by plasma = 0.96 * serum + 0 U/L (r = 0.999, n = 56, range: 9-936 U/L).

**Conclusion:** The creatine kinase, alanine aminotransferase (with P5P), and aspartate aminotransferase (with P5P) assays tested on the Atellica CH Analyzer demonstrated acceptable performance in all tested areas. *Under development. Not available for sale.

## B-351

### Performance Evaluation of the ADVIA Chemistry System Creatine Kinase Liquid Assay

J. Thomas[1], T. Johnson[1], S. Janas[1], S. A. Lewisch[1], K. Piper[2], L. Sinclair[2], Z. Burnside[2]. *[1]Siemens Healthineers, Newark, DE, [2]Randox Laboratories Ltd., Crumlin, United Kingdom*

**Background:** The ADVIA Chemistry Liquid Stable Creatine Kinase Liquid assay (CK_L) is a calibrated, IFCC traceable, chemistry assay. This assay is an improvement over the ADVIA CKNAC assay by virtue of its calibration, ready to use reagents, and longer onboard stability. Performance of the Creatine Kinase assay is described below.

**Methods:** Creatine Kinase reacts with creatine phosphate and adenosine diphosphate (ADP) to form adenosine triphosphate (ATP), which is coupled to the hexokinase-G6PD (glucose-6-phosphate dehydrogenase) reaction, generating NADPH (reduced nicotinamide adenine dinucleotide phosphate). The concentration of NADPH is measured by the increase in absorbance at 340/596 nm. The resulting reaction rate signal is proportional to the concentration of analyte in the sample. The ADVIA Chemistry CK_L assay is an adaptation of the IFCC Reference Method. The ADVIA Creatine Kinase assay provides IFCC reference assay traceable results within the analytical range of 15-1300 U/L undiluted (up to 7800 U/L with dilution) on both serum and plasma. The assay accomplishes this with a 4.5 µL sample size at a time to first result of 10 minutes. The assay has an onboard stability and calibration interval of 30 days - an increase of 10 days from those of the CKNAC assay.

**Results:** Observed agreement in patient sample method comparison studies versus two different systems: ADVIA Chemistry CK_L = 1.05 * IFCC Reference Assay - 6.9 U/L (r = 1.00, n = 100, range=16-1245 U/L), ADVIA Chemistry CK_L = 1.01 * ADVIA Chemistry CKNAC - 1.8 U/L (r = 1.00, n = 116 range = 22-1280 U/L). Precision was evaluated across the assay range per CLSI EP05-A2 using serum/plasma pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 2.0 %CV and ≤ 3.4 %CV, respectively. Limit of Blank, Limit of Detection and Limit of Quantitation were observed to be 3, 6 and 15 U/L respectively. Minimal interference (≤ 10%) was observed with Hemolysate (125 mg/dL), Bilirubin (60 mg/dL), Lipemia (1000 mg/dL), Ascorbic Acid (6mg/dL), Sulfasalazine (300 mg/L), and Sulfapyridine (300 mg/L). Agreement of serum and plasma in this assay is represented by: Plasma = 1.01 * Serum - 0.6 U/L (r = 1.00, n = 55, range=37-1282 U/L).

**Conclusion:** The ADVIA Clinical Chemistry Creatine Kinase assay exhibits equivalent performance characteristics and shows improvement to the current Siemens Healthineers ADVIA CKNAC assay by virtue of its ready-to-use reagents, calibration and improved onboard stability.



## B-352

### A mutation at factor VII protease domain *N*-glycosylation site may contribute to the coagulation disorder

H. Wang, Q. Wu. *Cleveland State University, Cleveland, OH*

**Background:**

Factor VII is an important protein in coagulation. Plasma factor VII interacts with the tissue factor released from the injured blood vessel and initiates coagulation. Factor VII deficiency may cause the coagulation disorder and bleeding. In clinical laboratories, it is diagnosed by factor VII assay.

Factor VII is secreted from hepatocytes into the circulatory system. Impaired secretion of factor VII is a cause of factor VII deficiency. Factor VII mutations with poor secretion have been identified in patients with factor VII deficiency. Genetic testing for factor VII deficiency is offered clinically.

Factor VII has one *N*-glycosylation site in protease domain. Previous studies showed that lack of protease domain *N*-glycans impaired factor VII secretion. However, the underlying mechanism is unknown. Meanwhile, a mutation at factor VII protease domain *N*-glycosylation site has been reported in human, but it has not been characterized yet.

**Objectives:**

The aims of our study were to investigate the underlying mechanism of protease domain *N*-glycans promoted factor VII secretion and to characterize the reported mutation at factor VII protease domain *N*-glycosylation site.

**Methods:**

cDNA of factor VII was inserted into the expression vector containing C-terminal V5 tag to generate wild type (WT) factor VII plasmid. Plasmids expressing factor VII mutants, N360Q and N360D, were made by PCR-based mutagenesis. HEK293 cells were transfected with plasmids for WT factor VII and mutants, respectively. After one-day culture, proteins binding to factor VII in the cells were co-immunoprecipitated with factor VII and factor VII in the conditioned medium was immunoprecipitated. The accumulated proteins were analyzed by SDS-PAGE and Western blotting.

**Results:**

Factor VII levels in the cell lysate were similar for WT factor VII and mutants. Compared with WT, N360Q had reduced level of factor VII in the conditioned medium (45 ± 9% *vs*. WT). In the meantime, the level of calnexin binding to N360Q was higher than that in WT (124 ± 6% *vs*. WT). Moreover, the level of BiP binding to N360Q increased significantly (232 ± 45% *vs*. WT). N360D, which is corresponding to the reported human mutation, had a reduced level of factor VII in the conditioned medium (32 ± 9% *vs*. WT). N360Q and N360D in the cell lysate and conditioned medium migrated faster than WT, supporting that the two mutants lacked *N*-glycans.

**Conclusion:**

The mutations of asparagine at *N*-glycosylation site disrupted the attachment of *N*-glycans to protease domain of N360Q and N360D, hence, proteins migrated faster in SDS-PAGE. Without protease domain *N*-glycans, secretion of N360Q was impaired, showing protease domain *N*-glycans are important in factor VII secretion. At the same time, more factor VII was retained with calnexin and BiP, indicating lower efficiency of utilizing calnexin-mediated protein folding and more misfolded protein removed by BiP. These data reveal that factor VII protease domain *N*-glycans are critical for protein folding and impaired protein folding leads to poor protein secretion. The reported mutation in human also impaired factor VII secretion as shown in N360D, suggesting the mutation disrupting factor VII protease domain *N*-glycosylation exists in human and it may contribute to coagulation disorder.

## B-353

### Evaluation of serum zinc concentration using protein fraction waveform data

T. Hisahara[1], H. Kataoka[2], S. Yamanaka[1], K. Ogura[1], Y. Matsumura[1], T. Sugiura[1]. *[1]Kochi Medical School, Nankoku, Japan, [2]Kawasaki University of Medical Welfare, Kurashiki, Japan*

**Background:** Zinc (Zn), one of the essential trace elements of human body, has a wide role including metabolism of the skin and hair, cell division, cell metabolism, taste and immunity. In a case of elderly, fall in serum Zn concentration has a risk for appetite loss and malnutrition, and generation of pressure ulcer. On the other hand increase of serum Zn concentration is observed in hyperthyroidism, polycythemia, essential hypertension and hemolytic anemia. Therefore, measurement of serum Zn concentration is important, but as the cost of measurement is expensive frequent

measurement of Zn is unreasonable. The aim of this study was to examine serum Zn concentration using the protein fraction waveform with a relatively low cost, and to determine the clinical utility of this method for predicting serum Zn concentration.

**Methods:** Serum Zn concentration was measured (colorimetric method) using 277 specimens submitted to routine laboratory test. Protein electrophoresis was normalized using raw electrophoresis waveforms obtained by Sebia CAPILLARYS2 PROTEIN(E)6. Raw waveform data were normalized without eliminating the information between the albumin (ALB) and α1 fractions. Mobility data was corrected by positioning the ALB peak at 75, and N,N-dimethylformamide (DMF : used as internal standard in pre-examination) at 300 and β1 peak position at 203. ROC analysis (≦65μg/dL : 1,≧66μg/dL : 0) was conducted at each 300 point of the normalized mobility. Logistic regression analysis was performed using these data as explanatory variables and a prediction formula was obtained to detect serum Zn concentration.

**Results:** Decrease in serum Zn concentration was associated with drop (AUC = 0.737) in the tail position of preALB (mobility : M33), drop (AUC = 0.750) in the tail position of ALB (mobility : M77) and drop (AUC = 0.705) in the peak position of β1 (mobility : M203) by the ROC analysis. The logistic regression formula : 3.607+(-0.065) × M33+(-0.012) × M82+0.015 × M83 (AUC =0.791) was obtained.

**Conclusion:** A high diagnostic characteristic associated with decrease in serum Zn concentration existed at the mobility 33, 77 and 203 of the protein fraction pattern. In addition to evaluation of 5 to 6 fractions of the protein electrophoresis, detecting distortion of the wave form peak of the wave form mobility by applying the normalization technology was also important to predict serum Zn concentration.

## B-354

### Evaluation of Immunoturbidimetric Assays Using the $SPA_{PLUS}$: Comparison with Immunonephelometry

E. Üner[1], R. Turkal[2], T. Çevlik[2], G. Haklar[1], Ö. Sirikçi[1]. *[1]Department of Biochemistry, School of Medicine, Marmara University, İstanbul, Turkey, [2]Biochemistry Laboratory, Marmara University Pendik E&R Hospital, İstanbul, Turkey*

**Background:** Specific proteins have been traditionally analyzed with immunonephelometry. Improvements led immunoturbidimetry to be considered as a validated alternative to immunonephelometry. Before switching to turbidimetric assays, a laboratory should ensure that performance is acceptable for clinical needs, or "fit for purpose". We aimed to verify the performance claims for precision and trueness of IgA, $IgG_4$ and kappa (κ) / lambda (λ) free light chains (FLC) and total light chains (TLC) assays on $SPA_{PLUS}$, that were previously validated by the manufacturer. We also evaluated low values of IgA for patients diagnosed with selective IgA deficiency based on serum IgA concentration. According to the European Society for Immunodeficiency, the definitive diagnosis of (selective) IgA deficiency can be made in patients older than four years of age with a serum IgA of less than 0.07g/L. **Methods:** Assay imprecision was evaluated following the Clinical Laboratory Standards Institude (CLSI) protocol EP15-A2, using three replicates per day for two levels of serum pool over 5 days (n=15 per level.). In order to demonstrate trueness, we used materials of minimum of two analyte concentrations sent for external quality control program. These materials were measured by a number of laboratories, and their peer group mean value (assigned value) was used to assess agreement. In addition, results obtained with $SPA_{PLUS}$ were compared to those obtained with the nephelometer Immage 800. The comparison also included Ig A results less than 0.0667 g/L, which is the limit of quantitation (LOQ) of Immage 800. **Results:** The total precision (CV %) ranged from a low of 0.93% (Ig $G_4$) to a high of 11.86% (κ FLC). The precision was less than 2.0% for 4 of the 12 serum pool samples. As the estimated within-run and total imprecision for all parameters except Ig A, kappa and lambda TLC were less than or equal to the verification value, we concluded that the data were consistent with the manufacturer's claim. Total imprecision for Ig A, kappa and lambda TLC were checked to see whether they are within the acceptable desirable specifications on Westgard's website updated in 2014 and none were outside desirable limits of imprecision (2.4%, 2.7%, 2.7%; respectively). In addition, the verification of manufacturer's claimed bias was achieved as the verification interval included the assigned value for all parameters. The correlation coefficients of linear regressions were between 0.84 and 0.98 for the analytes measured. Among the 37 patients with Ig A values <0.0667 g/L on Immage 800, 21 had results below the LOQ of SPA plus (<0.02 g/L), 2 had results greater than 0.07 g/L and the 14 remaining patients' results were between 0.02 g/L - 0.0667 g/L. **Conclusion:** In conclusion, $SPA_{PLUS}$ immunoturbidimetric assay is suitable for routine use, and correlates well with representative immunonephelometric assays on the Beckman Immage 800 analyzer. It might also be an increasingly accepted alternative to nephelometry especially for some selected parameters like IgA.

## B-355

### Reference values capillary protein electrophoresis of a carefully selected healthy population adhering to IFCC recommendations

A. Regeniter[1], L. Kadriu[2], A. U. Monsch[1]. *[1]University of Basel, Basel, Switzerland, [2]Lucerne City hospital, Luzerne, Switzerland*

Objective: We established percentual and absolute reference values for electrophoresis fractions on the Sebia Capillarys system.

Methods: The study group consisted of healthy individuals from a geriatric study supplemented with working individuals (age range 19-89, n= 478). Malnutrition (transthyretin), immunological status (IgG, IgA, IgM), fatty acid metabolism (cholesterol, triglycerides), iron metabolism (Iron, ferritin, transferrin, soluble transferrin receptor), kidney (creatinine), liver function (ASAT; ALAT, GGT) and blood differential were used to rigidly biochemically classify the study participants.

Results: 42.7% (n=204) had all values in the reference range, at least one value was elevated in 48.5% (n=232) and 8.8% (n=42) showed a monoclonal gammopathy. Subjects above 60 presented with statistically significantly lower albumin. We found no sex related differences, but women using oral contraceptives (n=41) had a statistically higher alpha-1 (median increase: 0.5 g/L) and beta-1 fraction (median increase: 0.85 g/L). These were excluded, yielding 69 men and 106 women (n=175) with a median age of 63 years (range 19- 92)..

| Fractions | g/L | | % | |
|---|---|---|---|---|
| | P 2.5 | P 97.5 | P 2.5 | P 97.5 |
| **Albumin < 60 years** | 41 | 49.9 | 56.2 | 69.5 |
| **> 60 years** | 36.4 | 45.9 | 55.5 | 67.4 |
| **Alpha-1** | 2 | 3.5 | 2.7 | 4.7 |
| **Alpha-2** | 5.2 | 9.2 | 7.4 | 13 |
| **Beta** | 5.6 | 9.8 | 8.1 | 13.4 |
| **Beta-1** | 3.5 | 5.3 | 5 | 7.2 |
| **Beta-2** | 1.8 | 5.1 | 2.6 | 7 |
| **Gamma** | 6.2 | 15.3 | 9.6 | 19.6 |

Conclusion: To our knowledge this is the first study that establishes percentual and absolute electrophoresis reference ranges according to IFCC criteria. Our rigidly selected reference range group markedly surpasses the recommended IFCC sample limit of 120 and showed a normal Gaussian distribution in all fractions. Albumin age related differences or the effect of oral contraceptives, however, still need further verification with additional larger, rigidly classified subgroups.

## B-356

### Validation of Alkaline Phosphatase Isoenzyme Assay by Electrophoresis

K. Gonzalez, T. Daly, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Alkaline Phosphatase (ALP) is the enzyme that catalyzes hydrolysis of phosphate esters at an alkaline pH. The liver, bone, intestine, and placenta are of the most ALP-abundant, producing organ specific isoforms. Differentiating these ALP isoforms in circulation may provide valuable diagnostic information. Here we report performance characterization of alkaline phosphatase isoenzyme by electrophoresis.

Method: Validation testing was performed on the SPIFE 3000 with SPIFE alkaline phosphatase isoenzyme gels/reagent (Helena Laboratories, Beaumont, TX, USA) using leftover patient serum samples in our laboratory. Performance validation included precision, method comparison, stability, and reference intervals. Data was analyzed using EP Evaluator Version 10 (Data Innovations LLC, Burlington, VT, USA).

Results: Within-day coefficient of variation (CV) was assessed by analyzing two patient pools 10 times within a single batch, and was found to be 0.4-1.7% for the liver isoenzyme, 1.1-3.0% for the bone isoenzyme, and 17.2% for the intestine isoenzyme. Between-day CV was evaluated by assaying the same pools twice a day for 10 days, and was found to be 2.8%-3.5% for the liver isoenzyme, 3.0-3.6% for the bone isoenzyme, and 207.0-330.4% for the intestine isoenzyme. The large CV for intestine fraction was due to the small quantity in the specimens. This method was compared with a similar agarose electrophoresis method offered by a reference laboratory using 38 leftover patient samples. The comparison offered a slope of 0.988, an intercept of 2.11, and an R value of 0.9662 for the liver fraction, a slope of 1.059, an intercept of 1.96, and an R value of 0.9568 for the bone fraction, and a slope of 0.849, an intercept of 0.36, and an R value of 0.9877 for the intestine fraction. All

isoenzyme fractions were found stable in serum for 24 hours at room temperature, 7 days under refrigeration, and 14 days at -20 °C. Specimens (n=209) used for reference range studies had normal total ALP and no clinical indication for abnormal liver, bone, or intestine conditions. The reference intervals indicated in the package inserts were verified using the reference specimens: liver isoenzyme was 26.0-86.2% and the bone isoenzyme was 10.7-68.3% for both genders. We established reference intervals for the intestine isoenzyme fraction (0.0-24.2%) and calculated isoenzyme activities: 14.9-66.8 U/L (liver), 12.2-51.3 U/L (bone), and 0.0-15.3 U/L (intestinal).

Conclusion: The SPIFE 3000 alkaline phosphatase isoenzyme assay by electrophoresis was validated for patient testing. Reference intervals were either verified to the package inserts or established using the reference specimens collected locally.

## B-357

### Evaluation of free light chains and various protein assays on the Optilite® turbidimetric analyser

F. Leung, M. Spencer, I. M. Blasutig. *University of Toronto, Toronto, ON, Canada*

**Background:** The measurements of serum free light chains (FLC), immunoglobulins (Ig), complement factors 3 (C3) and 4 (C4), and acute-phase proteins are used routinely for the management of various diseases. Serum levels of intact Igs and FLC can indicate plasma cell dyscrasias or immunodeficiencies while levels of complement and acute-phase proteins can reflect the presence of autoimmune disorders, hematologic syndromes or rare diseases such as Wilson's disease.

**Objectives:** To evaluate the performance of the IgG, IgA, IgM, kappa FLC (κ-FLC), lambda FLC (λ-FLC), C3, C4, haptoglobin (HG), and ceruloplasmin (CP) assays on the Optilite® turbidimetric analyser.

**Methods:** Precision was assessed using three levels of third-party quality control material tested over 10 working days in duplicate once per day. Correlation studies were performed with a minimum of 40 patient samples compared against the Siemens BN™II Nephelometer or the Binding Site SPAPLUS® Analyser (FLCs only). Furthermore, the FLC assays were evaluated for antigen excess detection and carryover.

**Results:** All assays gave imprecision of <8% CV at every level. Deming regression showed good agreement with slopes of 1.00 ± 0.2 with the exception of κ-FLC (0.72), HG (0.89), and CP (1.38). All analytes displayed R>0.9. For the FLC assays, antigen excess detection was confirmed using n = 7 (κ-FLC) and n = 3 (λ-FLC) samples and carryover was not observed.

**Conclusions:** The Optilite® analyser provides robust measurements for Igs, FLCs and acute-phase proteins.

Table 1 - Precision and correlation statistics

| Study | Metric | | IgG | IgA | IgM | κ-FLC | λ-FLC | C3 | C4 | HG | CP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Precision | Total CV (%) | Level 1 | 3.5 | 3.5 | 2.4 | 2.9 | 0 | 5.1 | 3.6 | 7.2 | 7.5 |
| | | Level 2 | 3.2 | 3.0 | 2.1 | 1.4 | 3.7 | 4.0 | 4.4 | 3.9 | 6.1 |
| | | Level 3 | 2.4 | 2.9 | 1.4 | 2.2 | 4.7 | 2.9 | 4.4 | 3.1 | 4.1 |
| Correlation | Deming slope | | 1.08 | 1.05 | 1.08 | 0.72 | 1.21 | 1.13 | 1.06 | 0.89 | 1.38 |
| | Deming y-intercept | | -1.02 | -0.12 | 0 | 11.02 | -21.94 | 0.13 | 0 | 0.06 | -0.04 |
| | R | | 0.992 | 0.997 | 0.999 | 0.976 | 0.988 | 0.975 | 0.990 | 0.987 | 0.914 |
| | n | | 57 | 57 | 59 | 54 | 55 | 76 | 72 | 52 | 59 |

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

TDM/Toxicology/DAU

## B-358

### Toxicity by Hair Dye in Upper Egypt

H. A. Ahmed. *Ministry of Justice, Assuit, Egypt*

**Background**: Hair-dye containing paraphenylenediamine is widely used in Middle East and some Asian countries. Many cases of toxicity and mortality either due to accidental or deliberate ingestion of hair dyes were reported. The aim of the present work was the chemical analysis of the black hair dye, to analyze the various aspects of acute poisoning through a retrospective study of fatalities reported in seven governors in Upper in Egypt as a result of its ingestion and if there is a dose-effect relationship. **Method**: the records of acute poisoning cases of seven governors in Upper Egypt investigated by Assiut Forensic Chemical Laboratory in the period from January 2002 to December 2008 were examined as regards type of poison, pattern, incidence, age, sex, geographical distribution and mode of poisoning. The studying of the systemic effects of ingestion of hair dye was conducted by oral administration of hair dye in different doses (500, 200, 100, and 50) to four groups of albino rats. The clinical manifestation was observed and the light microscopic examination of sections of vital organs was done. **The result**: The result revealed that about a fifth of the acute poisoning fatalities investigated by Assiut forensic chemical laboratory were due to ingestion of hair dye. The highest majority of them were suicide cases, particularly in Kenya, Sohag and Aswan Governorates respectively, with a female predominance. The highest percentage was found in the age group (31-40) years, followed by (21-30) years. Death occurred within five minutes in the first group, within ten minutes in the second group and within an hour in the third group. The animals of the fourth group survived until sacrificed after one week. The most common histopathological changes in all studied organs were vascular congestion and lymphocyte infiltration, with degenerative changes in the hepatocytes and the destruction of the renal tubules. **Conclusion**: Deliberate self-poisoning by hair dye is a major problem in Upper Egypt particularly in females. The main toxic effects were directed to the liver and kidneys while the other studied organs were affected to a mild extent. Also, there was a well-established dose-effect relationship.

**Keyword:** paraphenylenediamine, Upper Egypt, Assiut forensic chemical laboratory

## B-359

### Evaluation of the semi-automated electrochemiluminescence immunoassay for cyclosporine, tacrolimus, and sirolimus

A. W. S. Fung[1], M. J. Knauer[2], I. M. Blasutig[3], D. A. Colantonio[2], V. Kulasingam[3]. *[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada, [3]University Health Network, Toronto, ON, Canada*

**Background:** Therapeutic drug monitoring (TDM) of immunosuppressant drugs are used to monitor drug efficacy and toxicity and to prevent organ transplantation rejection. The evaluation of immunoassay-based immunosuppressant drugs methods at a major transplant hospital is important to identify method suitability and limitations. This study evaluates the analytical performance of the recently available semi-automated competitive electrochemiluminescence immunoassay (ECLIA) for immunosuppressant drugs (ISDs) including cyclosporine (CSA), tacrolimus (TAC), and sirolimus (SRL) on the Roche cobas e411 platform. To our knowledge, this is the first report on method evaluation of sirolimus ECLIA method. **Methods:** Residual EDTA whole blood samples that spans the analytical measuring range from patients undergoing immunosuppressant therapy were used to validate the electrochemiluminescence immunoassays for CSA, TAC, and SRL on the Roche cobas e411 platform. Total imprecision was evaluated using manufacturer and third party Bio-Rad whole blood immunosuppressant controls. Linearity was evaluated by measuring CAP linearity material or a series of dilutions spanning the analytical measuring range using a patient sample with concentration above the upper measuring range. Method comparison was assessed by comparing Roche cobas ECLIA ISD assays with Abbott ARCHITECT i2000 chemiluminescent immunoassay (CMIA) ISD assays and an ESI-LC-MS/MS ISD assays using residual patient samples spanning the analytical measuring range. Functional sensitivity and lot-to-lot comparison were also evaluated. **Results:** Total imprecision ranged from 3.3 to 7.1% for CSA, 3.9 to 9.4% for TAC, and 4.6 to 8.2% for SRL (CV goal of ≤7%). Linearity was verified from 30.0 to 960.9 ng/mL for CSA (claimed AMR 30.0-2000.0 ng/mL), from 1.1 to 27.1 ng/mL for TAC (claimed AMR 0.5-40.0 ng/mL), and from 0.5 to 32.3 ng/mL for SRL (claimed AMR 0.5-30.0 ng/mL). The functional sensitivity (CV ≤20%) met the manufacturer's claims and were determined to be <6.5 ng/mL for CSA (claimed LoQ 50.0 ng/mL), 1.1 ng/mL for TAC (claimed LoQ 1.0 ng/mL), and <0.1 ng/mL for SRL (claimed LoQ 1.5 ng/mL). Deming regression analysis of method comparisons of Roche ECLIA with the Abbott CMIA (n=100) yielded the following. For CSA, slope of 0.917 (95%CI: 0.885-0.949), intercept -15.2 (95%CI: -39.4-9.0) and *r* of 0.985. For Tac, slope of 0.938 (95%CI: 0.895-0.981), intercept of 0.2 (95%CI: -0.4-0.8) and *r* of 0.974. For SRL, slope of 0.842 (0.810-1.110), intercept of 0.9 (95%CI: 0.4-1.4) and *r* of 0.982. Deming regression analysis of comparisons of Roche ECLIA with an ESI-LC-MS/MS method (n=20) yielded the following. For CSA, slope of 1.331 (95%CI: 1.167-1.496), intercept of -68.0 (95%CI: -167.7-31.8) and *r* of 0.969. For TAC, slope of 0.924 (95%CI: 0.843-1.005), intercept of -0.2 (95%CI:-2.3-1.9) and *r* of 0.984. For SRL, slope of 0.971 (95%CI: 0.913-1.030), intercept of 2.4 (95%CI:0.5-2.4) and *r* of 0.993. A general positive bias is observed when ECLIA and CMIA are compared to LC-MS/MS assays suggesting analytical interference possibly due to cross-reactivity with drug metabolites. **Conclusions:** The Roche Elecsys ECLIA-based ISD assays have acceptable precision, linearity, and functional sensitivity. The method comparisons demonstrated the method is comparable to Abbott ARCHITECT immunoassays and LC-MS/MS and is fit for purpose for therapeutic drug monitoring of immunosuppressant drugs (CSA, TAC, and SRL).

## B-360

### Distribution of mephedrone in blood and brain tissue of rabbit after two different sublethal doses

K. M. Mohamed[1], R. M. Elsayed[2], R. A. Radwan[2]. *[1]Forensic Chemistry Department, College of Forensic Sciences, Naif Arab University for Security Sciences, Riyadh, Saudi Arabia, [2]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Sohag University, Sohag, Egypt*

**Introduction**: Abuse of mephedrone (4-methylmethcathinone) has been frequently emerged among the last few years. Intoxication and fatality associated with mephedrone have been reported in literature.

**Objective**: The aim of this study was to investigate the distribution of mephedrone in whole blood and brain tissue after two different sublethal doses.

**Methods:** Mephedrone was administered to male white Newzeland rabbits orally (40mg/kg) and (100 mg/kg) and then euthanized after three hours. Samples were analyzed by gas chromatography-mass spectrometry (GC-MS). Pretreatment of the sample was involved protein precipitation of the blood (1.0 mL) with acetonitrile and tissue homogenization of the brain with 1.0 N HCl. Isolation of mephedrone from biological sample was required one step derivatization-extraction method by ethyl chloroformate (ECF) as derivatizing reagent and ethyl acetate as extracting solvent. Methamphetamine-d5 was used as internal standard. The method was validated for linearity, sensitivity, precision and accuracy prior to rabbit samples analysis.

**Results and discussion**: Calibration curves were found to be linear over the concentration ranges of 10-2000 ng/mL (blood) and 25-2000 ng/mL (brain). The precision and accuracy date were within the acceptable limits (< 15%). The average concentrations of mephedrone in blood after 1/10 and 1/14 of the lethal dosage were 41.52 and 353.71 ng/mL, respectively, while the brain was 37.79 and 258.92 ng/mL, respectively. The concentration of mephedrone in blood was nearly closed from the brain.

**Keywords**: Mephedrone; GC-MS; Validation; Rabbit blood and brain; Concentration levels

## B-361

### The Effect of Electronic Waste Leachate on Liver Function of Wistar Albino Rats

B. C. Esogwah[1], G. R. E. E. Ana[2], O. M. Bolaji[2]. *[1]University College Hospital, Ibadan, Nigeria, [2]University of Ibadan, Ibadan, Nigeria*

**Background:** Electronic Wastes (E-wastes) Leachate (EWL) releases heavy metals and other persistent toxins into the environment when burnt . As rain water percolates through landfills and dumpsites, toxic substances escape as leachate into soil thereby posing risk to public health. This study was carried out to determine the *invivo* effect of EWL on liver of albino rat.

**Methods:** EWL was obtained from Oke-padre, Ibadan, Nigeria dump site and simulated using the American society for testing and materials (ASTM) method. Forty (40) male strain albino rats were randomly assigned into 8 groups of 5 rats each. Rats were fed on pellets and water ad-libitum. Group one - Control Group (CG) were given deionized water while the Experimental Groups (EG) two to six were treated with ascending concentrations (20%, 40%, 60%, 80% and 100%) of the leachates respectively and groups seven and eight were given 20mg/kg of $PbCl_2$ and 40mg/kg of $CuCl_2$ per body weight respectively, orally for 14 days. The rats were sacrificed 24 hours after the last administration of treatment. Blood was collected for biochemical analysis of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline phosphatase (ALP) using International Federation for Clinical Chemistry method. Total protein and Albumin were analysed using biuret and bromocresol green methods respectively. The results were analysed using descriptive statistics, t-test and ANOVA at p=0.05.

**Results:** Mean concentration of AST (158.4±24.1iu/l) and ALT (62.6±9.7iu/l) were significantly higher in EG2 when compared to the AST (99.0±41.6iu/l) and ALT (46.2±12.7iu/l) in CG. While a significant decrease was observed for ALP in EG3 (234.20±120.4iu/l) and EG4 (138±40.7iu/l) when compared with the CG (422.00±111.7iu/l). There was a significant increase in the total protein (8.32±0.4g/dl) and albumin (4.60±0.2g/dl) in EG4 when compared with CG (7.18±0.4g/dl and 3.86±0.4g/dl) respectively.

**Conclusion:** Leachate from the electronic waste dumpsite from Oke Padre Ibadan, Nigeria induced liver dysfunction in rats. Proper treatment of electronic waste is imperative to prevent possible health risks to humans.

## B-362

### Determination of posaconazole in plasma/serum by high-performance liquid chromatography with fluorescence detection

P. H. Tang. *Cincinnati Children's Hospital Medical enter, Cincinnati, OH*

**Background:** The objective of this study was to develop a sensitive high-performance liquid chromatographic (HPLC) method with fluorescence (FL) detection for the determination of posaconazole in human plasma/serum. Posaconazole is an extended-spectrum triazole antifungal agent with enhanced activity against major microorganisms. It is FDA-approved for the prophylaxis of invasive Aspergillus and Candida infections in patients who are at high risk of developing these infections due to being immunocompromised, such as hematopoietic stem cell transplantation recipients with graft versus host disease or those with hematologic malignancies with prolonged neutropenia from chemotherapy. **Methods:** Plasma/serum samples were deproteinized using methanol as extraction solvent in a single dilution step. A methanolic solution of ketoconazole was served as the internal standard for the assay. Following protein precipitation supernatant was transferred to an autosampler vial, the methanol extract was direct injected onto the HPLC system. The mobile phase consisted of a mixture of 0.1 M ammonium acetate, acetonitrile, and trifluoroacetic acid (440:560:1, v/v/v). The isocratic run was carried out at a flow rate of 1.1 mL/min. Separation of internal standard and posaconazole were achieved within 8 min by using a reversed-phase C18 column (250 x 4.6 mm, 5-µm) and column temperature at 45°C. Fluorescent measurements were performed at an excitation wavelength of 245 nm and emission wavelength of 380 nm. **Results:** The method achieved a linear concentration range of 0.1-10 mg/L, which adequately covered the therapeutic range for appropriate patient monitoring. The limit of detection was 0.04 mg/L. Both the within-run and between-run precisions were lower than 5%. A single dilution step produced mean recoveries of 92.4±1.3% and 91.6±1.3% for posaconazole and ketoconazole, respectively. No interferences with other substances in plasma/serum were observed. The method's limit of quantitation, linearity, imprecision, and accuracy met all criteria required by the Guidance for Industry Bioanalytical Method Validation.**Conclusion:** This method is sensitive, simple and easy to perform with excellent reproducibility, only requires a single dilution step and one centrifugation step prior to the HPLC analysis. The instrument time is less than 8 min per injection, an improvement over most published HPLC/FL and HPLC/UV methods using tedious and labor-intensive preparation and lengthy chromatography.

## B-363

### Automated solid phase extraction LC-MS/MS procedure for measuring fentanyl in clinical and medical legal blood specimens.

D. W. S. Stephen[1], I. Clunie[1], A. Dunlop[2]. *[1]NHS Grampian, ABERDEEN, United Kingdom, [2]Queen Elizabeth University Hospital, GLASGOW, United Kingdom*

**Background:** Fentanyl is a synthetic µ-opioid agonist with potency up to 50-100 times greater than that of morphine. It has a short duration of action and is consequently used therapeutically for the induction and maintenance of surgical anaesthesia and analgesia. It is available as an injectable solution but other formulations of the drug include tablets, lozenges, nasal sprays and transdermal patches for the management of chronic pain. Subsequently, fentanyl is not an uncommon finding following general drug screening. Low therapeutic blood concentrations and often limited blood specimen can compromise the analysis of this drug. We present a rapid LC-MS/MS method for the measurement of fentanyl in blood samples prepared for analysis using an automated solid phase extraction (SPE) technique. **Methods**: Using an HTS-PAL autosampler robot and disposable ITSP C18 SPE cartridges preconditioned sequentially with 150 µL 100% methanol, 150 µL deionised water and 150 µL ammonium acetate, extraction of fentanyl from 400 µL of blood serum was achieved using 200 µL of 100% methanol. LC-MS/MS-ESI of fentanyl [m/z 337.25>188.13 (quan ion), 337.25>132.03 (qual ion)] and fentanyl-d5 [m/z 342.29>221.17], using a Waters Acquity BEH C8 column (1.7 µm, 50 x 2.1 mm i.d.) held at 30°C and gradient mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) [(ratio A:B): 0.25 min 85/15, 1.75 min 55/45, 0.5 min 50/50, 1.5 min 0/100] at 0.35 mL/min, was used for chromatographic separation and quantification. **Results:** The assay was linear (*curve fit: $y = mx + c$*) over the analytical range 0.5-1,000 ng/mL. Lower limits of detection (LLOD) and quantification (LLOQ) of fentanyl were 0.12 ng/mL and 0.21 ng/mL, respectively. Intra-assay (n = 5) and inter-assay (n = 5) imprecision of fentanyl in all samples were 0.81% relative standard deviation (RSD) ($r^2$ for slope of calibration curve 0.9905) and 0.80% RSD ($r^2$ for slope of calibration curve 0.9934), respectively. The analytical recovery of fentanyl spiked into blood (QC levels 0.5 and 10 ng/mL) was >95%. Matrix effect in blood was -7.8% and extracted samples were stable for at least 14 days at 10°C. **Conclusion:** The described validated LC-MS/MS method for the detection of fentanyl in small volumes of SPE-prepared blood is a quick and easy procedure for the measurement of this drug in samples taken for toxicological purposes.

## B-364

### Analytical Evaluation of a New Biochip Array for the Simultaneous Screening of 20 Drugs of Abuse in Urine on the New Random Access Fully Automated Analyser Evidence Evolution

R. Keenan, L. Keery, J. Darragh, P. Sinha, R. I. McConnell, S. P. FitzGerald. *Randox Toxicology Ltd, Crumlin, United Kingdom*

**Background**

Maximising the reliable result output in the screening step during the drug testing process is advantageous. Biochip array technology enables the detection of multiple analytes from a single sample. With the aim of consolidate testing and increase the screening capacity of clinical laboratories, this study reports the analytical evaluation of the new DOA Ultra Urine biochip array for the simultaneous testing of 20 drugs of abuse on the new analyser Evidence Evolution. This system is a fully automated, high throughput, random access with STAT capability system. The time to first result is 36 minutes, 60 samples can be loaded per hour and 20 different biochip arrays can be on-board at any one time.

**Methodology**

Competitive semi-quantitative chemiluminescent biochip-based immunoassays applied to the Evidence Evolution analyser were employed. The signal output was is inversely proportional to the concentration of drug in the sample. The system incorporated dedicated software to process, report and archive the multiple data produced. Ligands were immobilized and stabilized to the biochip surface defining an array of twenty discrete test sites including: amphetamine, methamphetamine, barbiturates, benzodiazepine class 1, benzodiazepine class 2, cannabinoids (THC), buprenorphine, cocaine/benzoylecgonine, dextromethorphan, fentanyl, ketamine, meprobamate, methadone, opiates, oxycodone 1, oxycodone 2, phencyclidine, tramadol, tricyclic antidepressants and zolpidem. The sample volume required is 6 µL of neat urine.

**Results**

The 20 simultaneous immunoassays presented the following cut-off values in urine: amphetamine 200ng/mL, methamphetamine 200ng/mL, barbiturate 200ng/ml, benzodiazepine class 1 100ng/mL benzodiazepine class 2 100ng/mL, buprenorphine 5ng/mL, cannabinoids 50ng/mL, cocaine/benzoylecgonine 150ng/mL, dextromethorphan 20ng/mL, fentanyl 2ng/mL, ketamine 750ng/mL, meprobamate 500ng/mL, methadone 300ng/mL, opiates 200ng/mL, oxycodone 1 100ng/mL, oxycodone 2 100ng/mL, phencyclidine 25ng/mL, tramadol 5ng/mL, tricyclic antidepressants 100ng/mL and zolpidem 10ng/mL. Limit of detection was also determined by running 20 negative urine samples, the resultant mean concentrations plus three standard deviations were less than 50% of the cut-offs required. The cut-off values were further validated by assessing inter-assay precision. Urine samples were spiked with the appropriate drug compound 50% below, at the cut-off, and 50% above the recommended cut-off. Three replicates were assessed over 5 separate runs and the inter-assay precision calculated to be less than 20% for all levels across all assays. Intra-assay precision was also assessed with 20 replicates of a low, mid and high concentration sample within one run; resultant intra-assay precision was also calculated to be less than 20% for all levels across all assays.

**Conclusion**

The results indicate optimal analytical performance of the twenty immunoassays on the DOA ULTRA biochip array and applied to the new Evidence Evolution analyser for the simultaneous comprehensive semi-quantitative screening of drugs in urine samples. For this application no sample preparation is required and the use of the Evidence Evolution analyser allows high through put testing, random access and STAT capability. Toxicology tests can be run alongside various clinical tests making this system a new reliable multi-analytical tool for test consolidation.

## B-365

### Multicenter Evaluation of new ONLINE TDM Vancomycin Gen.3 (VANC3) assay on Roche Clinical Chemistry Analyzers

B. Loehr[1], D. Dot Bach[2], A. Arbiol Roca[2], C. Imperiali[2], A. Puertas González[2], B. Fernández Cidón[2], V. Stove[3], K. de Herde[3], C. Lemahieu[3], D. Meyer zum Büschenfelde[4], A. Schoenknecht[4], S. Kühn[4], G. Steinbach[5], S. Eichner[5], K. Stöhrer[5], C. Rudtke[5], A. Haeussler[5], N. Pfeifer[5], T. Pfeiler[5], S. Bockel[5], U. Osswald[5], J. Kaufmann[6]. *[1]Roche Diagnostics GmbH, Mannheim, Germany, [2]Bellvitge University Hospital, Clinical Laboratory, Barcelona, Spain, [3]University Hospital Gent, Laboratory for Clinical Biology, Gent, Belgium, [4]Labor Berlin - Charité Vivantes Services GmbH, Berlin, Germany, [5]University Hospital Ulm, Central Institute Clinical Chemistry, Ulm, Germany, [6]Roche Diagnostics GmbH, Penzberg, Germany*

**Objectives:**

The new ONLINE TDM Vancomycin Gen.3 (VANC3) assay of Roche Diagnostics was developed to increase comparability to other vancomycin assays and to improve calibration stability. The analytical performance of the assay was tested in three laboratories.

**Medical Background:**

Vancomycin is an antibiotic recommended for intravenous administration as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant Staphylococcus aureus. Blood levels have to be monitored closely due to several side effects.

**Assay principle:**

The kinetic interaction of microparticles in solutions (KIMS) is induced by binding of drugconjugate to the antibody on the microparticles. The competitive reaction is inhibited by the presence of Vancomycin in the sample. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

**Study Design:**

The analytical performance of the new VANC3 assay was evaluated in four independent laboratories using **cobas c** 702, **cobas c** 502 and **cobas c** 501 instruments.

Study program: Recovery of Roche TDM Control set, recovery of Ring Trial samples from RfB and Instand e.V., within-run precision of human sample pools, precision according to CLSI EP5-A3, method comparisons VANC2 vs. VANC3, instrument-to-instrument comparability (**cobas c** 701 vs **cobas c** 502), and calibration stability.

**Methods and Results:**

Repeatability and intermediate precision were measured according to the CLSI EP5-A3 protocol using three Roche controls and five human serum pools in the concentration range from 6.8 - 61 µg/mL. For the repeatability the coefficients of variation (CVs) were determined to be less than 3.5 % and for intermediate precision yielded CVs ranging between 2.0 and 5.3 % (two runs/day, 21 days). The recovery of three controls (Roche Diagnostics) was determined in three independent runs measuring 3 aliquots. The recovery of target values ranged from 95.9 to 105.5 %.

More than 115 samples in the concentration range from 2.8 to 72.6 µg/mL were used for method comparison experiments measured according to CLSI EP09-A3 protocol. Passing-Bablok regression analysis of between VANC2 (x) and VANC3 (y) assay resulted in slopes in a range of 0.92 to 1.03, intercepts of -0.4 to 1.0 µg/mL, and Pearson correlation coefficients ≥ 0.985. Interchangeable values were measured in method comparisons of **cobas c** 502 (x) versus **cobas c** 701 (y) analyzers using VANC3 reagent. Passing-Bablok regression resulted in slopes of 0.98 and 1.04, intercepts of +0.1 and +0.3 µg/mL, and Pearson correlation coefficients ≥ 0.992. At least 14 days calibration stability was shown on Roche analyzers.

**Conclusions:**

The results of the multicenter evaluation study prove a good analytical performance of the new VANC3 assay as well as an increased calibration stability. The assay is well-suitable for routine use.

## B-366

### Development and Validation of a LC-MS/MS Method for Measuring Erythrocyte Thiopurine Metabolites in Patients on Thiopurine Drug Therapy

L. Yang[1], D. Loomes[2], G. Ball[1], G. Hoag[1]. *[1]Department of Laboratory Medicine, Pathology and Medical Genetics, Island Health, Victoria, BC, Canada, [2]Pacific Digestive Health, Royal Jubilee Hospital, Island Health, Victoria, BC, Canada*

**Background**: Thiopurine drugs including azathioprine, mercaptopurine, and thioguanine, are commonly used in post-organ transplantation regiments, to treat inflammatory bowel disease (IBD) and many other autoimmune diseases, acute lymphoblastic leukemia. Thiopurines are metabolized to 6-thioguanine nucleotides (6-TGNs) and 6-methyl-mercaptopurine nucleotides (6-MMPNs). Elevated levels of 6-TGN and 6-MMPN are associated with myelosuppression and hepatotoxicity, respectively. Clinical trials and practice guidelines suggest that thiopurine metabolites testing can assist physicians in optimizing clinical efficacy, minimizing drug toxicity, and modifying clinical management. In order to facilitate patient care, we developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of thiopurine metabolites, 6-TGN and 6-MMPN, in erythrocytes. **Methods:** 6-TGNs and 6-MMPNs were extracted from 50 µL washed erythrocytes with 70% perchloric acid in a 300 µL mixed solution of DTT and the internal standards (IS) 6-TG-$^{13}C^{215}N$ and 6-MMP-d3. The supernatants were heated for 60 min at 98°C and converted to 6-thioguanine (6-TG) and a 6-methylmercaptopurine (6-MMP) derivative respectively. The cooled supernatant was diluted with water and injected (5 µL) for analysis on a Shimadzu Prominence HPLC coupled to a Sciex 4000 QTRAP mass spectrometer. The separation of the analytes was achieved using an Allure PFP propyl 5µm (50 x 2.1 mm) analytical column at 40°C by gradient elution with a 0.6 ml/min flow rate. The mass spectrometer was operated in positive polarity using an electrospray ionization (ESI) source. Sample analysis was performed in the multiple-reaction monitoring mode with the transitions *m/z* 168/151(quantifier) vs 168/134 (qualifier) for 6-TG, *m/z* 171/154 for 6-TG IS, *m/z* 158/110 (quantifier) vs 158/82 (qualifier) for the 6-MMP derivative, and *m/z* 161 > 110 for the 6-MMP derivative IS. This method was evaluated for precision, limit of quantification, linearity, and ion suppression. Forty-seven samples from IBD patients receiving thiopurine drug therapy were assayed and compared with an established HPLC method. The results were standardized to pmol/8 x $10^8$ RBCs. **Results:** Total run time was 2.8 min. The intra- and inter-assay precisions for two levels of quality control were less than 10 % for both the 6-TG and 6-MMP derivative. The limits of quantification for 6-TG and 6-MMP derivative were 0.1 µmol/L and 1.0 µmol/L respectively. The calibration curves exhibited linearity and reproducibility in the range 0.1-25 µmol/L for 6-TG and 1.0-250 µmol/L for the 6-MMP derivative. No significant ion suppression effects were noted. The 47 clinical sample comparison with an HPLC method demonstrated good agreement, for 6-TG (LC/MS/MS = 1.26 HPLC - 6.88, $R^2$ = 0.975; mean difference 16.5%) and 6-MMP derivative (LC/MS/MS = 1.07 HPLC - 121, $R^2$ = 0.982; mean difference -2.4%). **Conclusion:** This rapid and reliable LC-MS/MS method is suitable for routine monitoring of 6-TGN and 6-MMPN concentrations in erythrocytes samples from patients receiving thiopurine drug therapy.

## B-367

### Evaluation of quantitative microsampling for immunosuppressant drug monitoring

V. Gruzdys[1], S. D. Merrigan[2], K. L. Johnson-Davis[1]. *[1]University of Utah/ ARUP Laboratories, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT*

**Background:** Immunosuppressant drugs (sirolimus, everolimus, tacrolimus, and cyclosporine A) are used during chemotherapy, pre- and post- organ transplant, and other conditions. They are characterized by narrow therapeutic window and serious implications with hypo- therapeutic (tissue rejection or remission) and hyper-therapeutic doses (toxicity, immunodeficiency). A commercial volumetric absorptive microsampling device (VAMD) for fixed-volume blood collection is available. It enables quantitative analysis while minimizing sample volume requirements. VAMDs provide the potential for significant cost saving by elimination of phlebotomy and simplification of transport. Our objective was to evaluate a method for quantifying four immunosuppressant drugs in whole blood using VAMD by LC-MS/MS.

**Methods:** Calibrators, controls, and samples were equilibrated at room temperature while mixing on a rocker before collection by VAMD. Before drying 20 µL of whole blood EDTA samples were collected by contacting VAMD to 100 µL of sample spotted on parafilm. Samples were then extracted using 200 µL of MeOH/ water mixture containing the internal standard. Subsequently, they were sonicated and vortexed followed by evaporation. Samples were then reconstituted with 100µL of 5mM ammonium acetate containing 0.2% Formic acid in ACN/MeOH/water, followed by centrifugation, and transfer of 80 µL to a vial for injection (35 µL) into the LC system. Samples were chromatographically separated using a C18 analytical column on Agilent 1200 series HPLC system and detected by AB Sciex API 4000 mass spectrometer operating in positive MRM mode. This method was compared to a validated in-house LC-MS/MS method. Accuracy experiments were performed using residual de-identified patient whole blood samples for tacrolimus. Spiked samples were used to perform sensitivity and linearity experiments. Low (L) and High (H) quality control samples containing all four analytes were analyzed five times on a single run to evaluate imprecision.

**Results:** Tacrolimus VAMD sample extraction method compared to our in-house conventional extraction with Deming regression and 95% confidence intervals (±): tacrolimus VAMD= 1.153 ±0.121(in-house) + 0.743 ±1.5 ng/mL, R=0.99, (n=11). Other analytes will be evaluated. Limits of quantitation for tacrolimus (2.6 ng/mL), everolimus (2.6 ng/mL), cyclosporine A (27 ng/mL), and sirolimus (1.7 ng/mL) were achieved, which are comparable to conventional extraction. Calibrators sampled and extracted through VAMD showed linearity within the specified AMR for tacrolimus (up to 37 ng/mL), everolimus (up to 42 ng/mL), cyclosporine A (up to 896 ng/mL), and sirolimus (up to 48 ng/mL) with correlation coefficients greater than 0.995 for all analytes (quantitative and qualitative fragment ions). Coefficients of variation for tacrolimus (L: 4% H: 3%), everolimus (L: 5% H: 1%), cyclosporine A (L: 5% H: 3%), and sirolimus (L: 6% H: 3%) were obtained, indicating acceptable reproducibility. In addition, conventional sample processing was compared to VAMD sample collection and extraction for all four analytes.

**Conclusion:** This sample preparation method was able to achieve comparable limits of quantitation, linearity, and precision to the conventional sample preparation method. This sample collection and processing approach will require studies comparing VAMD sampling methodology in patient populations but represents an applicable method for simplified and more patient-friendly approach to therapeutic drug management.

## B-368

### Aluminum toxicity: Evaluation of 16-year trend among 14,919 patients

R. Bell[1], R. Schifman[2]. *[1]University of Arizona College of Medicine, Tucson, AZ, [2]Southern AZ VA Healthcare System, Tucson, AZ*

**Background:** Chronic exposure to aluminum from contaminated dialysis water and the use of aluminum-containing binders has historically been a major problem in patients undergoing dialysis. Aluminum exposure has been linked to iron-refractory microcytic anemia, osteomalacia, and even fatal encephalopathic disorders. Recognition of this problem has led to successful preventative measures by reverse osmosis and aluminum-level monitoring, which has led to aluminum toxicity becoming uncommon. Despite this trend, guidelines from the National Kidney foundation continue to recommend annual serum aluminum testing for ongoing surveillance. Aluminum is a ubiquitous ultratrace element and exogenous sources may lead to false elevations due to contamination during collection, processing or analysis. As the rate of true aluminum toxicity declines, the relative incidence of false elevations due to contamination would be expected to increase. The objective of this study was to investigate long-term trends in serum aluminum levels in a large Veteran population and estimate the frequency of falsely elevated values presumably due to contamination.

**Methods:** A retrospective observational study covering a 16-year period through October 2016 was conducted with information extracted from the Veterans Affairs corporate data warehouse. Serum aluminum concentrations >60 g/L were considered false positives, presumably due to contamination, if another specimen retested within 45 days was <20 g/L. Serum aluminum concentrations less than 20 g/L were considered normal. **Results:** A total of 45,480 serum aluminum results involving 14,919 patients and 119 Veteran Affairs 16 facilities over a 16-year period ending in October 2016 were evaluated. The percentage of elevated (>2017 g/L) serum aluminum results declined from 31.5% in 2000 to 2.0% in 2015. Average testing intervals changed from every 159 days in 2000 to every 238 days in 2015. Of 529 patients with serum aluminum concentrations >60 g/L, 216 (40.8%) were retested within 45 days (average=21 days) of which 83 (38.4%) were below 20 g/L after repeat measurements. **Conclusion:** Aluminum toxicity, as assessed by serum levels, has substantially declined over time and is now rare. In addition, the frequency of serum aluminum testing has declined in accordance with guidelines for routine surveillance. Our study revealed, that despite NKF guidelines recommending annual screening, testing is still performed more frequently (1.5 times per year). Further, testing intervals have remained relatively constant since 2011 despite declining overall rate in abnormal serum aluminum levels. Our study also revealed that a relatively high frequency of elevated serum aluminum concentrations were false positives and most likely the result of contamination. As a consequence, the incidence of aluminum toxicity is likely frequently overestimated. Therefore, patients with elevated serum aluminum concentrations should be retested before undergoing treatment or more investigation into sources of exposure should be performed to exclude false elevations.

## B-369

### Modeling nicotine in oral fluid: a possible tool to assess nicotine use and exposure

S. Feng, O. Cummings, G. McIntire. *Ameritox Ltd., Greensboro, NC*

**Background:** Oral fluid collection is non-invasive and easy to handle making it an ideal matrix for determining nicotine and hence smoking status. Various smoking related compounds including nicotine and cotinine have been used as markers for smoking status assessment. In the present study, we develop a transformed and normalized mathematic model of nicotine historical data for a quick assessment of nicotine exposure.

**Methods:** A historical distribution "model" of nicotine in oral fluid was developed using data collected from quantitative liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis. A data set of 6158 independent nicotine positive saliva specimens (10 ng/mL cutoff) collected over a 2 year period (2015-2017) is the foundation of this near Gaussian model. Demographic data were used to calculate patient specific parameters (i.e., calculated blood volume (CBV), lean body weight (LBW), and body surface area (BSA)) for the transformation and normalization of the oral fluid nicotine data. An earlier study indicated that using 10 ng/mL cutoff for nicotine identified 88% of self-reported smokers in a controlled clinical setting (Scheidweiler et al. *Ther Drug Monit.* 2011; 33(5): 609-618). Hence, this model is likely specific for active smokers.

**Results:** After transformation and normalization, the resulting model derived from positive nicotine test results shows a near Gaussian distribution. A separate population of patients who used the nicotine transdermal patch was used to test whether this mode of nicotine administration matches active smoking. This assessment found 96% of nicotine patch users fall within two standard deviations from the mean of the distribution consistent with a true Gaussian distribution. Transformation and normalization of nicotine results below 10 ng/mL exhibit a separate distribution from the overall nicotine positive consistent with results from a controlled clinical trial for patients "exposed" to environmental nicotine.

**Conclusion:** This model of nicotine historical distribution is consistent with nicotine administered via patch or directly from smoking. It is conceivable that any "active" form of nicotine administration (e.g., gum, patch, etc.) will be consistent with this model. Combined with patient interviews and prescription history, this model can provide information about patients' potential adherence to nicotine replacement therapy.

## B-370

### Quantitative Analysis of Ethyl β-D-glucuronide (EtG) in Human Umbilical Cord Tissue by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

S. L. Wabuyele[1], G. A. McMillin[2]. *[1]ARUP Institute for Clinical & Experimental Pathology, Salt Lake City, UT, [2]University of Utah, School of Medicine, Salt Lake City, UT*

**Background:** Prenatal alcohol exposure can significantly affect neonatal development and may result in adverse outcomes known as fetal alcohol spectrum disorders (FASD) which encompass a range of physical, behavioral and cognitive impairments. Since gestational alcohol consumption is often underreported, a biomarker for identification of *in utero* alcohol exposure is needed. The aim of this study was to develop a method for determination of Ethyl β-D-glucuronide (EtG), a direct ethanol metabolite, in umbilical cord tissue as a biomarker for *in utero* alcohol exposure.

**Methods:** Residual umbilical cord tissue specimens submitted to ARUP Laboratories for drug testing were de-identified and qualitatively screened for the presence of EtG using the LC-MS/MS method described here. Specimens with undetectable EtG were used to prepare six calibrators and control samples. Both spiked and authentic positive specimens were sliced and weighed (1.0g ±0.025). 3.0 ml acetonitrile, deuterated internal standard (IS) EtG-d5 and stainless steel UFO beads were added for homogenization on the Bead Ruptor, followed by centrifugation at 0°C. The supernatants were loaded on UCT Clean-Up® solid phase extraction (SPE) columns preconditioned with methanol and water. The columns were washed with water and methanol before elution with 2% formic acid in methanol. The eluate was dried under nitrogen (40°C) using a TurboVap® (Biotage) and reconstituted in the aqueous mobile phase. Chromatographic separation of EtG and the IS was achieved on a Phenomenex Hydro-RP C18 column (50mm x 2.1mm i.d., 2.5um) with a binary mobile phase of 0.1% formic acid in water and 0.1% formic acid in acetonitrile with gradient elution at a flow rate of 0.350 mL/min, with a total run time of 5 minutes. Quantification of the analytes was performed by AB Sciex Triple Quad 5500 mass spectrometer using positive turbo-ion-spray ionization in multiple-reaction-monitoring (MRM) mode with two mass transitions m/z 220.9 → 84.9 (quantifier) and 220.9 → 74.7 (qualifier) for EtG, and m/z 226.1 → 84.8 (quantifier) and 226.1 → 74.8 (qualifier) for the IS. A calibration range of 5.00-220 ng/g was established in umbilical cord tissue by a weighted ($1/x^2$) least square method. Accuracy, imprecision (% coefficient of variation), matrix effect, interference, carryover and stability were evaluated to determine the overall method performance.

**Results:** Good linearity was obtained (r = 0.9996). The limit of detection (LOD) was 1ng/g and the limit of quantitation (LOQ) was 5 ng/g with inaccuracy of <± 20% and CV of <20%. The within-run and between-run bias and imprecision of the method were <±15% and <15%, respectively. The relative matrix effect was <5% and the absolute extraction efficiencies for EtG ranged from 72%-89.2%. The selectivity of the assay was acceptable and no carryover was detected. Extracted samples were stable for 1 week at room temperature, 3 weeks at 4 °C and to freeze-thaw conditions. The method has been successfully used to quantify EtG in authentic umbilical cord tissue samples.

**Conclusion:** SPE-based sample preparation and an LC-MS/MS method for quantification of EtG in umbilical cord tissue homogenates have been developed and described here. This assay could be utilized for routine detection of *in utero* alcohol exposure.

## B-371

### Novel 5-plex Panel for the Detection of Acute Kidney Injury with Improved Analytical Performance

C. Adamo[1], T. Carlson[1], E. McCole[2], M. McGgarve[2], C. Richardson[2], P. Fitzgerald[3], J. Lamont[3], A. Sethi[1]. *[1]Pacific Biomakers, Seattle, WA, [2]Randox Teoranta, Dungloe, Ireland, [3]Randox Laboratories, Crumlin, United Kingdom*

**Background:** Acute kidney injury (AKI) is currently classified by the KDIGO guidelines based on serum creatinine and urine output. However, since creatinine is a lagging index of impending AKI, a novel set of more sensitive AKI biomarkers has been qualified in rodents. Studies are now underway to qualify a similar set of human biomarkers, but no high-throughput multiplex for these biomarkers is currently available to support an earlier and more robust classification of AKI. This work describes the development and optimization of a novel multiplex immunoassay panel comparing its performance with predicate ELISA methods for KIM-1, NGAL, cystatin C, clusterin, and osteopontin.

**Methods:** Randox Biochip technology was used to develop a multiplex immunoassay panel for the urinary biomarkers mentioned above using proprietary and commercially available antibodies. The analytical performance of this method was compared against singlicate ELISA methods using about 30 normal subjects, and data extracted from testing urine of each analyte by ELISA from >1000 subjects with normal kidney function or potential AKI.

**Results:** The functional sensitivities of assays, as revealed by the precision (%CV) of the lowest non-zero standard (n=6 each) were 24.4pg/mL (%CV=14.0), 0.89ng/mL (%CV=3.3), 1.22ng/mL (%CV=8.8), 7.02ng/mL (%CV=13.6), and 50.9ng/mL (%CV=8.5) for KIM-1, NGAL, cystatin C, clusterin, and osteopontin, respectively. These sensitivities were compared with the ideal lower limits of the dynamic ranges determined by ELISA from >1000 patient samples (40pg/mL, 1.0ng/mL, 1.5ng/mL, 10ng/mL, and 80ng/mL, respectively). The ideal upper limits of the dynamic range were 4000pg/mL, 100ng/mL, 150ng/mL, 1000ng/mL, and 8000ng/mL, for KIM-1, NGAL, cystatin C, clusterin and osteopontin, respectively. The effective upper limits of measurement and precision (%CV) were 4067.4pg/mL (3.8%), 97.8ng/mL (3.9%), 184.7ng/mL (8.0%), 1021.0ng/mL (2.9%), and 8028.7ng/mL (9.9%), respectively. Cross reactivity was tested for each analyte at the midpoint of the standard curve when spiked with x10 concentration of the highest standard of the other panel antigens, producing no significant cross-reactivity. Cross reactivity from various related, but non-panel proteins that may be present in urine were also tested for cystatin C, clusterin and KIM-1, again producing no significant cross-reactivity. Cross-reactivity of non-panel proteins for NGAL and osteopontin are pending. Correlation ($r^2$) of 29 urine samples for the KIM-1 multiplex assay vs ELISA (range 46 to 1447pg/mL) was 0.994; correlation of NGAL multiplex vs ELISA (n=28; range 1.1 to 197ng/mL) was 0.962; cystatin C multiplex vs ELISA (n=28; range 2.6 to 80.3ng/mL) was 0.872; clusterin multiplex vs ELISA (n=27; range 24 to 710ng/mL) was 0.812; and osteopontin multiplex vs ELISA (n=29; range 58 to 4517ng/mL) was 0.899. Slopes were 0.994, 0.544, 0.636, 1.67, and 0.685, respectively.

**Conclusion:** The development and optimization of the Randox Biochip 5-plex panel suggests that KIM-1, NGAL, cystatin C, clusterin and osteopontin will be suitable to replace the slower, more laborious, and presumably less sensitive ELISA methods for clinical trial and diagnostic use when the ongoing full analytical validation and clinical confirmations are completed.

## B-372

### Influence of function and co-administered calcineurin inhibitors on monitoring the trough mycophenolic acid concentration using particle enhanced turbidimetric inhibition immunoassay

K. Nakano[1], D. Iwami[2], T. Yamada[3], K. Morita[4], K. Yasuda[1], H. Shibuya[1], K. Kahata[1], N. Shinohara[2], C. Shimizu[1]. *[1]Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan, [2]Departments of Urology, Hokkaido University Hospital, Sapporo, Japan, [3]Department of Pharmacy, Hokkaido University Hospital, Sapporo, Japan, [4]Departments of Urology, Kushiro City General Hospital, Kushiro, Japan*

**Background:** Mycophenolate mofetil (MMF) is an immunosuppressive drug widely used in the patients after solid organ transplantation. Mycophenolic acid (MPA), major pharmacologically active metabolite of MMF, has been monitored by high performance liquid chromatography (HPLC). Recently, immunological methods such as the enzyme-multiplied immunoassay technique and homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINA) has been widely used due to their simplicity. However, there is a concern about cross-reactions with MPA-glucuronide (MPAG) as well as other minor metabolites in these immunoassays. The aim of this study was to evaluate the influence of the renal function and the co-administration of calcineurin inhibitors (CNI) on the MPA and MPAG concentrations and then develop a formula that took into account these clinical factors.

**Methods:** Forty-two solid organ transplant patients receiving MMF in combination with tacrolimus (TAC) or cyclosporine A (CsA) were enrolled in this study. Blood samples were collected from the recipients prior to receiving any MMF doses. HPLC separations of MPA and MPAG were performed independently. MPA or MPAG concentrations measured by HPLC using an ERC ODS-1161 column and Shimadzu LC-10ADLP system (Shimadzu, Kyoto, Japan). In addition to the HPLC assay, the MPA concentrations in the same samples were also measured by PETINA, using the Flex reagent cartridge MPAT and the Dimension Xpand Plus system (Siemens Healthcare Diagnostics, NY, US). All patient laboratory data were obtained from the medical records.

**Results:** There were no significant differences between MPA plasma concentrations and MMF dosage per body weight. In contrast, MPAG plasma concentrations were positively correlated with the MMF dosage per body weight (r=0.79, p<0.001). Moreover, while the MPAG concentration was negatively correlated with the

estimated glomerular filtration rate (eGFR) (r=-0.56, p<0.001), there was no correlation between the MPA concentrations and the eGFR. There was no significant difference in the MPA and MPAG concentration per MMF dose between the patients who were co-administered TAC versus CsA. While there was a trend for the patients co-administered CsA to have higher MPAG concentrations than those who were co-administered TAC, this difference did not reach significance. After taking the renal function and co-administration of CNI into account, we developed a formula to correct the PETINA values to more precisely reflect the HPLC values for MPA. The multiple regression equations for the model is as follows: Estimated MPA concentration = -0.010 + 0.8882×MPA (PETINA) + 0.001×eGFR -0.013×CNI (TAC=0 or CsA=1).

**Conclusion:** Although MPAG accumulates in conjunction with the worsening of the renal function, the MPA concentration is not affected by the renal function. Thus, it is difficult to predict the MPA concentration based on the MMF dosage or renal function. By being able to successfully develop a formula that excluded the influences of cross-reactions with the accumulated MPA metabolites, this made it possible to correct the PETINA data to values that more precisely reflected the HPLC values for MPA.

## B-373

### Determination of Benzoylecgonine in urine with dilute-and-shoot and LCMS/MS analysis.

B. F. P. Paulo, E. Mateo, A. C. S. Ferreira, M. E. R. Diniz. *Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

Cocaine (COC) is one of the alkaloids present in leaves of two species of the genus Erytroxylum, commonly known as coca, with contents between 0.17 and 0.76% of COC. It is a potent local anesthetic and powerful sympathomimetic agent with stimulating effects on the central nervous system and has being considered the most potent stimulant of central nervous system of natural occurrence, which is why it is used as an abuse drug. The main biotransformation product of COC is benzoylecgonine (BE), which is excreted in urine and used as a biomarker for exposure to the drug. For many years, GC-MS has been considered the gold standard method for the analysis confirmatory of drugs of abuse. However, the LC-MS/MS have gained space in this field, mainly after the evolution of the instrumentation. The objective of this work was to develop a simple and fast method for the determination of BE in urine by LCMS/MS. Chromatographic separation were performed on Poroshell 120 EC-C18 column (50 mm x 4.6 mm x 2.7 µm) using a guard column Poroshell 120 EC-C18 (5 mm x 4.6 mm). Isocratic separation was obtained with mobile phase constituted by 50% of methanol and 0.1% of formic acid at a flow rate of 0.500 mL min-1.The chromatographic run time was 2.0 min. All experiments were performed on an Agilent 6460C (Santa Clara, CA) triple quadrupole LC-MS/MS system, with an Agilent 1290 Infinity LC system. The source was operated in a positive mode. The sample preparation was performed by centrifuge of 100 µL of sample, calibrator or internal quality control, at 14.000 rpm for 10 minutes. 25 µL of supernatant were transferred to a 2.0 mL glass vial and diluted with 960 µL of aqueous solution containing 5.0% of methanol and 0.1% of formic acid. 15 µL of deuterated internal standard (BE-D3) solution was added. The mixture was shaken and 1.0 µL was injected into the chromatographic system. The method was validated achieving a LoQ of 12.0 ng mL1, linearity of 12.0 to 1,000.0 ng mL-1 and imprecision within-run and total were less than 5.3%. The method was compared to other GC-MS method by analysis of 20 fortified urine samples and achieve 0.99 of coefficient correlation. The accuracy was observed by two samples of CAP UDC proficiency test, and was achieved a standard deviation index (SDI) of 0.1 and 0.6. In conclusion, a simple method based of dilute-and-shoot extraction has been developed and validated successfully, with a good precision and a good correlation with the gold standard GC-MS method.

## B-374

### Development and validation of nicotine and cotinine in human serum by liquid chromatography electrospray ionization tandem mass spectrometry.

M. E. R. Diniz, F. V. Andrade, L. N. Dias, E. Mateo, A. C. S. Ferreira. *Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

The use of tobacco is one of the main preventable risk factors leading to major diseases, like cancers and death worldwide. Concentrations of nicotine and its metabolites in blood are indicative of tobacco exposure. A simple, rapid and sensitive Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was developed and validated for quantification of nicotine and its major metabolite cotinine in serum. The sample extraction is a simple protein precipitation with trichloroacetic acid 10% using only 100 µL of sample and 25 µL of internal standard (2-Phenylimidazole). Detection was performed on a 6460 MS system (Agilent Technologies) operate in a positive mode. Chromatographic separation was obtained on a Zorbax Eclipse Plus C18 RRHD column (2,1 X 50 mm 1,8 um) - Agilent with an isocratic mobile phase containing methanol, water, acetonitrile, ammonium formate and formic acid at a flow rate of 600 µL.min$^{1}$. The method had a chromatographic run time of approximately 2.5 min. The linear range obtained for cotinine was 4.0-500.0 ng.mL$^{-1}$ and for nicotine was 3.0-40.0 ng.mL$^{-1}$. The limit of detection (LOD) were 2.0 ng.mL$^{-1}$ for cotinine and 1.5 ng.mL$^{-1}$ for Nicotine. Within-day and between-day imprecision was less than 8% for cotinine and less than 11% for Nicotine. The accuracy was between 87-104% for cotinine and 88-101% for Nicotine. The analytes were determinate with satisfactory sensitivity, accuracy, repeatability and linearity. In conclusion, the LC-MS/MS method was developed and validated for the quantitative analysis of Nicotine and Cotinine in serum and has been applied successfully evaluation of the tobacco exposure.

## B-375

### Performance Evaluation of Dimension TAC Assay and Comparison with Other Commercial Tacrolimus Assay

E. Cho, K. Park, H. Hong, E. Shin, W. Lee, S. Chun, W. Min. *Asan Medical Center, Seoul, Korea, Republic of*

**Background:** Therapeutic monitoring of tacrolimus (TAC) is essential for reducing the organ rejection and *adverse* effects. The measurements of TAC in whole blood is performed by immunoassays or liquid chromatography-tandem mass spectrometry (LC-MS/MS) and many automated platforms have been developed. The aim of the present study was to evaluate the analytical performance of Dimension TAC assay (Siemens Healthineers, USA) which was upgraded reagent from the previous Dimension TACR assay. **Methods:** The evaluation was performed based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. The evaluation consisted of determination of the precision, linearity, limit of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ), and reagent lot-to-lot using three lot number. A correlation study was conducted using Dimension TACR assay, Architect Immunoassay (Abbott Diagnostics), Elecsys (Roche Diagnostics), MassTrak LC-MS/MS (Waters Corporation). We collected each sample more than 40 from kidney, liver and heart transplant recipients. **Results:** The total CV for the low, middle and high level quality control materials were 7.3%, 5.1% and 5.7%, respectively. The linear range where the coefficient of determination was >0.99 of the Dimension TAC assay was 1.61-31.72 ng/mL. The LoB, LoD, and LoQ was 0.29 ng/mL, 0.47 ng/mL, and 1.02 ng/mL, respectively. Correlation analysis indicated that results of the Dimension TAC assay was comparable to Dimension TACR assay, Architect Immunoassay and Elecsys in liver and heart transplants [correlation coefficients (r)=0.856~0.982]. In kidney transplants, Dimension TAC assay showed the less correlation with Architect Immunoassay and Elecsys [r= 0.558 and 0.775]. The results of these assay were slightly higher than those of MassTrak (mean bias 1.563-2.619 ng/mL) in all transplant groups. And we found few lot-to-lot reagent variation in the reagents which were evaluated [r >0.993]. **Conclusion:** The overall analytical performance of Dimension TAC assay is acceptable for therapeutic monitoring in clinical practice. This assay showed the higher concentrations than mass spectrometry which was consistent with results in previous study.

## B-376

### Performance Characteristics of Capillary Blood Methotrexate Polyglutamates by Volumetric Absorptive Microsampling Collection Method Coupled with LC-MS/MS

K. J. Brady[1], R. Apilado[1], T. O'Malley[1], S. Reddy[2], P. Chitkara[3], C. Ibarra[1], R. V. Alexander[1], Y. Qu[1], T. Dervieux[1]. *[1]Research and Development, Exagen Diagnostics, Vista, CA, [2]Arthritis Care and Research Center, Poway, CA, [3]Center for Arthritis and Rheumatologic Excellence, Chula Vista, CA*

**Background:** Red blood cell (RBC) Methotrexate triglutamate ($MTXPG_3$) levels have utility in the dosing optimization of methotrexate therapy. We sought to 1) develop an LC-MS/MS method for quantifying RBC $MTXPG_3$ levels from capillary blood collected on volumetric absorptive microsampler (VAMS), and 2) compare the performance characteristics of this microsampling method to the reference method (venous blood collected by venipuncture).

**Methods:** Rheumatoid arthritis (RA) subjects (n=101) under methotrexate therapy consented to donate capillary (10 µl per specimen on VAMS) and venous blood (10 ml per specimen). RBCs from venous blood were isolated and stored at -80°C before analysis. Dried capillary blood was eluted with water containing deuterated

(d3) $MTXPG_3$ as the internal standard. Following deproteinization (70% perchloric acid) and centrifugation, a 20 µL aliquot of the supernatant extract was injected onto a pentafluorophenyl column (2.1x50 mm, 2.6 µm) connected to a TSQ Quantiva mass spectrometer with heated electrospray operating in positive mode. Transitions consisted of a 713->308 m/z transition, and 716->311 transition for $MTXPG_3$ and $MTXPG_3$-d3, respectively. RBC $MTXPG_3$ levels from venous blood were also measured by LC-MS/MS.

**Results:** The LC-MS/MS method presented with intra-day and inter-day coefficient of variation below 15%. In 72 consecutive specimens, RBC $MTXPG_3$ levels from venous blood (average [SEM]: 36.8±2.8 nmol/L packed RBCs) were proportionally 2.2 fold higher than those recovered from capillary blood (17.6±1.4 nmol/L) ($r^2$=0.87) and this conversion factor (2.2) was applied to convert capillary blood levels to their RBC equivalent. The method was prospectively validated in 29 RA subjects. Capillary $MTXPG_3$ blood levels were 17.2±1.1 nmol/L, 37.9±2.3 nmol/L RBC equivalent and similar to RBCs $MTXPG_3$ levels from venous blood (35.5±2.2 nmol/L RBC; $r^2$=0.9, Slope=1.1). Figure 1 illustrates the correlation between the two methods for the 101 subjects enrolled.

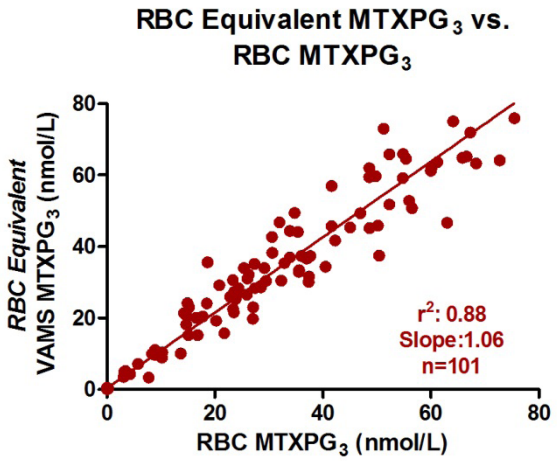



Figure 1.

**Conclusion:** We have validated a method for quantifying $MTXPG_3$ in capillary blood collected on VAMS.

## B-377

### Evaluation of Preanalytical Stability of Thiopurine metabolites in RBC and whole blood samples

O. JI. *Samsung Medical Center, Seoul, Korea, Republic of*

**Background:** Measurement of thiopurine metabolite is helpful to monitor adverse effects and to asses compliance in patients with thiopurine treatment. Maintaining stability of the metabolites in is necessary to get reliable test results. We evaluated the preanalytical stability of thiopurine metabolites in RBC and whole blood samples under various storage conditions to investigate the effects of preanalytical process

**Methods:** 6-thioguanine nucleotides (6-TG) and 6-methylmercaptopurine (6-MMP) were measured by the liquid chromatography-tandem mass spectrometry. Analyses were performed on an API 4000 tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an Agilent Technologies Series 1200 HPLC system (Agilent, USA). Quantitative analysis was performed in multiple reaction-monitoring mode (m/z 168.0>150.9 for 6-TG, m/z 158.0>110.0 for 6-MMP, m/z 171.0 >154.0 for 6-TG-$^{13}C_2{}^{15}N$, m/z 161.1 > 110.1 for 6-MMP-$d_3$) with a total running time of 3 minutes for each sample. Intra- and inter-day imprecisions were lower than CV 10%. To obtain pure RBC , we centrifuged the EDTA whole blood sample at 4000rpm for 10min followed by removing remnant plasma. After washing twice with 0.9% saline, isolated RBCs was stored in each condition until analysis. 6-TG and 6-MMP in RBC samples were measured in four aliquots at 2 concentrations to evaluate their stability under various conditions. Freeze-thaw stability (3 cycles at -70 °C), short-term stability (at room temperature and -4°C, 0 min, 30 min, 1, 2 and 4 hrs), and long-term stability (at -20°C and -70°C, 0, 1, 3, 7, 14, 21, 90 and 180 days) of the metabolites in preprocessed RBC specimens were evaluated. In addition, stability of the metabolites in whole blood samples without preprocessing was tested at room temperature and -4°C up to 7 days (0, 4, 8 hrs, 1, 2, 4 and 7 days). **Results:** The concentrations of 6-TG and 6-MMP in RBC samples showed no significant changes at room temperature and 4 ° C until 4 hours. In frozen preprocessed RBC samples, thiopurine metabolites were stable at -70° C for up to 6 months, but 6-TG concentrations were decreased to 70% of the initial values at -20 C. The concentrations of 2 metabolites (6-TG/6-MMP) were significantly decreased in comparison with the initial concentration.in whole blood samples; 74%/ 85% for 4 days and 35%/ 42% ° for 7 days at room temperature, 93%/ 95% for 4 days and 74/ 88% for 7 days at 4 ° C. The effect of 3 freeze- thaw cycles on the sample stability was negligible.

**Conclusion:** Sample storage and handling process are critical for accurate measurement of thiopurine metabolites. We recommended that the patient's whole blood sample should be processed as soon as possible and stored at -70 ° C until analysis.

## B-378

### Omecamtiv Mecarbil (OM) in Patients with Heart Failure: Development of an Immunoassay to Guide Omecamtiv Mecarbil Dose Adjustment

L. Ye[1], C. Lee[1], R. Ye[1], K. Yokobata[1], J. Donaldson[1], B. Bruenner[2], J. Clements[2], M. Boedigheimer[2], C. Kurtz[2], N. Honarpour[2], N. Go[2], T. Rigl[1], T. Prestigiacomo[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Amgen, Thousand Oaks, CA*

**Background:** Heart failure affects approximately 23 million people worldwide, including more than 5 million in the United States. Omecamtiv mecarbil (OM), a novel selective cardiac myosin activator, is being studied as a potential treatment for heart failure with reduced ejection fraction. The Phase 2 COSMIC-HF study indicated that a pharmacokinetic (PK)-based dose titration strategy was useful to adjust the treatment dose of OM for heart failure patients. GALACTIC-HF is a global Phase 3 double-blind, randomized, placebo-controlled study designed to enroll approximately 8000 heart failure patients that also implements a PK-based dose adjustment strategy. The QMS OM Immunoassay has been developed for the rapid (time to first result is approximately 10 minutes), quantitative determination of OM concentration in $K_3$-EDTA plasma. The assay is being investigated in central laboratories in the US, EU and Asia to support GALACTIC-HF.

**Methods:** The QMS OM assay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay. It is based on competition for anti-OM antibody binding sites between OM in the sample and OM coated onto microparticles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the OM concentration in the sample. The system has two reagent components, six calibrators that span 0 to 1200 ng/mL, as confirmed by an LC-MS/MS reference method, and multi-level controls.

**Results:** The following representative performance characteristics were obtained at the manufacturer laboratory. The studies were performed following CLSI guidelines, where applicable. The assay demonstrated a lower limit of quantitation of 85 ng/mL. Assay precision was determined by measuring controls, patient plasma pools and spiked plasma samples over the course of 20 days. Precision ranged from 1.0% to 3.1% CV (within) and 2.2% to 3.3% CV (total). The linear range of the assay was determined to be from 85 to 1200 ng/mL. The assay accurately recovered spiked OM samples at concentrations spanning this linear range. Specificity testing results suggested minimal to no cross-reactivity or interference with OM metabolites, medications potentially co-administered with OM in heart failure patients or endogenous substances commonly existing in human whole blood. One hundred forty-six (146) OM plasma samples measured by an LC-MS/MS reference method were tested by the QMS OM Immunoassay. The Passing-Bablok regression analysis yielded an equation of y = 1.04x - 6.4 and an R value of 0.99, indicating a strong correlation with the platform used in PK analyses (LC-MS/MS). The shelf life stabilities of reagents, calibrators and controls are greater than 12 months when stored as indicated. On-board reagent stability is 30 days. No sample carry-over was detected. To demonstrate Phase 3 clinical study readiness, the manufacturer's laboratory and two central laboratories in the US and EU demonstrated over a twenty (20)-day period lab-to-lab reproducibility less than 3% CV and bias versus LC-MS/MS less than 7%, among other performance characteristics.

**Conclusion:** QMS OM Immunoassay turn-around-time, precision, accuracy and other performance characteristics support its use in the GALACTIC-HF Phase 3 clinical study.

## B-379

### Comparison of drug testing rates and profiles in an urban vs a rural area in the province of Quebec, Canada

M. Habel[1], M. Bélanger[2], P. Hétu[2]. *[1]Hôpital Régional de Rimouski, CISSS Bas-St-Laurent, Rimouski, QC, Canada, [2]Centre hospitalier de l'Université de Montréal (CHUM), Montréal, QC, Canada*

**Background:** Drug addiction is often associated to a population living in underprivileged areas. Drug profiles may vary according to demographics, level of urbanization, crime rate and educational level. Few data exist on the patterns of drug abuse in urban and more rural communities in Quebec. In addition, many drug users and addicts consume more than one drug simultaneously. The objective of this project is to compare drug testing profiles of an underprivileged urban area to those of a semi-urban/rural area.

**Methods:** We compared drug testing in a major city's downtown area (Montreal, pop. 2 million) to the one of a small city in a remote area (Rimouski, pop. 50 thousand). Drug testing data for the year 2016 were extracted from Montreal's Laboratory Information System (LIS) and from Rimouski's laboratory. Incomplete results and screening in children less than 12 years old were excluded. Drugs of abuse screening panels (THC, opiates, cocaine, amphetamines, and benzodiazepines) were analyzed on Beckman-Coulter DxC 800 and 600. Reagents used (Beckman-Coulter) were the same except for the opiate analysis of the Montreal laboratory (CEDIA, Thermo Fisher). Data were analyzed according to sex and age, positive results for drug testing and frequency, origin of the request (emergency, outpatient, inpatient) as well as consumption profile.

**Results:** 68.5% (1980/2890) of drug screenings performed in downtown Montreal were positive compared to only 42% (640/1525) in Rimouski. In both cities, screening was more frequent in men (72% and 68%) than in women (28% and 32%), with similar rates of positivity for both sexes. The majority of positive results were found in individuals aged between 31 and 40 years old. Multiple drug abuse appeared to be more frequent in Montreal where 40.7% (806/1980) of screenings were positive for 2 to 3 drugs compared to 33.2% (212/640) in Rimouski. A similar pattern was observed for 4 drugs or more (5.2%, 49/1980 vs 1%, 5/640). In addition, Montreal's drug types differed from Rimouski's, with opiates and cocaine accounting respectively for 24% and 17.4% of all positive results (749 and 544/3126) in the former, compared to only 5.2% and 6.7% (47 and 61/911) in the latter, suggesting a different pattern of addiction between both populations. Rimouski had a slightly higher rate of positivity for THC (43.1% vs 33.6%) and benzodiazepines (26.9% vs 15.3%).

**Conclusion:** Clients screened for drug abuse were comparable between the two cities in terms of gender and age distribution. However, the rates of positivity and multiple drug abuse found in Montreal were much higher overall, with hard drugs such as opiates and cocaine being the main drugs of abuse. These results could indicate a higher drug addiction rate in Montreal's underprivileged neighbourhoods compared to Rimouski, a city known for it's relatively high quality of life. A deeper analysis of the results would be needed to confirm such a hypothesis.

## B-380

### Validation of a LC-MS/MS method for measuring Voriconazole with ABSciex 4500 and comparison to a GC-MS method

A. Smith, E. Enriquez, V. Leung-Pineda. *Cook Children's Medical Center, Fort Worth, TX*

**Background:** Voriconazole is an antifungal drug indicated for patients with active invasive infections and for prophylaxis to prevent new fungal infections in high-risk immunocompromised patients. Voriconazole pharmacokinetics are nonlinear and inter-individual variability is high. Therefore, there is great difficulty for clinicians to effectively keep the patient's serum concentrations in the therapeutic range. The need for therapeutic drug monitoring of voriconazole is great and turnaround time of results is crucial for effective dosing. As no FDA approved methods are available, we developed a method using GC-MS. However, as our laboratory acquired new LC-MS/MS equipment the decision was made to validate voriconazole in this platform, to improve method performance and turnaround time.

**Objective:** Our goal was to determine the performance specifications of a LC-MS/MS method for measuring Voriconazole, and to compare them to our previous GC-MS method.

**Methodology:** The following parameters were evaluated to validate the LC-MS/MS method: Linearity, LLMI, matrix effect, carryover, accuracy, dilution, precision, interferences and sample stability. Moreover, these parameter results were compared to our previous GC-MS method validation data, as well as proficiency testing results, QC statistics and sample run time.

**Results & Conclusions:** In general, the performance characteristics of our new LC-MS/MS method surpassed those of our previous GC-MS method. For the LC-MS/MS method, linearity was achieved from 0.2-10 µg/mL, and with dilution, we could measure up to 30 µg/mL. No matrix effect, extraction recovery or ion suppression issues were detected. Carryover was tested to be less than 0.5% and CVs for between runs was determined to be < 8% for 3 different concentrations. Accuracy was assessed by method comparison, testing of PT samples and spike and recovery, all acceptable.

Comparing the LC-MS/MS method to the GC-MS method, we saw a significant reduction in sample prep time and run time leading to quicker turnaround times.

Furthermore, comparing proficiency testing events, the LC-MS/MS method was more accurate, with an average bias%= 31% for the GC-MS method to 5.8% for the LC-MS/MS method. Precision statistics from routine QC also showed a decrease in the CVs from the GC-MS method (CV= 11.9%) to the LC-MS/MS (CV= 3.3%) method. Finally, method time from sample prep to result reporting was reduced from about 7 hrs with GC-MS to 4 hrs with LC-MS/MS method.

## B-381

### Exploration of Ion Ratio Challenges with Routine THC GC-MS Confirmation Assays

A. Brock, C. Gineste, L. Bazydlo. *University of Virginia, Charlottesville, VA*

Background: Mass spectrometric identification of drugs confers a high specificity, in part due to the use of ion ratios. Failure of ion ratios can be due to co-migrating interferences, matrix effects, or less than optimal peak shape resulting in poor integration. Ion ratio failures can lead to further work-up of the sample which can be time-consuming and costly, to verify that the identified analyte is accurate. We present a case study in which we investigated the cause of ion ratio failures in four cases and provide a solution that uses a different THC solid phase extraction column.

Methods: Four human urine specimens with prior ion ratio failures were analyzed by gas chromatography-mass spectrometry (GC-MS). Base hydrolysis, solid phase extraction, and derivatization were performed on the urine samples prior to GC-MS. Two batches of the four specimens were analyzed to compare Agilent Bond-Elut CERTIFY and United Chemical Technologies (UCT) Clean Screen THC solid phase extraction columns. GC-MS was performed quantitatively and qualitatively on two Agilent 7890A and 7890B gas chromatographs coupled to 5975C and 5977A inert mass spectrometers.

Results: All four specimens extracted with the Bond-Elut column had ion ratios outside of the acceptance criteria varying from 128 to 164% of the predicted ion ratio. In-depth analysis of the TIC scans for the specimens reveal the monitored ions at the appropriate retention time, but also an interfering shoulder on the 473 m/z qualifier ion that contributes to the increased ion ratio, an additional non-analyte peak with unexpectedly high derivatization, and a co-eluting, unanticipated 399 m/z ion demonstrating less predictable ionization patterns. Repeating the analysis with UCT-extracted specimens eliminated the ion ratio failures of all four specimens (92 – 114% of calibrators).

Conclusions: While urine drug confirmation with mass spectrometry is widely conducted, challenges with ion ratio failures are often mitigated by specimen dilution, sending out to other facilities, or overlooked altogether. Directly exploring these ion ratio difficulties reveal interesting correlations between specimen, solid phase extraction column, and ion ratio success.

## B-382

### A retrospective analysis of oxycodone metabolism in patients with different CYP2D6 genotypes

Z. Su. *Ameritox LLC, Greensboro, NC*

**Objective:** Oxycodone is one of the opioids being mostly abused and misused in pain management. In *vivo*, oxycodone is metabolized to oxymorphone and noroxycodone by cytochrome p450 enzymes, CYP2D6 and CYP3A4, respectively. The CYP2D6 gene is highly polymorphic, with more than 100 alleles having been determined, as a result affect its metabolic activity. Analysis of the correlation between CYP2D6 genotypes and urine concentrations of oxycodone and its metabolites will help us to understand the metabolism of the drug in human bodies.

**Methods:** CYP2D6 genotyping was performed using the QuantStudio 12K Flex Real-Time PCR system with buccal swab DNA. Urine concentrations of oxycodone,

oxymorphone and noroxycodone were tested with LC-MS/MS methods developed and validated in our laboratory.

**Results:** CYP2D6 genotyping has been done for total of 330 patients. 15 allelic variants (*1, *2, *3, *4, *5, *6, *9, *10, *13, *17,*29, *35, *41) were tested. The alleles with higher frequency are *1 (38%), *2 (16.5%), *4 (17.5), *5 (4.4%), *35 (5.0%), *41 (7.4%). The top four genotypes are: *1/*4 (10.9%), *1/*2 (10%), *2/*4 (6.4%), and *1/*41 (5.2%). CYP2D6 phenotypes were predicted based on the tested genotypes. Among 330 patients, 276 (83.6%) are extensive metabolizer/normal metabolizer (EM), 26 (7.9%) are poor metabolizer (PM), 18 (5.5%) are intermediate metabolizer (IM); and 10 (3.0%) are ultra-rapid metabolizer (UM).

Among the 330 patients, 116 were prescribed oxycodone and generated total of 476 urine samples in our database. 356 of the 476 urine samples (74.8 %) are from 95 extensive metabolizers; 68 urine samples (14.2%) are from 10 poor metabolizers, 26 urine samples (5.5%) are from 6 intermediate metabolizers, and 26 urine samples (5.5%) are from 5 ultra-rapid metabolizers. Ratios of oxymorphone and noroxycodone to the sum of parent drug and metabolites (oxycodone+oxymorphone+noroxyco done) were normalized by applying logarithmic transformation, respectively. An unpaired *t* test was used to analyze the normalized data. Our results indicated that urine concentrations of oxymorphone, a metabolite of oxycodone through the CYP2D6 enzyme pathway, are lower in CYP2D6 PM than those in EM, IM and UM significantly ($p = 0.0001$ for PM *vs* EM; PM *vs* IM; and

PM *vs* UM). However, there is no significant difference observed among EM, IM and UM (EM *vs* UM, $p=0.603$; EM *vs* IM, $p=0.237$; IM *vs* UM, $p=0.284$). Our data also indicated that there is no significant difference for urine concentrations of noroxycodone, another metabolite of oxycodone through CYP3A4 pathway, between CYP2D6 poor metabolizers and other metabolizers ($p>0.2$).

**Conclusions**: The metabolism of oxycodone to oxymorphone may be reduced in patients who are CYP2D6 poor metabolizer.

## B-383

### Ranitidine Interference in Roche Amphetamine Urine Drug Screen

K. Luckenbill[1], T. McManamon[2], J. Bartsch[1], H. Evans[1], E. Hanson[1]. *[1]Sanford Health Bismarck, Bismarck, ND, [2]Sanford Broadway Clinic, Fargo, ND*

**Background:** A urine specimen sent to our laboratory tested positive for amphetamines with the Roche Integra 400 amphetamines II immunoassay (500 ng/mL cutoff), negative for amphetamines with the Siemens Dimension EXL immunoassay (1000 ng/mL cutoff), and negative for amphetamines by LC-MS/MS performed at Mayo Medical Laboratories. The patient was taking 300 mg ranitidine daily, and no other prescription or over the counter drugs were declared. As of February 2017, Roche did not list ranitidine as a known interference in the Roche Integra 400 amphetamines II package insert. We investigated how much ranitidine was required to cause a false positive result using the Roche Integra 400 amphetamines II immunoassay with a 500 ng/mL cutoff.

**Methods:** Blank urine was spiked with ranitidine at concentrations ranging from 50-2500 ug/mL, and tested for amphetamines with the Roche Integra 400 amphetamines II immunoassay. The spiked samples were also tested with the Abbott Architect ci8200 amphetamine immunoassay (1000 ng/mL cutoff). In addition, a volunteer from the lab who was taking 150 mg ranitidine daily provided urine samples at 4 and 8 hours post-dose, and amphetamines were tested with the Roche Integra 400 amphetamines II immunoassay. Another volunteer from the lab took a single dose of 300 mg ranitidine, and urine and blood were collected at 4 and 8 hours post-dose. The urine was tested on the Roche Integra 400 amphetamines II immunoassay, and serum was sent to NMS Laboratories for ranitidine quantitation by HPLC.

**Results:** All ranitidine spiked samples, with the exception of the 50 ug/mL spiked sample, tested positive for amphetamines with the Roche Integra 400 amphetamines II immunoassay. The samples spiked with 2500 ug/mL and 1250 ug/mL tested positive on the Abbott Architect ci8200 amphetamines immunoassay, while the remainder of the samples, spiked with 625 ug/mL or less of ranitidine, were negative, which is consistent with the package insert. The lab volunteer who was taking 150 mg ranitidine daily tested negative for amphetamines at both 4 and 8 hours post-dose, while the lab volunteer who took 300 mg ranitidine, tested positive for amphetamines at both 4 and 8 hours post-dose, with the serum ranitidine concentration peaking at 390 ng/mL.

**Conclusion:** Standard doses of ranitidine, a frequently used prescription and over the counter H2-receptor antagonist, interfere with the Roche Integra 400 amphetamines II immunoassay, while much higher ranitidine doses are required to interfere with the Abbott Architect ci8200 amphetamines immunoassay. Laboratorians and clinicians need to be aware that ranitidine, a commonly used drug, interferes with amphetamine urine drug screens at varying concentrations depending on the manufacturer.

## B-384

### Improving the measurement of L-asparaginase: a standard-of-care drug used in pediatric oncology

A. W. S. Fung[1], D. A. Colantonio[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada*

**Background:** L-asparaginase (ASNase) is used as treatment for acute lymphoblastic leukemia (ALL) in children. ASNase catalyzes the hydrolysis of L-asparagine, depriving leukemic cells of this essential amino acid needed for growth. Despite improving survival outcomes, clinical hypersensitivity and silent inactivation remains a commonly reported adverse event (~30%). Therapeutic drug monitoring (TDM) of ASNase is essential to monitor treatment efficacy and identify patients with clinical hypersensitivity and silent inactivation. Currently, no commercial reagents are available, resulting in a lack of standardization of methods. Furthermore, there is little data on enzyme stability, interferences and dilution effects. This study shows the development and investigation of a microplate-indooxine method for measuring ASNase activity. Importantly, we investigated the use of inactivated vs neat serum for blanking and sample diluent (affects LoQ and patient samples); stability studies; and the potential interference due to endogenous ASNase activity, which is a recent discovery and never before reported in the literature. **Methods:** Two main preparations of ASNase are approved for ALL treatment: pegylated *Escherichia coli* ASNase (PEG-ASNase) and *Erwinia chrysanthemi* derived ASNase (ErASNase). Both differ in their pharmacokinetic and biochemical properties, requiring unique calibration curves. ASNase stock solutions were prepared by dissolving lyophilizates in 0.9% physiological saline solution, then mixed 1:1 with pooled blank human serum. Calibration standards and QC materials were diluted with pooled blank human serum to final working concentrations. Within-run and between-day imprecision, functional sensitivity (LoQ) and linearity were evaluated. Patient samples were split to perform a method comparison. Sample, QC and calibrator stability, the use of different matrices for blanking and sample dilution, and the frequency of endogenous ASNase activity, were investigated. **Results:** For PEG-ASNase, within-run and between-day imprecision ranged between 4.3-15.3% and 7.5-10.8%, respectively. For ErASNase, within-run and between-day imprecision ranged between 5.4-7.5% and 4.1-10.9%, respectively. Linearity was verified up to 300 IU/L for PEG-ASNase, and 250 IU/L for ErASNase. The LoQ is 15 IU/L for both. How results are interpreted clinically was assessed to determine what proportion of patients would be classified differently between methods; a few patients were classified differently, indicating comparability between methods. Stability of ASNase over several hours at room temperature, at 4°C, and over several freeze-thaw cycles were evaluated. Interestingly, samples were stable at room temperature over several hours as well as up to 3 freeze-thaw cycles. Blank serum with endogenous ASNase activity was investigated. Differences were observed between neat vs. inactivated serum when used for blanking or for dilutions, indicating that patient results can be greatly affected. Although rare, some patient's samples contain very low levels of endogenous ASNase activity. **Conclusions:** This in-house microplate-indooxine based ASNase activity assay for PEG-ASNase and ErASNase has acceptable precision, linearity, and functional sensitivity, and compares well to other methods. Our examination of the effects of sample stability, the use of inactivated serum for blanking and dilutions, the addition of glycerol to provide added sample stability to QC/calibrators, and endogenous ASNase activity demonstrate better patient testing and monitoring performance of the method over time.

## B-385

### Method validations for identification and quantification of fentanyl analogs

U. Uddayasankar[1], R. E. Ariano[1], C. Lee[2], C. Oleschuk[2]. *[1]University of Manitoba, Winnipeg, MB, Canada, [2]Diagnostic Services Manitoba, Winnipeg, MB, Canada*

**Background:** Fentanyl analogs are an emerging issue in clinical and forensic toxicology. While fentanyl is known to be 100 times as potent as morphine, the risk of lethal overdose appears to be exacerbated for fentanyl analogs, such as carfentanil, which has been postulated to be 10,000 times more potent than morphine. A targeted screening assay was developed to detect fentanyl analogs at low levels in both urine and post-mortem blood. Additionally, fentanyl immunoassay kits were studied for screening for fentanyl analogs. Finally, an LC-MS/MS assay was developed for quantitation of fentanyl analogs. This assay was used to determine sequential carfentanil levels in an overdosed patient enabling estimation of half-life of this drug.

**Methods and results:** Screening methods were evaluated for detection of carfentanil, furanyl fentanyl, U47700, AH-7921, MT-45, W-15, W-18, norcarfentanil and furanyl norfentanyl in urine and whole blood. Only carfentanil and furanyl fentanyl were

identified using Immunalysis and Thermo Fisher Scientific immunoassays, with limits of detection > 1 ng/mL. The LC-MS/MS screening method encompassed minimal sample preparation, reverse phase chromatography and MRM. Presence of a drug was determined by MRM ratios and retention time. The limit of detection for carfentanil and furanyl fentanyl were similar for urine and blood (0.2 ng/mL), while it was > 1 ng/mL for all other fentanyl analogs tested. The LC-MS/MS quantitative method was for analysis of carfentanil, furanyl fentanyl, MT-45, AH-7921 and U47700 in whole blood. It encompassed liquid-liquid extraction and lipid-removal procedures, reverse phase chromatography and MRM. The calibration range was 0.05 to 40 ng/mL. The assay was validated in keeping with SWGTOX guidelines. With the exception of MT-45, accuracy of analysis was within 10% of spiked values and %CV was < 10% at 0.4 and 5 ng/mL. For carfentanil half-life determination, heparinized whole blood from a carfentanil-overdosed patient was collected on admission, day 1 and 2 post-admission and analyzed for carfentanil. Medical treatment involved the administration of 2 mg of i.v. naloxone by first responders at the scene, however the patient remained unresponsive. The patient received supportive breathing by bag and was intubated at hospital. Upon admission, carfentanil concentration in blood was 22.4 ng/mL. The patient awoke 34 h after admission with an estimated corresponding carfentanil level of 0.45 ng/mL.

**Conclusions:** The LC-MS/MS screening method achieved a limit of detection of 0.2 ng/mL for carfentanil and furanyl fentanyl and had a lower limit of detection than either immunoassay evaluated. Neither immunoassay had reactivity with fentanyl analogs U47700, MT-45 or AH-7921 in addition to metabolites norfentanyl and furanyl norfentanyl. For these fentanyl analogs, the LC-MS/MS screening method had a limit of detection > 1 ng/mL. Using a more involved sample preparation, an LC-MS/MS method was developed with limit of quantitation of 0.05 ng/mL for carfentanil and furanyl fentanyl, which has been a satisfactory limit of quantitation for all cases of toxic exposure examined to date. Based on our analysis of an overdosed patient, the half-life for carfentanil in humans is 5 – 6 hours.

## B-386

### Falsely elevated total phenytoin by EIA in a uremic patient treated with fosphenytoin

A. W. S. Fung[1], D. A. Colantonio[2]. [1]*University of Toronto, Toronto, ON, Canada,* [2]*The Hospital for Sick Children, Toronto, ON, Canada*

**Background:** Phenytoin is a commonly prescribed anticonvulsant drug for partial and generalized tonic-clonic seizure. Fosphenytoin, a phosphate ester prodrug of phenytoin, is readily water-soluble and can be administered via intravenous or intramuscular injections resulting in less severe side effects than phenytoin. Fosphenytoin is pharmacologically inactive and metabolized to the active phenytoin *in vivo* by phosphatases in liver, erythrocytes, and other tissues. Fosphenytoin can also be dephosphorylated *in vitro* by serum alkaline phosphatase. Here we report a case of a uremic patient, who was treated with fosphenytoin, showing falsely elevated total phenytoin results by both Abbott ARCHITECT EIA and Roche Modular EIA methods compared to an HPLC method. We also report the results of cross-reactivity studies for fosphenytoin by ARCHITECT EIA and HPLC phenytoin methods. **Methods:** For the investigation of the index patient, we measured total phenytoin by HPLC, Abbott ARCHITECT EIA, and Roche Modular EIA, along with free phenytoin by HPLC. For the cross-reactivity study, stock fosphenytoin solution was diluted in 0.9% physiological saline and spiked into pooled human serum or saline at a range of concentrations. Samples were then incubated at room temperature for 0, 2, and 17 hours and phenytoin concentrations measured by ARCHITECT EIA and HPLC. To further elucidate whether high urea in patient specimens affect phenytoin immunoassays *in vitro*, fosphenytoin was also spiked into pooled serum containing normal or high urea levels. Samples were then incubated at room temperature for 0, 2, and 6 hours. Urea concentrations were measured by Ortho Vitros colorimetric method and phenytoin concentrations measured by ARCHITECT EIA and HPLC methods. Percent cross-reactivity were calculated. **Results:** In the index patient, total phenytoin differs between ARCHITECT EIA and HPLC by 120 to 215% in different specimens. Investigation of discordant result by Roche Modular EIA also show a discrepancy with HPLC values. Cross-reactivity studies showed that fosphenytoin cross reacts with the phenytoin immunoassay depending on the presence of serum alkaline phosphatase, and does not depend on *in vitro* urea concentrations. Comparisons between spiked pooled serum and spiked saline showed a 10% cross reactivity of fosphenytoin by the ARCHITECT phenytoin EIA assay. This was not reported in the manufacturer package insert. **Conclusions:** Fosphenytoin may crossreact with phenytoin immunoassays. This results in unpredictable discordant phenytoin results with specimens containing fosphenytoin. Therefore, it is recommended that for patients treated with fosphenytoin, therapeutic drug monitoring of phenytoin should be measured 2-4 hr post-dose to ensure all fosphenytoin have been metabolized into phenytoin. Additionally, the index patient is uremic and may contain other metabolites that are immunoreactive to phenytoin immunoassay antibodies. This is consistent with a previously reported observation that falsely elevated phenytoin results may be due to the oxymethylglucuronide metabolite that is accumulated in patients with renal insufficiency. Therefore, free fraction of phenytoin by HPLC is recommended for therapeutic drug monitoring for uremic patients, patients with chronic liver disease, and patients with hypercholesterolemia to prevent misinterpretation.

## B-387

### Development and Validation of an LC-MS/MS Method for the Quantification of Capillary Blood Hydroxychloroquine Levels Collected on Volumetric Absorptive Microsampler

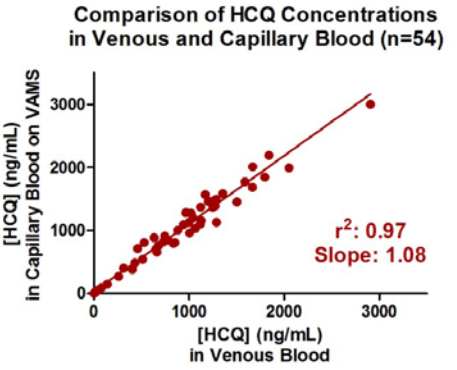
R. Apilado[1], K. Brady[1], T. O'Malley[1], P. Chitkara[2], S. Reddy[3], C. Ibarra[1], R. V. Alexander[1], Y. Qu[1], T. Dervieux[1]. [1]*Exagen Diagnostics, Vista, CA,* [2]*Center for Arthritis and Rheumatologic Excellence, Chula Vista, CA,* [3]*Arthritis Care and Research Center, Poway, CA*

**Background:** Hydroxychloroquine (HCQ) blood levels have established utility in patients with chronic autoimmune rheumatic diseases. We sought to develop and validate an LC-MS/MS method for capillary blood hydroxychloroquine (HCQ) levels collected on volumetric absorptive microsampler (VAMS).

**Methods:** Capillary blood on VAMS (10 µL per specimen) and venous blood in EDTA (10 ml per specimen) were collected in 54 subjects receiving HCQ (200-400 mg/day). VAMS were eluted into water containing deuterated (d4) HCQ internal standard (IS). Following deproteinization with perchloric acid, supernatants were injected onto a Kinetex C8 column coupled to a TSQ Quantiva mass spectrometer with a heated electrospray ionization source operating in positive mode. Mobile phase was 0.1% formic acid and 0.01% triethylamine in water with an acetonitrile gradient. Transitions consisted of m/z 336 → 247 and m/z 340 → 251 for HCQ and IS, respectively. Venous blood HCQ levels were also measured using LC-MS/MS.

**Results:** Lower limit of quantification for capillary blood HCQ levels on VAMS was 10 ng/mL and was linear from 10 to 2000 ng/mL. Accuracy was within 15% of target value and intra/inter-day coefficients of variation were below 15%. No carry over, matrix effect, or interference from endogenous or exogenous substances were observed. HCQ concentrations recovered from capillary blood (day 3 elution, average 970±90 ng/ml) were similar to those observed in venous whole blood (average 873±83 ng/ml) ($r^2 = 0.97$; Deming slope = 1.08). HCQ collected on VAMS was stable for 10 days at ambient temperature and at least for 24 hours at 50°C**.**

**Conclusion:** HCQ can be accurately measured in capillary blood collected on VAMS. Our method offers a valuable alternative to venous blood for therapeutic drug monitoring of HCQ.




## B-388

### Quantitation of Gabapentin, Pregabalin, Lamotrigine, Topiramate and Clobazam in Serum by LC- MSMS Using Low Sensitivity Ions to Expand the Linear Range

P. Rutter, M. Jarrells, R. Whitley. *University of Kentucky, Lexington, KY*

**Background**: Blood drug testing is an effective tool for compliance monitoring, dose correlation and clinical tolerance. The aim of this study was the development of a fast and cost-efficient LC-MSMS method for simultaneously detecting and quantifying five anticonvulsant drugs in serum covering a projected quantitation range of 2 to

50,000 ng/mL (4+ orders of magnitude). Calibration curves constructed using primary product ions for gabapentin, pregabalin, lamotrigine and topiramate demonstrated signal saturation and deviation from linearity. To solve this problem, a strategy for expanding the linear range using secondary 'low sensitivity' product ions was developed. Deuterated analogs of pregabalin and topiramate were used as internal standards.

**Method**: Ten microliters of a 50 µg/mL internal standard mixture, containing deuterated standards in methanol, was added to fifty microliters of patient serum followed by 400 µL of acetonitrile. The solution was mixed for 15 seconds and centrifuged at 13,000 rpm for 5 min. at room temperature. The supernatant was transferred to a glass tube and evaporated to dryness at 37° C for 10 min using a gentle stream of air. The extracted sample was reconstituted with 200 µL of mobile phase A, mixed, transferred to an autosampler vial and injected onto a RESTEK Ultra bi-phenyl analytical column (5 µm, 50x2.1 mm) maintained at 40° C. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in acetonitrile. The acquisition method utilized 10 µl injection volume, 0.6 mL/min flow rate, and a gradient program of 98% A, increased to 80% B over 4 min. Run time was 5 min (injection to injection). The HPLC system consisted of Shimadzu pumps and autosampler. MSMS was performed on a Sciex 4500 triple quadruple mass spectrometer with TurboIonSpray© source monitored in positive and negative modes using primary (high sensitivity) and secondary (low sensitivity) product ions.

**Results**: Specificity was assessed by retention times and unique quantifier/qualifier transition peak area ratios. Intra-assay imprecision at two concentrations averaged 7.8% CV. A primary product ion was used to quantitate clobazam in the low (nanogram) range; linearity ranged from 2 to 1,000 ng/mL. Secondary product ions were used to quantitate gabapentin, pregabalin, lamotrigine and topiramate in the microgram range; linearities ranged from 100 to 50,000 ng/mL. Extraction efficiencies were greater than 90%. Matrix effects of native analytes were similar to deuterated analogs and did not affect quantitation. No carryover, endogenous or exogenous interferences were observed, with analyte stability at room temperature for 24 hrs. Qualitative correlations between our procedure and commercial LC-MSMS methods showed 100% agreement at cutoffs. Quantitative correlations showed less than 10% differences.

**Conclusion**: We present the development and validation of a LC-MSMS procedure for the quantitative determination of five anticonvulsants in serum at therapeutic levels employing small amount of a single specimen, deuterated internal standards, and a single extraction - without derivatization and additional chromatographic resolution. By monitoring both primary and secondary product ions, the linear measurement range is expanded, and the need for re-assay with dilution or pre-dilution is eliminated. This novel method is suitable for routine clinical use.

## B-389

### Development of a Plazomicin Immunoassay as a Potential Aid for Therapeutic Drug Management (TDM) in the Potential Treatment of Serious Bacterial Infections Due to Multi-drug Resistant (MDR) Enterobacteriaceae, including Carbapenem-Resistant Enterobacteriaceae (CRE)

A. Thao[1], R. Ye[1], A. Tsai[1], J. Huang[1], K. Bender[2], R. Theolis[2], N. Sandon[2], T. Brotz[2], L. Ruiz-Taylor[2], L. Ye[1], T. Rigl[1], T. Prestigiacomo[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Achaogen, South San Francisco, CA*

**Background:** Rapid spread of multi-drug resistant (MDR) bacteria is rendering current antibiotic therapies less effective. Plazomicin is a next generation aminoglycoside being developed for the treatment of serious bacterial infections due to MDR Enterobacteriaceae, including carbapenem-resistant Enterobacteriaceae (CRE). Plazomicin is synthetically derived from sisomicin and contains structural modifications allowing it to maintain activity in the presence of common aminoglycoside-modifying enzymes that inactivate existing aminoglycosides. The QMS® Plazomicin Immunoassay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay under development for the quantification of plazomicin in human plasma and serum using automated clinical analyzers to help monitor plazomicin concentrations in patients receiving plazomicin.

**Methods:** The QMS Plazomicin Immunoassay consists of two reagent components, namely a monoclonal anti-plazomicin antibody and plazomicin-coated microparticles, six calibrators spanning from 0 to 40 µg/mL and tri-level controls. The immunoassay is based on competition between free plazomicin present in a sample and plazomicin derivative coated onto microparticles for antibody binding sites. The plazomicin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-plazomicin antibody reagent and the rate of absorbance change is measured photometrically. When a sample containing plazomicin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent agglutination inhibition curve is obtained with maximum rate of agglutination at the lowest plazomicin concentration and lowest agglutination rate at the highest plazomicin concentration. The QMS Plazomicin Immunoassay performance characteristics were evaluated on the Beckman Olympus AU680 analyzer in both plasma and serum through a set of analytical studies following CLSI guidelines.

**Results:** The assay range was determined to be 0.4-40 µg/mL. The lower limit of quantitation (LLOQ), was 0.4 µg/mL. The assay was linear with less than 6.3% error for plazomicin concentrations between 0.8-40 µg/mL. Assay precision and accuracy in plasma and serum spiked with 2, 4, 15, and 30 µg/mL of plazomicin were determined by testing twice per run, two runs per day for 5 days. This yielded within-run precision between 1.8 and 3.2 %CV and total-run precision between 3.1 and 6.7 %CV, with accuracy greater than or equal to 95.6%. No significant interference was observed with various endogenous substances, aminoglycosides, or common concomitant medications. No known plazomicin metabolites have been identified to test for potential cross reactivity with the assay. Sample carry-over was 0.158 µg/mL or below. Method correlation studies comparing the QMS Plazomicin Immunoassay and a LC-MS/MS reference method using clinical plazomicin plasma samples yielded a Passing-Bablok's regression equation of y = 1.03x - 0.122 and a correlation coefficient of 0.9770 (n=82). Onboard reagents were stable for a minimum of 30 days and the calibration curve was stable for 7 days.

**Conclusion:** The QMS Plazomicin Immunoassay enables measurement of plazomicin with acceptable precision and accuracy between 0.4 - 40 µg/mL. The assay's ability to accurately measure plazomicin has the potential to enable individualized dosing for patients receiving plazomicin, a novel antibiotic also under development. This project has been partially funded under BARDA Contract No. HHSO100201000046C.

## B-390

### CEDIA® Heroin Metabolite (6-AM) Application for the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

T. Prestigiacomo, T. Huynh, M. Aquino, K. Cheung. *Thermo Fisher Scientific, Fremont, CA*

**Introduction**: Heroin (3, 6-diacetylmorphine) is a Schedule 1 substance and commonly abused opioid within the United States and in the rest of the world. It is generally synthesized through chemical modification of morphine, a naturally occurring alkaloid. Heroin can be administered by intravenous and subcutaneous injection or by nasal insufflation. It is rapidly metabolized (half life of 9 minutes) to Monoacetylmorphine (6-AM) by various esterases in the blood; then it is converted to morphine within the liver through hydrolysis. 6-AM is a distinctive metabolic marker for heroin use because it cannot be formed by acetylation of morphine within the body. Use and possession of heroin is illegal and is associated with a number of adverse effects including lung complications, kidney disease, and bacterial infection of blood vessels. The ability to detect heroin within overdose patients and drug offenders proves vital for health practitioners and the members of the criminal justice system. **Method:** The CEDIA 6-AM Assay utilizes the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments (EA and ED). The heroin metabolite present within human urine samples and the 6-AM conjugated to an inactive enzyme fragment (ED-LC) compete for antibody binding sites. Because 6-AM inhibits the binding of ED-LC to the antibody, its presence allows the two inactive enzyme fragments to better re-associate into an active enzyme. The concentration of 6-AM within the sample will affect the complementation of the enzymes fragments. Enzyme activity results in an absorbance change that is directly proportional to the concentration of 6-AM in the sample; this change can be measured spectrophotometrically. The VITROS 4600 Chemistry and 5600 Integrated Systems are new applications for the CEDIA 6-AM Assay for the qualitative detection of heroin metabolite in human urine with a cutoff of 10 ng/mL. Analyzer performance was determined for precision, limit of blank, and accuracy. Correlation studies using the two instruments were conducted in comparison to liquid chromatography-mass spectrometry (LC-MS) values.

**Results:** All studies were evaluated using CLSI guidelines. Three levels of 6-AM controls were used in the studies. The within-run precision ranged from 0.2 to 0.5% CV and the total precision, 1.5 to 2.6%CV. The limit of blank on the VITROS 4600 Chemistry and 5600 Integrated Systems resulted in 0.43 ng/mL and 0.35 ng/mL, respectively. Accuracy was measured using patient correlation against LC-MS values. The VITROS 4600 Chemistry System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, $n_{total}$=100). The VITROS 5600 Integrated System yielded 92.7% Positive Agreement, 97.8% Negative Agreement, and 95.0% Total Agreement ($n_{negative}$=45, $n_{positive}$=55, $n_{total}$=100).
**Conclusions:** All measured studies demonstrated acceptable performance, validating

use of the CEDIA Heroin Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry and 5600 Integrated Systems. The assay will provide an effective detection system to screen individuals who use heroin in its various forms.

## B-391

### DRI® Hydrocodone Application on the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

T. Prestigiacomo, M. Aquino, T. Huynh, K. Cheung. *Thermo Fisher Scientific, Fremont, CA*

**Introduction:** Hydrocodone is a commonly used semi-synthetic opioid derivative. It is synthesized from codeine, a naturally occurring alkaloid. Due to its ability to relieve moderate and severe pain, hydrocodone has grown in usage, particularly within the United States. The analgesic effect of the drug begins within 20-30 minutes of taking it and can last up to 8 hours. Furthermore, an increase in the abuse of hydrocodone in its various forms has been observed in recent years. Thus, an effective monitoring system is necessary for clinicians and law enforcement to determine drug levels within addicts and criminals. **Method:** The DRI Hydrocodone Assay utilizes a drug-labeled variant of glucose-6-phosphate dehydrogenase (G6PDH) and the effects of competitive inhibition. When in the presence of select antibodies, G6PDH competes with free drug present within a sample for antibody binding sites. When the drug-labeled enzyme binds to the antibodies, enzyme activity is decreased as a result. Thus, drug concentration and enzyme activity are directly proportional. This relationship can be determined by monitoring the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 Chemistry System and VITROS 5600 Integrated System are new applications for the DRI Hydrocodone Assay for the qualitative and semiquantitative determination of hydrocodone in human urine at a cutoff of 300 ng/mL. The analyzers were subjected to precision, limit of blank (calculated as three times the standard deviation of the negative calibrator), and linearity studies. **Results:** All studies were evaluated in adherence to CLSI guidelines. Total precision was conducted over the span of 20 days. In this timeframe, the low and high control values were compared to the cutoff calibrator in both qualitative and semiquantitative methods. Within-run precision results ranged from 0.1% to 0.3% CV qualitatively and 1.1% to 1.9% CV semiquantitatively between the two instruments. Total precision results ranged from 0.2% to 0.5% CV qualitatively and 1.9% to 3.2% CV semiquantitatively between the two instruments. The limit of blank was determined to be 3.01 ng/mL on the VITROS 4600 Chemistry System and 3.17 ng/mL on the VITROS 5600 Integrated System. Additionally, linearity was evaluated by comparing calibrator blends to their nominal values. The VITROS 4600 Chemistry System yielded 95.2% to 102.0% recovery, and the VITROS 5600 Integrated System yielded 94.5% to 101.7% recovery. **Conclusions:** All studies aforementioned demonstrate acceptable performance of the DRI Hydrocodone Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and 5600 Integrated System. Further studies characterizing positive and negative agreement between methods will be conducted.

## B-392

### DRI® Methadone Metabolite (EDDP) Application on the Ortho Clinical Diagnostics VITROS® 4600 Chemistry System and VITROS 5600 Integrated System

T. Prestigiacomo, M. Aquino, T. Huynh, K. Cheung. *Thermo Fisher Scientific, Fremont, CA*

**Introduction:** Methadone is a synthetic opioid that has been used for decades by clinics and other addiction-treatment facilities to manage opioid dependency. After administration, methadone is metabolized to normethadone by N-demethylation, which is converted by dehydration to the primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)[1]. Effective monitoring of methadone and its metabolite is essential for ensuring compliance. **Method:** The DRI Methadone Metabolite Assay is a competitive assay between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug in the urine sample for a fixed number of antibody binding sites. Antibodies detect EDDP without crossreactivity to the parent drug, methadone. Drug concentration and enzyme activity are directly proportional to the conversion of NAD to NADH, which is measured spectrophotometrically at 340 nm. The VITROS 4600 Chemistry and 5600 Integrated Systems are new applications for the DRI Methadone Metabolite Assay for the qualitative and semiquantitative determination of methadone metabolite in human urine at a cutoff of 1000 ng/mL. **Results:** All studies were evaluated using CLSI guidelines. Total precision was evaluated for 20 days in which low and high control levels were compared to the cutoff calibrator level in both qualitative and semiquantitative modes. For the VITROS 4600 Chemistry System, qualitative %CV results ranged from 0.3-0.4% and semiquantitative %CV results ranged from 1.4-1.7%. For the VITROS 5600 Integrated System, qualitative %CV results ranged from 0.6-0.9% and semiquantitative %CV results ranged from 1.3-1.7%. The Limit of Blank (i.e., 3 times the SD of the negative calibrator) was determined to be 29.4 ng/mL on the VITROS 4600 Chemistry System and 21.6 ng/mL on the VITROS 5600 Integrated System. One hundred and ten (110) patient samples were analyzed for positive and negative agreement compared to liquid chromatography-mass spectrometry (LC-MS). Qualitative positive and negative agreement was 100% and 90%, respectively, on both instruments. Semiquantitative positive agreement was 98% on the VITROS 4600 Chemistry System and 100% on the VITROS 5600 Integrated System. Semiquantitative negative agreement was 91.7% on both instruments. **Conclusions:** All studies demonstrated acceptable performance, validating the use of the DRI Methadone Metabolite Assay on the Ortho Clinical Diagnostics VITROS 4600 Chemistry System and VITROS 5600 Integrated System.

## B-393

### ARK Fentanyl Assay for the Beckman Coulter AU680 Automated Clinical Chemistry Analyzer

K. Chung, M. Abney, L. Hodges, B. Moon, J. Valdez. *ARK Diagnostics, inc, Fremont, CA*

**Background:** Fentanyl is a highly addictive potent synthetic opioid that is widely used for chronic pain management and surgical anesthesia. The drug is a controlled Schedule II substance and was introduced in 1960 as a replacement for other opioids in cardiac surgery. Currently, the drug is available as injectable solution for surgical anesthesia and transdermal patches at concentrations of 25, 50, 75, 100 mg/h for chronic pain management. Other than medical applications, fentanyl has also been sold to drug users, primarily heroin abusers and resulted in hundreds of overdoses. The severity of the situation became apparent when the above average numbers of overdoses were observed in many regions in United States. Since fentanyl is 50-100 times more potent than heroin and present in biological samples at very low concentrations, administration and monitoring of fentanyl present a great challenge in clinical and forensic laboratories. There is an increasing need for a high throughput screening method for the detection of fentanyl in human urine.

**Methods:** The ARK Fentanyl Assay is a liquid stable, homogeneous enzyme immunoassay, intended for the qualitative and/or semi-quantitative determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL on automated clinical chemistry analyzers. Two reagents, calibrators (0.0, 1.0, 2.0, 4.0, and 10.0 ng/mL) and controls (0.5 and 1.5 ng/mL) compose the test system. The 1.0 ng/mL Calibrator is the Cutoff for distinguishing "positive" from "negative" samples. Precision over 20 days, histogram overlap analysis of Control and Cutoff concentrations, recovery and specificity were evaluated on the Beckman Coulter AU680.

**Results:** Semi-quantitative precision was determined for 0.5 (11.3%CV), 1.0 (5.5%CV) and 1.5 (6.0%CV) ng/mL. Qualitative determination of fentanyl in Low and High controls did not overlap with the Cutoff by histogram analysis. Recovery of fentanyl ranged from 91.6% (0.75 ng/mL) to 104.7% (6.0 ng/mL). Norfentanyl metabolite tested positive at 300.0 ng/mL. Fentanyl analogues despropionylfentanyl, hydroxyfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, and sufentanil tested positive at 75.0, 1.0, 1.0, 2.0, 500.0 and 600.0 ng/mL respectively. Other opiates were not crossreactive. The sensitivity (true positive, 100 samples) and specificity (true negative, 50 samples) was 96.2% and 98.0%, respectively, versus LC-MS/MS (fentanyl cutoff 0.2 ng/mL).

**Conclusion:** ARK Fentanyl Assay determines fentanyl in human urine accurately and sensitively in either semi-quantitative or qualitative modes with fast turn-around times. Detection of fentanyl use in pain management, compliance or misuse/abuse with a superior cutoff concentration for a screening assay is an important new addition to clinical chemistry.

## B-394

### Two efficient sample preparations for opiates testing in urine

X. Zang[1], S. Milasinovic[1], P. Reddy[1], A. Oroskar[1], Y. Duperron[2]. *[1]Orochem Technologies Inc., Naperville, IL, [2]KURA Biotec-La Piedra Biotecnologia SpA, Puerto Varas, Chile*

**Background:**

In 2014, nearly two million Americans either abused or were dependent on prescription opioid pain relievers. 91 Americans die every day from an overdose involving either prescription opioid or heroin. Today´s abuse profile combine both clinical and street drugs. Combining efficient hydrolysis based on purified beta-glucuronidases with an automatable clean-up or extraction method enables provides robust and defendable results.

**Methods:**

Two different sample preparation methods were tested for recovery, linearity, precision and enzyme hydrolysis. Three analytes (6-MAM, codeine, and morphine) and their deuterated internal standards (IS) were spiked to blank urine at different concentrations for extraction method validation. Codeine-6-beta-D-glucuronide, morphine-3-beta-D-glucuronide and 6-MAM were spiked to blank urine at ULOQ level (equal to 2000 ng/ml free drug level) for enzyme hydrolysis recovery test.

For filtration method, 0.2 ml of master mix (including ammonium acetate buffer , 66 µL of EBG beta-glucuronidase and IS ) and 0.1 ml of fortified urine was directly added to RubyPro protein crash plate (2cc/well 96-well plate). After incubation at 50°C for 15 minutes, 0.05 ml of methanol was added to each well. After 5 minutes, positive pressure was applied to elute the analytes, and eluate was injected to LC-MS/MS (Shimadzu 10AD-API 3000) directly.

For solid phase extraction (SPE) method, mixture of 0.25 ml of master mix(ammonium acetate buffer, 25 µl of BG100 beta-glucuronidase and IS) and 0.1 ml of fortified urine was incubated at 68°C for 30 minutes. Orpheus C18 SPE plate(50mg/well, 2cc/well plate) was pre-conditioned with 1 ml of methanol, then 1 ml of water. Hydrolyzed urine solution was loaded to C18 and then washed with 1 ml of each water and 5% methanol. Analytes were eluted with 0.5 ml of methanol, and directly injected to LC-MS/MS. LC-MS/MS was operated in positive ion mode. Reliasil C18 3 micron, 2.1x50 mm column was used for separation with acetonitrile and water gradient mobile phases. LC run time is 5 minutes.

**Results:**

For both methods, all analytes are linear in the range of 20-2,000 ng/ml, with precision within 15% and 6-MAM integrity is preserved with above 90% recovery. Recoveries of codeine and morphine are similar for both methods, above 85% for codeine and above 90% for morphine.

**Conclusion:** Robust and defendable results are achieved. Two accurate and automated sample prep methods for opiates drug testing are validated. Technically, the methods are a proof of concept solving three analytical challenges: hard-to-cleave analyte (codeine-6-glucuronide), protein-binding (morphine), labile analytes ( 6-MAM). Hydrolysis can be conducted directly on the Protein crash filter plate providing a faster, simpler to operate method, at a lower cost. It meets the typical screening requirement of PDM and pain-management. C18 SPE method removes more endogenous in the urine, and increases the lifetime of LC column and mass spectrometer. With 0.5 ml of elution, we eliminate evaporation time. This method may be preferred for opiates/opioids confirmation assays,e.g. in workplace drug-testing. Both method should now be challenged with the addition of further analytes to complete a broad pain-panel.

## B-395

### Predicting Drug Exposure in Breast-Feeding Infants: Using Physiologically-Based Pharmacokinetic Modeling of Escitalopram in Breast Milk to Simulate Infant Plasma Concentrations

S. Delaney[1], P. Malik[2], M. Takeuchi[3], C. Stefan[4], S. Ito[3], A. Edginton[2], D. Colantonio[3]. *[1]University of Toronto, Toronto, ON, Canada, [2]University of Waterloo, Waterloo, ON, Canada, [3]Hospital for Sick Children, Toronto, ON, Canada, [4]Centre for Addiction and Mental Health, Toronto, ON, Canada*

**Background:** Postpartum depression is a common disorder affecting between 10-15% of new mothers. Escitalopram (ESC) is considered to be the first line treatment for postpartum depression; however safety data about its use during breastfeeding is limited and the risk of drug exposure to the nursing infant remains unknown. To ensure new mothers are compliant to their medication regimen, along with safeguarding the nursing infant from potential drug exposure, it is essential to determine the safety profile of ESC during lactation and investigate the extent of drug exposure to the nursing infant. The objectives of this study are: i) develop an LC-MS/MS method to measure ESC in breast milk, ii) perform population pharmacokinetics (popPK) and physiologically-based pharmacokinetic modeling (PBPK) to simulate drug concentrations in milk and infant plasma so to estimate the risk of adverse events. **Methods**: Eighteen women receiving ESC therapy were recruited through the Drugs in Lactation Analysis Consortium (DLAC) framework at the Hospital for Sick Children. Breast milk was collected at 3-5 time points, post-dose, across the patient's dosing interval (24h). An LC-MS/MS method was developed for ESC and its metabolite, desmethylescitalopram (D-ESC), and validated in accordance with CLSI guidelines. To simulate the infant dose based on the concentration of drug in breast milk, popPK modeling was performed using the non-linear mixed effects program, NONMEM, using stochastic approximation expectation maximization and the ADVAN5 subroutine. After the infant dose was generated, PBPK modeling was then performed to simulate infant plasma drug concentrations using PK-SIM software. **Results**: Results from the development and validation of the ESC/D-ESC LC-MS/MS method will be presented. There was no significant difference seen between ESC concentrations in the foremilk and hindmilk. A one-compartment model (+ absorption) with a proportional residual error model was selected to best describe the time-concentration profile of ESC in milk. Based on this final popPK model, ESC concentrations in milk were simulated at steady-state in 1000 women and the median dosage an infant would be exposed to, via milk, was 9 µg/kg/day. The infant dose at the 99th percentile was 8.8% of the weight-adjusted maternal daily dose, which was 18 µg/kg/day. Using a PBPK approach, the median simulated infant $AUC_{0\text{-}inf}$ following ESC exposure through breast milk was 12 µg.h/L, which is 3.2% of the maternal $AUC_{0\text{-}inf}$. Both the dosage through breast milk and the median infant $AUC_{0\text{-}inf}$ fall well below the therapeutic range. **Conclusion**: The data from this study demonstrates that infant exposure to ESC through breast milk is less than 10% of the maternal weight-adjusted dose. These results indicate that the risk of an adverse events in nursing infants is low. Therapeutic drug monitoring using a robust analytical method, followed by pharmacokinetic modeling and simulation data, provide a comprehensive view of ESC excretion into milk that will help guide decision making for drug use during lactation. Filling in the gaps of knowledge that exist in this area is essential to understanding drug safety and reducing the risk of adverse events in infants exposed to drugs through breast milk.

## B-396

### Increasing Sample Throughput for Quantitation of Anticonvulsant Drugs for Clinical Research Use

K. Hassell[1], S. Mundodi[2]. *[1]ThermoFisher Scientific, Somerset, NJ, [2]ThermoFisher Scientific, San Jose, CA*

Anticonvulsant drugs such as zonisamide, lacosamide, topiramate, levetiracetam, lamotrigine, clozapine, norclozapine, and oxcarbazepine metabolite, 10,11-Dihydro-10-Hydroxycarbamazepine have been measured in biological fluids in both clinical research and forensic analysis. Better techniques for monitoring concentrations of these drugs are needed to help optimize drug therapies in healthcare. In this study we evaluate an analytical method to measure multiple anticonvulsant drugs within a very narrow range of detection and sensitivity to meet clinical research laboratory requirements for analytical efficiency. Detection in biological fluids can be time consuming however, by utilizing online sample cleanup and multi-channel LC/MS detection, a fast cost effective analysis of anticonvulsant drugs can be performed. All standards were obtained from Cerilliant (Round Rock, TX) and analyzed as received. Serum curves were prepared in human serum, isotopic labeled standards were added, vortexed, and spun. Supernatant was put in autosampler vials and injected into the LC/MS. A volume of 5 uL was injected onto a Thermo Scientific™ TurboFlow™ Cyclone™ column, which was then focused onto an analytical column, Thermo Scientific™ Hypersil™ GOLD aQ. Compound separation was accomplished using a reverse-phase gradient with the Thermo Scientific™ Transcend™ II system with Thermo Scientific™ UltiMate™ 3000 RSLC pumps in a duration of seven minutes. The Thermo Scientific™ TSQ Endura™ was employed to detect the target analytes using electrospray ionization. Timed selected reaction monitoring (SRM) was utilized to maximize detection efficiency for the compounds analyzed. In order to increase throughput, multiplexing two LC channels was employed. In order to maximize efficiency, data windows are made as small as possible so the detector can always be acquiring data. With the analytical method developed here utilizing two-dimensional chromatography, 80-90% recovery off the TurboFlow column was obtained. Accuracy and precision data were collected in neat and serum matrices**.** The analytical performance gave RSD values that were less than 15.0% for all compounds tested. Additionally, accuracy was ±15% of the theoretical value for all the assays. The correlation coefficient values for all the compounds were below 0.995, showing linearity throughout all concentrations and analytes. Detection levels below

50 ng/mL can be obtained which meets clinical research laboratory requirements. In addition, multiple compounds can be analyzed in one run and can be separated chromatographically.

## B-397

### Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Assay for the Simultaneous Quantitation of 5 Azole Antifungals and 1 Active Metabolite

A. J. McShane, S. Wang. *Cleveland Clinic, Cleveland, OH*

**Background**: Invasive fungal infections are deadly and prevalent in certain high-risk patient populations: patients with hematological malignancies, the immunosuppressed, and the critically ill. Successful azole antifungal medication therapy can be life-saving for these patients. To support azole therapeutic drug monitoring at our hospital, a liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated for 6 analytes: fluconazole, voriconazole, posaconazole, isavuconazole, itraconazole, and its active metabolite hydroxyitraconazole. **Methods**: During preparation, 50 µL of sample was precipitated via mixing with 250 µL of an acidified acetonitrile solution [0.1% (v/v) 1M HCl] containing the internal standards for all 6 analytes (1 µg/mL). A 50 µL aliquot was then diluted with 200 µL 0.1% (v/v) formic acid in ultrapure water. Utilizing a Transcend LC system (Thermo Scientific), 20 µL of the sample was injected onto a reversed-phase column (Accucore RP-MS, 50 x 2.1 mm, 2.6 µm), coupled to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer. To validate the method, the following experiments were performed: ion suppression, mixing study, interference, analytical measuring range (AMR), carryover, stability, precision, and method comparison. **Results**: *Ion suppression* Only fluconazole exhibited signal suppression versus a blank sample. *Mixing study* The mean percent difference between pooled-patient serum and charcoal-stripped serum (the calibrator matrix) ranged from -5.2% to 4.3%. A mixing study was also performed between pooled-patient serum and pooled-patient lithium heparin plasma, to expand the acceptable sample types. The mean percent difference ranged from -7.5% to 5.5%. *Interferences* The method was found to be free (percent difference <20%) from potential endogenous (hemolysis, icterus, lipemia, and uremia) and exogenous (>100 therapeutic drugs and common analytes) interferences. *AMR* The AMR for all analytes, except fluconazole, was established by triplicate analysis at 7 levels in spiked, pooled-patient serum: 0.2, 0.3, 0.6, 1.3, 2.5, 5.0, and 10.0 µg/mL. Fluconazole's AMR was evaluated in the same matrix, but at higher levels to be consistent with therapeutic target ranges: 0.5, 0.9, 1.9, 3.8, 7.5, 15.0, and 30.0 µg/mL. The analytical recovery ranged from 91.6% to 121.3%, with coefficients of variation less than 14.3%. *Carryover* No carryover was found (percent difference <1.8%) at twice the upper limits of the AMR. *Stability* The unextracted samples were found to be stable (percent difference <8%) for 15 days at room temperature and 4 °C. At -20 °C, all analytes were stable for 60 days (percent difference <8%). The extracted stability at 4 °C (percent difference <8%) was 7 days. *Precision* The intraday CVs ranged from 1.5% to 3.4%. The total CVs ranged from 1.8% to 3.6%. *Method comparison* Approximately 40 patient samples (spiked or endogenous) were compared to reference laboratories, for each analyte. The correlation coefficients ranged from 0.9658 to 0.9981. The range for the slopes and intercepts were 0.947 to 1.105 and -0.296 to 0.127, respectively. **Conclusion**: This LC-MS/MS method has been validated to support therapeutic drug monitoring of patients undergoing azole antifungal medication treatment. The method only requires simple sample preparation and a sub 2 minute analysis time.

## B-398

### Comparison of Centrifugal Filter Performance for Free Phenytoin Assays

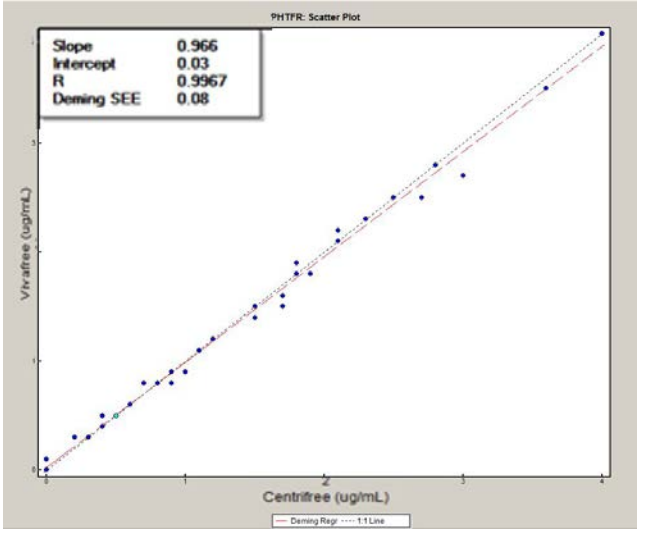
J. Filippi, E. Reineks. *Cleveland Clinic, Cleveland, OH*

**Background:** Free phenytoin concentration samples require a preanalytic spin through a filtration system to separate free from bound drug. A good filtration system needs to produce an accurate result as well as a high yield. The objective of this study was to compare the performance of the Vivafree™ 2 centrifugal filters from VivaProducts versus the Centrifree® Ultrafiltration Device from Millipore for free phenytoin testing. Performance was evaluated by comparing the analytical results for the filtered samples and the yield of filtrate.

**Methods:** 40 samples were created using patient pools and the Roche COBAS FP Free Phenytoin Calibrators. Mixtures were created to span the AMR of 0.1-4.0 µg/mL. 500 µL of each mixture was then transferred to a filter from each manufacturer and spun for 20 minutes at 1810 g. The samples were transferred to cobas micro sample cups and processed on a Roche cobas Integra 800. Filtration yield was investigated by making a patient pool and spinning 3 of each manufacturers' filters with a range of volumes up to 1 mL. Also, 2 mL of sample in the Vivafree 2 filters was tested.

**Results:** Values of <0.1 were interpreted as 0.0 and values >4.0 were interpreted as 4.0. The correlation, slope and intercept are shown in the plot. Comparing the yields, the Centrifree filter produces a higher yield at lower sample volumes, producing 130 µL with 250 µL of sample vs. 50 µL yielded by the Vivafree. At 1000 µL of sample, the filters yield 180 vs. 187 µL respectively.

**Conclusion:** The Vivafree 2 and Centrifree filtration systems produce comparable analytical results. The Centrifree system has a higher yield at lower volumes, but the volumes are comparable at higher sample volumes. If larger volume yields are needed, the Vivafree filters could be utilized.




## B-399

### Validation of dilute and shoot and evaluation of SPE method for barbiturates and THC carboxylic acid panel in urine

S. Milasinovic, X. Zang, P. L. Reddy, A. Oroskar, A. Oroskar. *Orochem Technologies Inc., Naperville, IL*

**Background:** Barbiturates and THCA panel is commonly tested in clinical labs by dilute and shoot approach which comes with high matrix interference at low concentration, and long term deterioration of the LC-MS system. The purpose of study reported here was to validate in lab developed dilute and shoot assay and evaluate solid phase extraction (SPE) approach as alternative. **Methods:** ExionLC - Triple Quad 4500 MS was operated in negative ion mode. Xenobiotics and buffer chemicals were purchased from Sigma Aldrich. *E. Coli* beta-glucoronidase (BG) was purchased from Campbell and Kura Biotec. HPLC-MS grade solvents were purchased from Pharmco-Aaper. Gazelle biphenyl column (1.8 µm, 50 × 2.1 mm) was used with a 30-95% methanol (0.1% FA) gradient mobile phase. Dilute and shoot procedure was as follows: 100 µL of urine was spiked with ISs, vortexed with pH 6.8 phosphoric buffer, mixed with 20 µL of BG solution and incubated (55 deg C, 30 min). 150 µL of ammonium acetate buffer in 40/60 water/methanol was added prior to vortexing and centrifuging. Assay was validated for accuracy, precision, linearity, carryover limit, matrix interference, xenobiotic interference and analyte stability across three validation batches on three different days. To develop an SPE method we screened Orpheus C18, and two types of polymeric DVB SPE plates (Celerity and Panthera Deluxe): each plate was conditioned with 1 ml of methanol and 1ml of water, loaded with 100 µL of fortified urine (spiked at cut-off and 30% of ULOQ concentration), while washing and elution procedures were varied. Elutes were evaporated, reconstituted (when needed) and analyzed. Recovery and reproducibility were tested along with different wash protocols using varying concentrations of methanol with or without 0.1-1% formic acid instead of water and varying elution solutions and volumes. At the end, we verified the final procedure by comparing one validation batch against the batch of the previously validated dilute and shoot method. **Results:** Dilute and shoot assay conformed to industry standards: accuracy and precision were within 20% of the target (25% at LLOQ) while linearity was assessed for calibrators across three validation batches and showed back-calculated concentrations of all calibrators (n=6) within 20% of target. AMR was determined to be 10 - 2500 ng/mL

for all barbiturates and 4 - 500 ng/mL for THCA. Optimized SPE method showed recoveries above 90% for barbiturates and 70% for THCA and improvement of matrix interference, ion suppression and enhancement. We could skip evaporation step easily by using 0.5 ml of methanol elution. Cross validation of two bathes of dilute and shoot assay against the optimized SPE procedure showed that SPE procedure gives comparable results with desired improvement pertaining to better sample clean up.

**Conclusion:** We developed a fast SPE method with about 10 minutes per plate processing time. This improved method reduced matrix interference more than double while it could easily be compared against the validated dilute and shoot method.

## B-400

### Automated blood sampling with paper spray ionization mass spectrometry: improving workflow and the safe handling of human blood for personalized medicine and clinical trials

K. E. Yannell, C. B. Kissinger, R. G. Cooks, P. T. Kissinger. *Purdue University, West Lafayette, IN*

Traditional, manual methods of blood collection are imprecise with regard to the time of collection and volume of blood removed from the patient. Collection times are significant in pharmacokinetic studies and in personalized medicine where a patient's rate of absorption and clearance of a drug is evaluated. If each blood draw collects an excess of sample, then frequent sampling can induce anemia. In addition, with the use of sharps common to manual collection, there is a risk of exposure to blood-borne pathogens for the person collecting the sample. Likewise, there is a risk of nosocomial infection to immune-compromised patients through repeated access to indwelling catheters. Improvements are justified for both sample collection and analysis. Herein, a workflow is demonstrated that utilizes a device (Phlebot) which automatically and painlessly collects small (0.025 to 1.000 mL) programmable volumes of whole blood from an intravenous catheter at preset time points and deposits them in refrigerated, sealed vials pretreated with an anticoagulant. The procedure is painless and the device is as mobile as an infusion pump.

After centrifugation and collection of plasma, each sample was spotted onto paper and dried at room temperature to deactivate blood-borne pathogens and permit safe handling of samples. The paper was then analyzed by paper spray (PS) ionization tandem mass spectrometry (MS/MS). PS is an ambient ionization technique amenable to point of care analysis of biofluids. It quantitates small analytes in whole untreated biofluids in under one minute and has successfully been demonstrated with opioids, benzodiazepines, illicit drugs, immunosuppressants, and others. This technology allows for pharmacokinetic and pharmacodynamic measurements to be readily taken for personalized medicine in a painless manner for improved patient care.

For this proof of workflow study, acetaminophen was measured in human plasma. A healthy male subject (n=1) was dosed orally with 500 mg acetaminophen. Blood collections of 250 µL were programmed for 5 minute intervals over 2.5 hours into cold, presealed, heparinized vials held at 4°C. Plasma was recovered from the blood and transferred to vials with a random code which blinded their identity to the analyst. Samples were stored frozen. For analysis they were thawed, mixed with an internal standard solution, and then 10 µL of the sample was pipetted onto paper triangles. The sample was dried for 30 minutes at room temperature before being placed in front of the MS inlet where a solvent and high voltage were applied to the paper to ionize the analytes. The method has a limit of detection of 50 ng/mL, was linear ($R^2$ = 0.990), precise (CV < 9%), and accurate (QC error < 11%). A time vs. concentration plot of the data demonstrated an absorption and excretion profile consistent with the pharmacokinetics of acetaminophen in a healthy volunteer after a single oral dose. This proof of concept study demonstrates that this blood collection device and analysis technique allow for an improved workflow of blood collection and analysis for personalized medicine and clinical studies.

## B-401

### Introduction of Anti-Fungal Triazoles on an Agilent High-Performance Liquid Chromatography System in the Clinical Biochemistry Laboratory

S. Y. Wong, F. Tiau, C. P. Yeo. *Singapore General Hospital, Department of Clinical Pathology, Clinical Biochemistry Laboratory, Singapore, Singapore*

**Background:**

Triazole antifungals (Voriconazole, Posaconazole, Itraconazole) are synthetic antifungal drugs that are widely used for the management of fungal infections. As a result of their broad spectrum antifungal activity and fewer side effects, these benefits of Triazoles antifungals make them appropriate to the prevention and therapy of systemic fungal infections. However, Triazole antifungals demonstrate marked variability in patient plasma drug concentrations as a result of wide inter-individuality variability in absorption, metabolism, elimination, or interaction with concomitant medication. Therapeutic drug monitoring of these antifungals is therefore beneficial for optimizing clinical efficacy and reducing drug toxicity in patients receiving antifungal prophylaxis or therapy. A commercial assay kit for Itraconazole, Posaconazole and Voriconazole in Serum/Plasma by Chromsystems™ using HPLC methodology has been adapted for evaluation in our laboratory on the Agilent 1260 Infinity HPLC system.

**Methods:**

The Chromsystems Triazoles assay kit consisted of commercial mobile phases, precipitation reagents, internal standard, HPLC column, lyohilized calibrators and quality controls. The assay is able to quantitate Voriconazole, Posaconazole, Itraconazole and its Hydroxy-Itraconazole metabolite. Performance validation parameters of the assay included assay imprecision (within and total), linearity, recovery, limits of detection (LOD) and quantification (LOQ), and carry-over. Sample preparation was performed according to manufacturer's instructions using a 2-step protein precipitation method with kit precipitation reagents, vortex-mixed, followed by centrifugation and analysis of the supernatant on the Agilent 1260 Infinity HPLC system. Imprecision was assessed using manufacturer's quality control materials measured in 6 replicates over 5 days. Linearity, recovery and carry-over studies were performed using appropriately spiked Cerilliant Antifungal Certified Spiking Solutions® into drug-free patient serum matrix. Limits of detection and quantification were assessed by determining HPLC signals from drug-free patient serum matrix and serial dilutions of a low concentration Triazole sample respectively.

**Results:**

Within run and total imprecision for all 4 Triazole analytes were determined to be ≤6.0% on the Agilent HPLC. The assay demonstrated linearity across the analytical measurement range up to 20 mg/L for Voriconazole and up to 10 mg/L for Posaconazole, Itraconazole and Hydroxy-Itraconazole. Analytical recoveries obtained for all Triazoles ranged between 89-116%. The LOD was assessed to 0.2 mg/L for Voriconazole and ≤ 0.06 mg/L for Posaconazole, Itraconazole and Hydroxy-Itraconazole. LOQ was assessed to be ≤ 0.5 mg/L for all Triazoles. Results of carry-over studies were insignificant. Preliminary participation in a trial run in the United Kingdom National Quality External Quality Assessment program (UK NEQAS) for Anti-fungals showed good agreement with other HPLC peers.

**Conclusions:**

Overall, the Chromsystems Triazoles kit performed in agreement with the manufacturer's specifications except for LOQs which were determined at a higher concentration on our HPLC system. The kit method is easily adaptable to contemporary HPLC systems and benefits from the advantages of simple preparation procedures, simple isocratic elution, commercially prepared mobile phases and readily available calibrator standards, quality control materials and internal standard. The commercial kit enables workflow efficiency and ease of staff training in our laboratory and has been validated to be a suitable choice for high volume and busy clinical laboratories.

## B-402

### Targeting Drug Transport: Using Vitamins to Inhibit Bcrp-Mediated Transport of Methotrexate into Milk

S. Delaney[1], S. Ito[2], D. Colantonio[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada*

**Background:** : The health benefits of breastfeeding are well known for both infant and mother. During lactation, the Breast Cancer Resistance Protein (Bcrp) is highly induced in the mammary epithelium and is known to actively transport various toxins, drugs, and nutrients into milk. Use of medication in the postpartum period has been reported to be as high as 80%, causing concern for infant exposure of drugs through breast milk. The potential risk of infant drug exposure via breast milk causes women to discontinue breastfeeding prematurely or decide not to initiate breastfeeding altogether. Methotrexate (MTX) is an anchor drug used in various inflammatory disorders; however, it is considered to be contraindicated during lactation, largely due to the lack of safety information. The objective of this study was to develop a strategy to improve the safety profile of MTX during lactation by investigating if the administering of vitamins and isoflavonoids, which are known to interact with Bcrp, can competitively inhibit MTX efflux into milk, resulting in a lower level of drug in milk.

**Methods:** To determine the influence of co-administering a nutritional Bcrp substrate on MTX efflux into milk, FVB mice (lactation day 8-12) were administered either 20

mg/kg riboflavin (vitamin $B_2$), 100 mg/kg flavin mononucleotide (FMN), 20 mg/kg genistein (soy isoflavonoid) or 20 mg/kg of the synthetic Bcrp inhibitor, ko143, 30 minutes prior to administering 0.75 mg/kg MTX. Milk and plasma were collected 60 minutes post-MTX dose and analyzed by LC-MS/MS and the Abbott ARCHITECT, respectively.

**Results:** Genistein administration decreased milk MTX concentrations and increased MTX plasma levels, but not significantly. Administration of the synthetic Bcrp inhibitor, ko143, significantly decreased MTX concentrations in milk; however, it also significantly increased plasma MTX levels, indicating that ko143 alters drug elimination pathways non-selectively throughout the body. Conversely, a significant decrease in MTX milk concentrations was seen after injecting mice with riboflavin or FMN, without increasing plasma MTX concentrations; suggesting that mammary BCRP transporter were primarily inhibited while systemic BCRP transporters where not inhibited.

**Conclusion:** It is known that Bcrp is a major transport mechanism involved in the excretion of MTX into milk and in clearing the drug from the body through renal and hepatic mechanisms. Our data shows that MTX efflux into milk can be modulated using riboflavin or FMN, without increasing MTX plasma concentration. This is important as this data suggests co-administering high-dose vitamins can reduce milk drug levels without reducing systemic clearance so that the drug can be cleared from the body. This data may have clinical implications and could help reduce MTX exposure to the infant via breast milk. The data obtained from this study may help improve the safety profiles of drugs with Bcrp-mediated excretion into milk.

## B-403

### Performance of the Thermo Scientific QMS everolimus assay based on transplant type, metabolite differences and assay platform following everolimus immunosuppression.

V. Buggs[1], B. Schniedewind[2], S. Albeiroti[1], J. Huss[1], K. Sobhani[3], U. Christians[2], A. W. Butch[1], K. A. Kelly[1]. *[1]UCLA Medical Center, Los Angeles, CA, [2]University of Colorado Anschutz Medical Campus, Aurora, CO, [3]Cedars-Sinai Medical Center, Los Angeles, CA*

**Background:** Everolimus has a narrow therapeutic window and significant intra-individual variability, which requires a rapid turn-around-time and in-house measurement for dosing titration and adjustments. The Thermo Scientific Quantitiative Microsphere System (QMS®) everolimus immunoassay is the only FDA-approved immunoassay for everolimus measurement and is a homogeneous, particle-enhanced turbidimetric immunoassay. **Objective:** The analytical performance of the Thermo Scientific QMS® everolimus immunoassay on Indiko Plus was evaluated and compared to an LC-MS/MS method and QMS® immunoassay on AU680 based on transplant type and everolimus metabolites**.** We determined if significant metabolite cross-reactivity differences occur between LC-MS/MS and QMS® immunoassays. **Methods**: Whole blood EDTA-anticoagulated patient samples were pretreated with methanol and precipitation reagent (immunoassay) or zinc sulfate (LC-MS/MS) to remove proteins before analysis. An LC-MS/MS method for measuring everolimus and major metabolites (39-O-des-methyl, 16-O-des-methyl, 24-hydroxy, 25-hydroxy, 46-hydroxy and 11-hydroxy) was used and the transition *m/z* 980.9→389.1 was monitored for everolimus, with *m/z* 980.9→409.5 as qualifier. Total imprecision of the QMS® everolimus assay was determined by analyzing patient pools and commercial controls in duplicate with everolimus concentrations ranging from 4.6 - 16.9 ng/mL. Linearity was determined using diluted liver and kidney transplant patient pools of known everolimus concentrations (determined by LC-MS/MS). Functional sensitivity was determined by analyzing patient samples (duplicate analysis, 5 days) with everolimus concentrations ranging from 1.5-4.5 ng/mL. Thirty-four transplant samples (20 liver, 14 kidney) were used for the initial method comparison. Passing-Bablock regression, Spearman correlation coefficient, and Bland-Altman plot analysis were performed using EP Evaluator® software. **Validation and Results:** Total imprecision (%CV) for QMS® everolimus assay was 3.1% (mean=4.2 ng/mL), 3.3% (mean=9.3 ng/mL) and 2.8% (mean=14.3 ng/mL) for patient pools from liver samples. The kidney transplant samples showed a similar imprecision of 2.3% (mean=4.8 ng/mL), 3.2% (mean=8.5 ng/mL) and 5.5% (mean 14.7 ng/mL). Assay using patient pools from liver or kidney samples was linear from 2-15 ng/mL with equivalent slopes (1.000 and 1.001 respectively) and functional sensitivity was 1.5 ng/mL at <15%CV. Patient comparison studies revealed the following relationships: QMS® on the Indiko Plus=1.130(LC-MS/MS)-0.02 (r=0.91, average bias=0.51ng/mL); QMS® on the AU680=0.897(LC-MS/MS)-1.36 (r=0.90, average bias=(-)0.34 ng/mL. The methods were also compared in liver and kidney transplants with similar outcomes. Although the immunoassay methods compared favorably with LC-MS/MS, accuracy studies showed the QMS® immunoassay underestimated kidney transplant samples >10% and overestimated liver transplant samples >10% at low therapeutic values of 3.0 ng/mL. Evaluation of everolimus metabolites (39-O-des-methyl, 16-O-des-methyl, 24-hydroxy, 25-hydroxy, 46-hydroxy and 11-hydroxy) measured by LC-MS/MS found that the distribution of the everolimus metabolites was similar between liver and kidney transplant samples. However, the ratio of [pg] metabolite / [ng] everolimus was higher in kidney transplant patients and varied by metabolite (3-42%), especially for 24-hydroxy metabolite. **Conclusions:** Thermo Scientific QMS® everolimus immunoassay produces everolimus concentrations in patient samples that are comparable to LC-MS/MS values except at low therapeutic values. Accuracy studies revealed differences between liver and kidney transplant samples and may be due to differences in the concentrations of everolimus metabolites in liver versus kidney transplant samples.

## B-404

### miR-26a and miR-15b expression profiles as a potential early biomarker for clopidogrel-induced hepatotoxicity

R. C. C. Freitas[1], R. H. Bortolin[1], L. Meneguello[2], L. Tamborlin[3], M. B. Lopes[1], V. N. Silbiger[1], R. D. C. Hirata[4], M. H. Hirata[4], A. D. Luchessi[3], A. D. Luchessi[1]. *[1]Universidade Federal do Rio Grande do Norte, NATAL, Brazil, [2]Universidade Estadual Paulista, Rio Claro, Brazil, [3]Universidade Estadual de Campinas, Limeira, Brazil, [4]Universidade de São Paulo, São Paulo, Brazil*

**Background:** Antiplatelet therapy, especially clopidogrel, is essential for prevention of thrombosis and atherosclerosis. Among clopidogrel adverse effects, the hepatotoxicity is a potential adverse effect related to liver damage. In a scenario of lack of early diagnostic for clopidogrel-induced hepatoxicity, new exosomes-derived miRNAs may be useful for improve the safety of this antiplatelet. Therefore, the miR-26a-5p, miR-145-5p, miR-15b-5p and miR-4701-3p in exosomes were evaluated *in vitro* aiming use then as potential biomarkers of clopidogrel-induced hepatotoxicity. **Methods:** HepG2 cells were cultured in RPMI containing 5% Exosome-Depleted Fetal Bovine Serum and supplemented with penicillin (10.000 UI/mL), streptomycin (10.000 UI/mL), and sodium bicarbonate (44 mmols/L) at 37°C in 5% $CO_2$ air. Clopidogrel treatments were performed during 24 and 48 h using the concentrations of 6.25, 12.5, 25, 50, and 100 µM. The cytotoxicity was evaluated by flow cytometry to analyze DNA fragmentation and cell cycle. Relative expression of exosomes-derived miRNAs was evaluated by RT-qPCR. **Results:** Our results revealed that in both periods of treatment the concentrations of 6.25 e 12.5 µM had similar profile that those observed in the control. In relation to the concentrations 25, 50, and 100 µM, it was observed a dose-dependent increase in DNA fragmentation. The miR-26a-5p was upregulated in concentration of 100 µM of clopidogrel and miR-15b-5p were downregulated in this toxic concentration. **Conclusion:** The increased DNA fragmentation in high clopidogrel concentration associated with miR-26a-5p upregulation and miR-15b downregulation are suggestive that these miRNAs profiles may be useful as an innovative diagnostic tool for early detection of clopidogrel-induced hepatotoxicity.

## B-405

### Pilot Study for determination of Infliximab Levels in Inflammatory Bowel Disease Patients

T. A. Agbor[1], S. Shajib[1], J. Marshall[1], B. Selena[1], S. Halder[1], A. Gils[2], W. Khan[1]. *[1]McMaster University, Hamilton, ON, Canada, [2]KUL, Leuven, Belgium*

**Objectives:** Therapeutics for Inflammatory bowel disease (IBD) have recently switched to the use of monoclonal antibodies against TNF-α (anti-TNF-α) to modulate inflammation and mucosal healing. One such drug, a chimeric IgG1 antibody (Infliximab) targets circulating and cell surface TNF-α. These agents have improved management of IBD patients especially those who did not respond to conventional IBD therapies. However, approximately a third of IBD patients lose response to anti-TNF-α drug therapy over time due to development of immunogenicity (produce anti-drug neutralizing antibodies), which accelerate systemic drug clearance leading to treatment failures. Generation of antibodies against anti-TNF-α agent is drug-specific. Measurements of anti-TNF-α drug trough and anti-drug antibodies levels have been utilized to evaluate clinical efficacy, safety and cost of anti-TNF-α therapeutics. Furthermore, there is a correlation between anti-TNF-α drug levels and their clinical efficacy. As these drugs have a narrow therapeutic window, knowledge of circulating trough levels can help clinicians in dose adjustments and/or switching to an alternative anti-TNF- agent in case of high clearance or lose of response. Thus, drug monitoring can help in treatment optimization which has been associated with improved clinical

outcome. In this pilot study, we evaluated a commercially available ELISA assay to measure therapeutic drug levels of Infliximab and anti-infliximab antibody levels and compared that with an established in-house assay in IBD patients undergoing treatment.

**Design and Methods:** Nine patients with IBD and on Infliximab were recruited for this study after obtaining ethics approval from the Hamilton Integrated Research Ethics Board (HIREB). Blood samples were collected in serum tubes just before infusion of their next Infliximab dose (trough levels) and 15 minutes after drug infusion (peak levels). Blood was left to clot at room temperature before centrifuging at 1500 x g. Serum was collected and stored at -20°C until use. Infliximab and anti-infliximab antibody levels were measured in duplicate using the Theradiag Duo Infliximab III ELISA kit.

**Results:** Five males and 4 females with age ranging from 21-56 years old were included in this study. The intra-run CV between duplicates ranged from 1.7-6.1% (maximum for assay = 8.8%) for both calibration standards and patient samples. The calibration curve was curvilinear as described by the manufacturer along the assay range of 0-16 µg/ml of Infliximab. Trough drug levels for patient samples ranged from 1.6-13.9 µg/ml while all peak samples were above the upper assay range of 16 µg/ml. We compared our patient sample data (trough levels only) with those of an in-house well established ELISA assay in Leuven, Belgium and found a good agreement between the two methods. The anti-infliximab antibody levels in patient samples were below the limit of detection of both assays.

**Conclusions:** This pilot study demonstrates that the Theradiag Duo Infliximab assay has a good precision and linearity within the assay measurement range. There was a good agreement between this method and an established in-house ELISA method in Leuven, Belgium. We are currently recruiting patients for a complete method validation study with a view to commence this test in clinical diagnostic laboratory.

## B-406

### Stimulant Drug Analysis by Chiral Liquid Chromatography Tandem Mass Spectrometry

A. Nguyen, P. Nguyen, P. Audon, T. Nguyen, M. Bach, D. Bach. *Bach Diagnostics, Santa Ana, CA*

**Background:** Amphetamine-type stimulants have often been abused as recreational drugs or doping agents in sports. However, the less active enantiomers are often used in over-the-counter medication, such as nasal decongestants. Traditional screening methods such as immunoassays used in clinical diagnostics can exhibit interference from cross reactivity with drugs such phentermine and pseudoephedrine, resulting in false positives that can have legal and medical consequences for patients. This paper presents a chiral chromatography-tandem mass spectrometry method for quantification of methylphenidate, phentermine, phenylpropanolamine, and pseudoephedrine and chiral separation and quantification of D-amphetamine, L-amphetamine, D-methamphetamine, and L-methamphetamine in urine matrix. The method is used to both identify possible sources of unexpected positive screens due to over-the-counter medication and differentiate chiral enantiomers in order to eliminate potential errors during the analysis of patient samples.

**Methods:** Analytical Standards for methylphenidate, phentermine, phenylpropanolamine, pseudoephedrine, D-amphetamine, L-amphetamine, D-methamphetamine, and L-methamphetamine as well as deuterated internal standards were purchased from Cerriliant. Our method was automated using a Hamilton Microlab STAR liquid handling system. DPX CO-RE tips containing reverse phase (1 mg) and weak-anion exchange (2 mg) resins were used for sample preparation. Drug separation is performed using Shimadzu 20A Prominence LC. Phenomenex Lux AMP column (150mm x 3.0 mm, 3 µm) is used to achieve well-defined separation. Mobile phase A consist of 5mM ammonium bicarbonate, adjusted to pH 11 with ammonium hydroxide and mobile phase B consist of methanol. Sample are analyzed on ABSciex API 4000 and results are generated using Analyst 1.6.2 software.

**Results:** The precision for all stimulant drugs and enantiomers were less 15% CV. Accuracy for all drugs and enantiomers were less than 15% bias. The linear quantifiable range is 40 ng/mL to 2000 ng/mL with correlation coefficient of greater than 0.995.

**Conclusion:** The method was successful in quantification of individual peaks for the (D)- and (L)- enantiomers of amphetamines and methamphetamines, methylphenidate, phentermine, phenylpropanolamine, and pseudoephedrine with performance that was satisfactory in terms of linearity, precision, accuracy and limits of detection.

## B-407

### Evaluation of the Thermo Scientific CEDIA Cyclosporine PLUS assay on the Abbott Architect c8000 analyzer

M. Dill[1], A. Iwanski[1], S. A. Hackenmueller[2]. *[1]UW Health, Madison, WI, [2]UW Madison School of Medicine and Public Health, Madison, WI*

Background: Cyclosporine is an immunosuppressive drug commonly used post-transplantation. Both immunoassay and chromatographic methods are used for quantitation of cyclosporine. One advantage of immunoassays is the greater availability of assays on automated chemistry analyzers and the potential for 24/7 testing service. Another advantage compared to chromatographic analysis, typically requiring manual sample extraction, is the faster turnaround time. A third advantage is that testing can be set up and/or consolidated onto a main chemistry platforms rather than maintaining a separate instrument. The Thermo Scientific CEDIA cyclosporine PLUS assay is an enzyme immunoassay utilizing a mouse monoclonal antibody against cyclosporine. The objective of this study was to evaluate the Thermo Scientific CEDIA cyclosporine PLUS assay on the Abbott Architect c8000.

Methods: Studies included evaluating linearity, intra- and inter-assay imprecision, and accuracy with respect to a currently in-use method. The comparison method was a whole blood chemiluminescent immunoassay on the Siemens Dimension EXL analyzer.

Results: The assay was linear across the measurement range of 25-450 ng/mL (slope = 0.989, correlation coefficient (r) = 0.998). Within run imprecision was 5.4% at 48 ng/mL (n=10), 3.3% at 108 ng/mL (n=20), and 1.4% at 429 ng/mL (n=10). Day-to-day imprecision was 16.6% at 60 ng/mL, 3.6% at 187 ng/mL and 3.4% at 350 ng/mL (n=22 for each). Samples ranging from 43 to 420 ng/mL (n = 45) were analyzed over at least 5 days and indicated overall acceptable agreement between the Thermo Scientific CEDIA and Siemens Dimension EXL methods ($y = 1.05x – 8.99$, $r = 0.988$). The average bias between methods was -2.94%, but the method-to-method difference for individual samples was as high as 31%.

Conclusion: The Thermo Scientific CEDIA Cyclosporine PLUS assay on the Abbott Architect c8000 shows acceptable average bias compared to the Siemens Dimension EXL analyzer, but unacceptably high (>16%) day-to-day imprecision at a low concentration.

(Word limit: 500)

## B-408

### Use of Urine Drug Screening Positivity Rates from Qualitative Liquid Chromatography Tandem Mass Spectrometry Definitive Testing to Identify Annual Trends in Drug Use

A. Ptolemy[1], H. Li[2], D. Bailey[1], G. Waite[1]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

**Background:** Comprehensive and specific information regarding trends in the prevalence of licit and illicit drug use within a specific region is often not widely available or current. Urine drug screening positivity rates for patients within a specific geographical area has recently been used to obtain this information. However, comprehensive studies of urine drug screening positivity rates for patients in Ontario, Canada have not yet been published. **Objective:** Identify multi-year trends in drug use by examining qualitative liquid chromatography tandem mass spectrometry (LC-MS/MS) based urine drug screening positivity rates. **Methods:** All LC-MS/MS urine drug screening results from 2014 (*N*=136,864), 2015 (*N*=153,329) and 2016 (*N*=106,687) were retrospectively reviewed. Following enzymatic hydrolysis and protein precipitation, all urine specimens received targeted LC-MS/MS screening which identified the presence of drugs within the following drug classes: anesthetic (ketamine); anticonvulsant (gabapentin); antidepressant (bupropion, trazodone); benzodiazepine (alprazolam, clonazepam, diazepam, flunitrazepam, flurazepem, lorazepam, nitrazepam, oxazepam, phenazepam, temazepam, triazolam); cannabinoid; opioid (buprenorphine, codeine, fentanyl, hydrocodone, hydromorphone, morphine, naltrexone, meperidine, methadone, oxycodone); stimulant (amphetamine, methylphenidate); and illicit (benzylpiperazine, cocaine, heroin, mephedrone, methamphetamine, MDPV, MDEA, MDMA). Relevant drug metabolites and related compounds (naloxone, levamisole) were also included in this test. A total of 63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing. **Results:** Over the examined three year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (diazepam, flunitrazepam, 7-aminoflunitrazepam, flurazepam, desalkylflurazepam, phenazepam, JWH018, JWH200, MDEA, MDPV and mephedrone). From 2014 to 2016 significant

(p≤0.05) increases in urine drug screening positivity rates were observed for: gabapentin (5.4% to 6.8%); alprazolam (0.3% to 0.5%); α-hydroxyalprazolam (0.5% to 0.7%); buprenorphine (7.1% to 8.7%); norbuprenorphine (8.5% to 10.0%); 6-acetylmorphine (0.6% to 1.1%); naloxone (7.0% to 8.5%); naltrexone (0.1% to 0.3%); amphetamine (3.4 to 5.8%); methamphetamine (2.9% to 4.8%); cocaine (2.8% to 3.5%); and benzoylecgonine (7.6% to 11.5%). From 2014 to 2016 significant (p≤0.05) decreases in urine drug screening positivity rates were observed for: bupropion (2.6% to 2.4%); 7-aminonitrazepam (8.5% to 7.0%); oxazepam (6.6% to 5.8%); temazepam (5.0% to 4.6%); methadone (57.4% to 38.1%); EDDP (47.0% to 38.8%); and benzylpiperazine (0.05% to 0.02%). All other analytes included in the LC-MS/MS screening panel did not show significant annual differences within the tested patient population. THCA positivity rate was 29.6%, 28.9% and 29.5% in 2014, 2015 and 2016, respectively. **Conclusion**: From 2014 to 2016, significant annual trends of increasing (gabapentin; heroin; naltrexone; methamphetamine; cocaine; alprazolam; buprenorphine) and decreasing (methadone; temazepam; oxazepam; nitrazepam; and bupropion) drug use were identified through the review of urine drug screening positivity rates of community-based patients in Ontario, Canada. The relative changes in drug use within the examined cohort may be reflective of the relative decrease of methadone patient testing and related positivity rates. Tabulating and communicating urine drug screening positivity rates from qualitative definitive LC-MS/MS testing would serve to educate physicians about drug use within their communities.

## B-409

### Drug Confirmation -- Method Consolidation Reduces Cost-to-Payer, Instrument Run Time, Labor Costs, and Result Turnaround Times

K. Doyle. *Intermountain Healthcare, Murray, UT*

**OBJECTIVE**: Drug class-specific LC-MS/MS methods are routinely performed to confirm urine drug immunoassay (IA) results. However, more than 50% of specimens are positive for more than one drug class requiring the sample to be run by multiple methods. Hospital laboratories often have limited resources and are behooved to simplify test workflows to minimize cost and turnaround time while maximizing sensitivity and specificity. We tested 15,197 specimens by a single-run LC-MS/MS method to quantitate 49 analytes known to trigger positive results in our 9 drugs of abuse IA drug screen and evaluated this workflow against conventional class-specific methods to determine differences in cost-to-payer, within-lab turnaround time, instrument run time, and technologist hands-on time.

**METHODS**: The single injection, consolidated workflow is based on an enzyme-hydrolysis, isotope-dilution LC-MS/MS method that employs positive/negative switching to quantitate 49 analytes that are known to trigger positive results in our 9 drugs of abuse IA drug screens (amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, opiates, oxycodone, methadone, and cannabinoids). We tested 15,197 specimens that were submitted to our laboratory from 22 hospital emergency departments that were positive for one or more of the IAs. The frequency of drug classes found positive per specimen, as well as the distribution of positive drug classes, were used to calculate costs, tech time, in-lab turnaround time, and instrument run time of the consolidated method. These same distributions were then used to determine estimated values for conventional workflows utilizing class-specific methods (i.e., amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, opiates/oxycodone, methadone, and THC).

**VALIDATION:** The frequencies of positive IAs by drug class are opiates/oxycodone, 30%; benzodiazepines, 21%; amphetamines, 19%; cannabinoids, 16%; buprenorphine,7%; methadone, 3%; cocaine, 3%; and barbiturates, 2%. The frequencies of specimens that were positive for drug classes by IA are: one class, 49%; two classes, 30%; three classes, 14%; four classes 6%; and >4 classes, 1%. Based on these frequencies, known costs, and the requirements of each analytical workflow, we determined that a consolidated method reduces cost-to-payer by 1.8 fold. The consolidated method was largely favored over the class-specific methods for average sample prep time (5.9 versus 9.4 minutes/ sample), instrument run time (includes calibrators and quality controls) (13.5 versus 18.5 minutes/sample), and total within-lab turnaround time (33.3 versus 22.4 minutes/sample), respectively. It is projected that cumulative hands-on technologist time was reduced by 1400 hours over a period of a year by employing the consolidated testing method.

**DISCUSSION**: LC-MS/MS enables laboratories to quantitate many drugs/metabolites simultaneously in both positive and negative ion modes over short run times. As labor and instrumentation are two of the largest costs of sample testing, a consolidated testing approach has a marked impact on these variables. However, we observed some challenges with the consolidated workflow including additional complexity of preparing calibration materials, internal standards, and controls; added complexity of data analysis of many analytes; QC or calibration failures for single analytes in a panel; and the large amount of data to move to the LIS. Solutions were implementer to address these added complexities and the benefits outweigh the challenges.

## B-410

### A Comparison of Methods for Measurement of Plasma Methotrexate in a Pediatric Population.

Y. Kataria, C. MacDonald, T. Law, M. D. Kellogg, R. W. Peake. *Boston Children's Hospital, Boston, MA*

**Introduction** Methotrexate (MTX) is an antineoplastic drug that prevents DNA synthesis via the competitive inhibition of the enzyme, dihydrofolate reductase. MTX is widely used to treat malignancies, such as osteosarcoma, acute lymphoblastic leukemia, and lymphoma. Following the administration of MTX, plasma levels are typically measured at 24, 48, and 72 hours post-dose, due to the inherent risk of nephrotoxicity. Traditional "random-access" methods for plasma MTX measurement include florescent polarization immunoassay (FPIA), and more recently, homogeneous immunoassay. Liquid-chromatography tandem mass spectrometry (LC-MS/MS) methods are also available, due to their utility for measurement of MTX in the context of carboxypeptidase treatment in cases of toxicity. In this study, we compared the performance of three methods for MTX: The Abbott TDx FPIA, the ARK™ homogeneous immunoassay, and an LC-MS/MS method. Performance characteristics were assessed in a subset of plasma specimens from a pediatric population. **Method** Assay imprecision on each platform was assessed by replicate measurement (n = 5) of MTX in patient plasma pools across the concentration range, 0 to 0.10 µmol/L. The lower limit of quantification (LLOQ) of each assay, determined by means of functional sensitivity, was assessed by replicate analysis (n = 5) of plasma pools created from pediatric patients (n = 5) across the concentration range 0.04 to 0.15 µmol/L. A direct comparison of the three methods was performed by measurement of patient specimens (n = 30) across the concentration range, 0.04 to 19.0 µmol/L. Comparison between patient samples using the three methods was assessed by Deming-regression analysis. **Results** Assay imprecision (%CV) assessed at MTX concentrations of 0.04, 0.08 and 0.10 µmol/L. The mean coefficient of variation (%CV) using the TDx and LC-MS/MS methods at 0.04, 0.08 and 0.11 µmol/L were 11.0%, 6.7% and 3.6% respectively. Similar data using the ARK™ assay produced %CV values of 13.6%, 4.4% and 3.1% at 0.06, 0.10 and 0.14 µmol/L respectively. Deming-regression across the concentration range, 0.0 to 1.0 µmol/L showed excellent correlation between the TDx and LC-MS/MS methods (r = 0.997). Values for slope ranged from 1.1844 to 1.2336. For ARK™ versus LC-MS/MS (r = 0.993), values were obtained for slope (range: 0.9923 - 1.009) and intercept (range: 0.022 - 0.039). The data suggested that the ARK™ method exhibits a slight positive bias compared with the other two methods. **Conclusions**: Our data suggests that there is a slight positive proportional bias with the ARK™ method relative to the other two methods. In conclusion, all three MTX methods are reproducible at concentrations used for medical decisions in plasma specimens obtained from pediatric patients.

# Wednesday, August 2, 2017

Poster Session: 9:30 AM - 5:00 PM

Technology/Design Development

## B-411

### A Novel 3D Microfluidic Culture Platform for Studying Cancer Cell Metastasis

L. Li[1], G. Hu[1], L. Qu[1], S. Torabi[2], R. Xu[3], W. Xu[1]. *[1]Department of Laboratory Medicine, The First Hospital of Jilin University, Changchun, China, [2]Department of Mechanical Engineering, University of Kentucky, Lexington, KY, [3]Markey Cancer Center, University of Kentucky, Lexington, KY*

**BACKGROUND:** There is a critical need in pharmaceutical development and medicine for biologically relevant *ex vivo* tissue models. Traditional 2D cell culture fails to reproduce the physiology of the *in vivo* cell microenvironment. However, animal models capture this complexity, but are expensive, difficult to observe in real time, and present potential ethical issues. One ideal solution is to use 3D cell culture techniques, which can provide a more mechanically and physiologically relevant microenvironment. When coupled with an integrated microfluidic network that mimics *in vivo* microcirculation, these 3D methods can gain even greater biological relevance and provide a platform for high resolution study of cell microenvironment with precise control over experimental parameters.

**OBJECTIVE:** To develop a microfluidic platform to provide a biomimetic environment to observe and analyze cell proliferation, cell response to chemical agents and cell migration within a three-dimensional structure. This model not only presents more physiological relevance to the in vivo microenvironment of cells, but also provides the capability for dynamic high-resolution study of cell survival, biochemistry and morphology.

**METHODS:** The microfluidic device contains a 5x5x4mm chamber to accommodate the 3D cell culture and 27 micro-channels, 100x200µm cross-section and 5mm in length, to represent the microvasculature. Fabrication of the device was done by SU-8 3050 photolithography to make the master mold and PDMS soft-lithography to fabricate microfluidics layer and the vacuum gland. Then, layers were bonded using oxygen plasma bonding.

After sterilization of the device by using 70% ethanol solution and 30 minutes of UV light exposure, the cell-hydrogel solution was prepared (80000 cells/ml) and cast in the 3D cell culture chamber and then cured in an incubator for 30 minutes. Then the device was sealed using the vacuum gland, inlet/outlet tubing were connected and the cell culture medium was pumped through the device at the flow rate of 2 µl/min continuously. The experiment was run for 4 days with a static 3D cell culture as the control. The luciferase activity was then measured to determine the number of cells in each sample.

**RESULTS:** Cells not only survived in the microfluidic device, but also proliferated and increased their population that illustrated the functionality of the device as a 3D cell culture platform. The cells in the microfluidic device showed a similar behavior as the ones in the control, with only a difference in replication rate. Also, the data shows more controllable and repeatable proliferation behavior in the cells in the microfluidic device than in the regular 3D cell culture method. In addition, it is important to note that, having micro-channels close to the culturing hydrogel, continuous feeding of the cells and constant take away of the waste products in the devices represented a more biomimetic model for culturing cells.

**CONCLUSIONS:** This study demonstrated a 3D biomimetic platform for cell culture and cancer metastasis, optimize the microfluidic device mechanical parameters such as flow rate, hydrodynamic resistance and microfluidic geometry to mimic cell microenvironment, develop a compatible hydrogel casting method to integrate cell culture with the device.

## B-412

### Comparison of ARCHITECT 2000 I SR for determination of carbamasepine with VITROS 5600

N. Serdarevic[1], R. Serdarevic[2], J. Coric[1], A. Memic[3]. *[1]Institute for Clinical Chemistry and Biochemistry; Clinical center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]Department of Ophthalmology, Clinical center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [3]Department of Psichiatry; Clinical center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*

Background: Carbamazepine is a commonly prescribed anti-convulsant used mainly in the treatment of seizure disorders, but is also used to treat trigeminal neuralgia. Carbamazepine has also been useful in the treatment of manic depressive patients as an alternative to lithium therapy. Carbamazepine measurements are used to monitor patient compliance and therapy, and to diagnose potential overdose.

Methods: The carbamasepine concentration of 80 serum samples were determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 and dry chemistry slide method is VITROS 5600. All patients were hospitalized at Department of Neurology at the University Clinics Center of Sarajevo. The reference serum range of carbamaseine in patients is 4-12 mg/L. The quality control, precision and accurancy of Architect i 2000 and VITROS 5600 were assessed.

Results: The quality control was done using quality control serums for low (= 5.37 mg/L), medium (= 9.41 mg/L) and high (= 16.9 mg/L). We have used commercial ABBOTT controls and got CV 1.23 % to 4.50 % for Architect i 2000. We done a quality control using quality control serums for low (= 4.41 mg/mL ) and high control (= 9.65 mg/L ) VITROS Diagnostic and got CV 1.44 % to 4.14 %). Mean value of carbamasepine serum concentration using ARCHITECT I 2000 (CMIA) was 6.09 +/- 1.54 mg/L and VITROS 5600 (dry chemistry slide method) was 8.47 +/- 2.01 mg/L. It was established that the main difference between Architect i 2000 and VITROS 5600 and it was statistically significant for $p < 0.0001$ according to Paired samples t-test. Correlation coefficient was r = 0.9549 to 0.9863 and regresion line had a slope 1.2741 (1.4433) and a y axis intercept of -0.3644 (0.7748). The ARCHITECT I 2000 carbamasepine assay was performed on the 80 human serum samples in the range 3.84 to 8.59 mg/L and VITROS 5600 5.30-11.7 mg/L. The average mg /L difference bias exhibited by ARCHITECT carbamasepine vs. VITROS carbamasepine assay in this study was -2.38 µg /mL. The 95 % confidence interval of the ng/ml difference bias is -2,1918 to -2,5628 mg/L.

Conclusion: The study shows that it was good agreement in using those methods for detection of carbamasepine. The patients should be monitored on a single method to avoid differences in the results. Different techniques for carbamasepine detection in human serum using different methods which leads to different results.

## B-413

### Preliminary study on a high-sensitivity hydrogen peroxide detection method using the metal chelating reagent, Chromazurol B (CAB)

E. Hokazono[1], S. Osawa[2], E. Ota[1], T. Tateishi[3], M. Kawano[4], M. Seimiya[2], Y. Kayamori[1]. *[1]Kyushu University, Fukuoka, Japan, [2]International University of Health and Welfare, Narita, Japan, [3]Kyushu University and Junshin Gakuen University, Fukuoka, Japan, [4]Kyushu University and International University of Health and Welfare, Fukuoka and Narita, Japan*

**Background:**

Quantitation of biological sample components and enzyme activities is indispensable for determining the pathological condition of patients. The rapid determination and return of accurate results is critical to clinical chemical analysis for clinical diagnosis. The sensitivity of analytical techniques is vital to ensuring the accuracy of analyses.

Currently, two detection techniques are used for the majority of clinical enzyme activity measurements. The first of these methods, NAD (P) H, has low sensitivity, but is largely unaffected by reducing compounds. Conversely, the hydrogen peroxide-peroxidase method is highly sensitive, but is adversely affected by the presence of reducing species; especially when measuring urinary constituents.

Therefore, in the present study, we developed a novel high-sensitivity measurement system for hydrogen peroxide ($H_2O_2$) using a metal chelating reagent, Chromazurol B (CAB).

**Methods:**

CAB develops an absorption band at 600 nm when chelating $Fe^{3+}$. In our novel method, $Fe^{2+}$ is oxidized to $Fe^{3+}$ by hydrogen peroxide originating from oxidizing enzymes under acidic conditions. Then, the absorbance of the CAB-$Fe^{3+}$ complex

is measured at 600 nm, and the increase in absorbance can be used to determine the quantity of hydrogen peroxide.

In this study, we used a Hitachi Model 7170 and 7600 (P module) automated analyzer to perform a two-point end assay at 37°C. The sample ($H_2O_2$, 10 µL) was mixed with 200 µL of reagent 1 (117 µmol/L iron (II) sulfate and 0.12 mol/L formic acid buffer (pH 4.0, 25°C)). The mixture was maintained at 37°C for 5 min. After the addition of 24 µL of reagent 2 (975 µmol/L CAB in distilled water), the absorbance was measured at 600/800 (main/sub) nm wavelengths.

**Results:**

The within-run CVs of the above method using 0.4 and 2.2 µmol/L $H_2O_2$ solutions were 3.78 and 1.74%, respectively ($n$ = 20). The results exhibited linearity from 0 to 3.0 µmol/L. The detection limit was 0.2 µmol/L. The molar absorption coefficient, indicating the measurement sensitivity, was 202,160 $L \cdot mol^{-1} \cdot cm^{-1}$. This is about seven times or over greater than the sensitivity of the current methods.

**Conclusion:**

The newly-developed method was highly sensitive to hydrogen peroxide, and may be applicable in a wide range of research and clinical laboratories. In the future, we will investigate the effects of reducing substances in biological samples, such as serum and urine, on the sensitivity of this method.

## B-414

### Improving Immunoassay Throughput with Spatial Proximity Analyte Capture Luminescence

M. Cameron. *Beckman Coulter, Southfield, MI*

**Background:** SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence) is a chemistry that allows for the quantification of proteins and antibodies from biological fluids in a homogeneous immunoassay format. The core of the novel chemistry is the interaction of horse radish peroxidase (HRP) and hydrogen peroxide and the release of free radicals that break the chemical bonds of acridan molecules, emitting light. The amount of light generated is proportional to the amount of analyte bound in a specific immune complexes. The chemistry is proximity based and an anti-oxidant is used to control the distance of free radical travel, limiting background signal. The chemistry is highly flexible and configurable noting that acridan and HRP must be in the assay design, allowing for the possibility of multiple immunoassay formats including competitive, sandwich and high throughput screening. Based on previous work (Clinical Chemistry, Vol 62, No. 10 Supplement, page S226) we sought to make improvements in the workflow by the investigation of anti-oxidant addition early in the sequence of the workflow with the goal of eliminating one pipetting step, thereby improving efficiencies. **Methods:** Anti-human IgG Fc antibodies were commercially sourced. Each antibody candidate was labeled with acridan and HRP. All antibody pair candidates were screened against purified human IgG. Assay development using the selected antibody was performed for the determination of dynamic range, duration of assay run time and optimization of key reagents (HRP and acridan conjugated antibodies and the anti-oxidant). Validation included the accuracy and precision determination of interpolated concentration values of QC samples (80, 20, and 5 ng/mL of IgG) for the existing and modified workflows.

**Results:** A modification in workflow (adding anti-oxidant during conjugated antibody incubation with IgG target and adding anti-oxidant after conjugated antibody incubation with target) was evaluated by assessing accuracy and precision of three QC samples for both immunoassay workflows. The total error (TE) (relative error (RE) plus the coefficient of variation (CV %)) was determined for each QC sample in each workflow. For the standard workflow, the TE for QC high was 13.6%, TE for QC middle was 6% and the TE for QC low was 12.4%. For the modified workflow, the TE for QC high was 4.6%, TE for QC middle was 4.9% and the TE for QC low was 6%. **Conclusion:** The novel chemistry is simple, allowing for rapid development and validation of a human IgG assay. The modification of the workflow resulted in improved accuracy and precision as compared to the standard workflow, allowing the elimination of a pipetting step and improving throughput and efficiency. The SPARCL chemistry may be applied to a broad range of immunoassay types (pharmacokinetics, high throughput screening, biomarkers and therapeutic antibody development) with the advantages of improved workflow, lower workload and faster turnaround time. As the signal generated is luminescent, no specialized equipment is needed beyond a luminometer. The SPARCL chemistry may be amenable to lab developed tests and point of care testing due to rapid test results, simple procedure, small sample volume requirements and the no washing format.

## B-415

### Development and Evaluation of the Novel Multi-Test VITROS® XT Chemistry Products TRIG-CHOL Slides

T. DiMagno, C. Graby, R. Novick, J. Scherer. *Ortho Clinical Diagnostics, Rochester, NY*

**Background:** A new Multi-Test technology is under development that enables more efficient clinical chemistry laboratory diagnostics. The new VITROS® XT MicroSlide technology incorporates two chemistry chips on single dry slide element. This new XT test element enables unique benefits such as higher throughput, reduced sample volume, less reagent storage space, less waste, and ease of use without compromising analytical performance. One example of this technology is a single dry slide with both a triglyceride test and a cholesterol test on a single slide. The VITROS XT Chemistry Products TRIG-CHOL Slides will be developed to quantitatively measure cholesterol concentration and triglycerides concentration in serum and plasma. The VITROS XT TRIG-CHOL Slides contains two multilayered, analytical elements coated on a polyester support. A drop of patient sample is deposited on each chemistry chip (2.5 uL for TRIG and 4.0 uL for CHOL) and is evenly distributed by the spreading layer to the underlying layers. The density of the dye formed through the reaction cascades is proportional to the triglyceride and cholesterol concentrations present in the sample on their respective chemistry chips and is measured by reflectance spectrophotometry.

**Methods and Results:** We evaluated the accuracy of 90 patient serum samples (TRIG: 21 - 504 mg/dL; CHOL: 69 - 277 mg/dL) on the VITROS XT 7600 Integrated System (in development) compared to the VITROS Chemistry Products CHOL Slides and VITROS Chemistry Products TRIG Slides on a VITROS 5600 Integrated System. The VITROS XT TRIG-CHOL Slides showed excellent correlation with the VITROS CHOL and TRIG Slides. VITROS XT TRIG-CHOL = 0.993 * VITROS TRIG - 1.97; (r) = 1.000 for TRIG; VITROS XT TRIG-CHOL = 1.023 * VITROS CHOL - 1.94; (r) = 0.998 for CHOL. A 50-replicate within day precision study conducted on the VITROS XT 7600 System showed excellent precision. Mean TRIG concentrations of 110.3 mg/dL, 262.0 mg/dL, and 437.1 mg/dL resulted in within-day percent coefficient of variation (%CV) of 0.72%, 0.70%, and 0.50% respectively. Mean CHOL concentrations of 134.8 mg/dL and 236.9 mg/dL resulted in within-day %CV of 0.92% and 0.76% respectively. The linearity for the VITROS XT TRIG-CHOL Slides was determined from an 18-level admixture series. The TRIG test was linear from 11.1 mg/dL to 556.2 mg/dL, and the CHOL test was linear from 24.6 mg/dL to 355.4 mg/dL. The Limit of Detection (LoD) for the VITROS XT TRIG-CHOL Slides was determined based on 240 determinations with 4 low-level samples. The LoD for the TRIG test was 9.7 mg/dL and for the CHOL test was 6.1 mg/dL. The measuring range for the VITROS XT TRIG-CHOL Slide exceeded 11-525 mg/dL for the TRIG test and 50-325 mg/dL for the CHOL test.

**Conclusion:** The VITROS XT TRIG-CHOL Slides has exhibited good correlation with serum samples across a broad measuring range with excellent precision, linearity, low end sensitivity, and measuring range on the VITROS XT 7600 Integrated System.

## B-416

### Comprehensive analysis of CYP2D6 variants and copy numbers using reverse-hybridization and real-time PCR based assays

A. Berndt, C. Oberkanins, H. Puehringer. *ViennaLab Diagnostics, Vienna, Austria*

**Background:** The cytochrome P450 2D6 (CYP2D6) is an important liver enzyme involved in the metabolism of up to 25% of clinically used drugs. The CYP2D6 gene is highly polymorphic, with numerous (sub)variants described in the Human Cytochrome P450 Database (www.cypalleles.ki.se). While the most frequent allelic variations are caused by single nucleotide polymorphisms and small insertions/deletions, highly homologous regions in the CYP2D6 gene locus facilitate unequal cross-over leading to large deletions, duplications and gene conversions.

**Methods:** We developed a reverse-hybridization assay (PGX-CYP2D6 XL StripAssay) for the simultaneous detection of 19 sequence variations in the CYP2D6 gene, which define the most prevalent alleles impacting enzyme activity in Caucasians. For the detection of copy number variations a real-time PCR based assay (CYP2D6 RealFast CNV Assay) was established. The StripAssay and real-time PCR assay were validated on 118 and 98 samples, respectively.

**Results:** The PGX-CYP2D6 XL StripAssay correctly identifies allelic variants with normal (*1, *2, *35, *39), reduced (*9, *10, *17, *29, *41) and no (*3 to *8, *11, *12, *14, *15, *40, *58) enzyme activity, and hence allows the classification of individuals into extensive (EM), intermediate (IM) and poor (PM) metabolizers. In addition, ultrarapid (UM) metabolizers and CYP2D6*5 carriers can be identified by

quantifiying their abnormal copy number status using the CYP2D6 RealFast CNV Assay. Both assays demonstrate a test accuracy of >0.99.

**Conclusion:** The metabolizer phenotype of patients treated with CYP2D6 substrates can be accurately determined by the combined use of both assays.

## B-417

### Performance of Total Protein, Ultra HDL, Alanine Aminotransferase, Urea Nitrogen and Phosphorus on the Alinity c Analyzer

A. E. Reeves, M. Berman, L. Templin, S. Syed, B. Diaconeasa. *Abbott, Abbott Park, IL*

**Objective:** To evaluate analytical performance utilizing photometric technologies for detection of analytes in human plasma/serum and urine on the Alinity c, Abbott's next-generation clinical chemistry analyzer. The Alinity c analyzer is a high throughput instrument testing up to 900 tests per hour. The sample is dispensed into a cuvette followed by reagents. The contents are mixed and incubated allowing for the reaction to occur. If a second reagent is required, the second reagent is added to the cuvette; the contents are mixed again and incubated. Absorbance readings of the sample are taken at regular intervals throughout the process at a primary and if applicable, a secondary wavelength. Data reduction generates a calculated absorbance based on the reaction mode of the assay (rate or end point) and measures the calculated absorbance using a calibration curve to generate a result.

**Methods:** Key performance testing including precision, linearity, limit of quantitation (LoQ), and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range across which acceptable performance for bias, imprecision and linearity was met.

**Results:** Total imprecision, LoQ and linearity results along with the defined measuring interval are shown for representative assays in the table below. Results versus the comparator assay demonstrated a slope 0.97 – 1.01 and r = 0.99 - 1.00.

| Assay | Total %CV | LoQ | Linearity | Measuring Interval |
|---|---|---|---|---|
| Total Protein | ≤ 3 | 0.76 g/dL | 0.2 - 22.5 g/dL | 0.8 – 18.4 g/dL |
| Ultra HDL | ≤ 4 | 5 mg/dL | 5 - 200 mg/dL | 5 - 180 mg/dL |
| ALT | ≤ 6 | 5 U/L | 2 - 3899 U/L | 5 - 3899 U/L |
| Phosphorus (Serum) | ≤ 4.6 | 0.62 mg/dL | 0.0 - 27.1 mg/dL | 0.7 – 25.3 mg/dL |
| Phosphorus (Urine) | ≤ 4.6 | 4.39 mg/dL | 0.3 - 227.2 mg/dL | 5.0 – 186.0 mg/dL |
| Urea Nitrogen (Serum) | ≤ 4.5 | 3 mg/dL | 2 - 128 mg/dL | 3 - 125 mg/dL |
| Urea Nitrogen (Urine) | ≤ 4.5 | 40 mg/dL | 13 - 2084 mgdL | 40 - 1991 mg/dL |

**Conclusion:** Representative clinical chemistry assays utilizing photometric technologies tested on the Alinity c analyzer demonstrated acceptable precision, sensitivity and linearity. Method comparison data showed excellent agreement.

## B-418

### Total Error Profiles - A New Method for Visualizing Product Performance

S. Schneider[1], V. Petrides[1], S. Westgard[2], B. Renley[1], A. Orzechowski[1]. *[1]Abbott, Abbott Park, IL, [2]Westgard QC Inc, Madison, WI*

**Introduction:** The sigma value is a commonly used metric for comparing products and is derived from the TEa, precision, and bias determined at a single critical point. In a clinical laboratory, the sigma value is one simple aid determining whether a product is fit-for-use. Often, however, laboratorians want to understand the performance of a product beyond a single critical point. A Total Error Profile is a valuable tool that can be used to visualize the performance of a product across a range of concentrations.

**Methods:** A Total Error Profile was created to visualize the performance for three immunoassays. For a given assay, the total error was estimated at multiple concentrations across the assay's measuring interval using the equation: *%total error = 2 x %CV + |%bias|*. To estimate the precision (%CV), a study was conducted at Abbott on each assay using the Alinity i-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, > 70 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity i-series and ARCHITECT $i2000_{SR}$ systems. The mean concentration of the Alinity i-series results were regressed versus the mean ARCHITECT $i2000_{SR}$ results and a weighted Deming regression model was fit. Using the regression model, the %bias was estimated at the same concentrations at which the precision samples were tested. A Total Error Profile was created by plotting the %total error values versus the mean concentration values for the Alinity i-series. Sigma values were calculated using the equation: *sigma = (%TEa - |%bias|) / %CV.* Additionally, a precision profile was created by plotting the within-laboratory %CV values versus the mean concentration values.

**Results:** The Total Error Profile is a continuous function across multiple concentrations for each assay. The Total Error Profile provides additional rigor beyond the sigma value and precision profiles by leveraging all total error estimates above, below, and near the critical decision points. In the example assay data, the total error decreased as the Total β-hCG concentration increased, whereas the total error increased slightly as the TSH concentration increased.

**Conclusion:** Total Error Profiles can be useful supplements to sigma metrics and precision profiles by providing a visual assessment of assay precision and bias performance across the measuring interval. Another benefit of the Total Error Profile is that it is not dependent on the TEa goal. This approach is particularly useful for assessing assays with more than one medical decision point and for assays with wide measuring intervals. By providing a detailed analytical understanding of the expected assay performance, these profiles can be useful tools for helping laboratorians understand the full dynamic performance characteristics of each assay and the potential impact to patient results.

## B-419

### An Aptamer Technology Platform for Cancer Diagnosis

S. Chen, X. Zhang, K. Tang, Q. Song, C. Kasbek, Y. Song, D. Quintanilha, N. Almalki, T. Wang, J. Huang. *Admera Health, South Plainfield, NJ*

**Background/Objectives:** The gold standard in cancer diagnosis is morphological and molecular pathology of a biopsy, which is crucial for timely and effective treatment of cancers. Molecular diagnoses of cancers using protein biomarkers are mostly antibody-based immunoassays. Development of immunoassays for cancer diagnosis has been hindered by the availability and inherent properties of antibodies. Thus alternative affinity reagents are in need for the development of cancer diagnostics. Aptamers are short (15-100 nt) ssDNAs/RNAs that bind their targets avidly and specifically. Aptamers are often called "chemical antibodies", due to its nature of *in vitro* selection and chemical synthesis. The advantages of aptamers compared to antibodies are broader target range (from small chemicals to macromolecules), better batch consistency, lower cost and they are more amenable to diverse assay formats. Here, we present an aptamer technology platform for cancer diagnostics.

**Methods:** Systematic Evolution of Ligands by EXponential enrichment (SELEX) was utilized to develop various schemes for *in vitro* selection of both DNA and RNA aptamers. The aptamer technology platform was then applied in a case study of a diagnostic assay for lung cancer.

**Results:** The variables of aptamer selection schemes were optimized including the library design, the target immobilization methodology, the enzymology for incorporating modified nucleotides into DNA/RNA and the bioinformatics pipeline for aptamer candidate identification. Through the optimization and development, we have established an aptamer selection platform integrating SELEX seamlessly with Next Generation Sequencing (NGS) with high success rate (>80%) of aptamer selection. The aptamer technology platform was applied to develop aptamers for ten biomarkers of lung cancer (e.g. follistatin) with nM affinity ($K_d$) (Fig 1). Aptasensors for early diagnosis of lung cancer are under development.

**Conclusion:** We have developed an aptamer technology platform with great potential in cancer diagnosis.

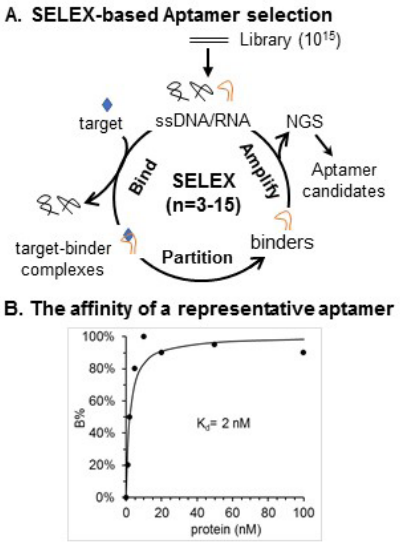



**Fig 1**. The scheme of aptamer selection based on SELEX (A) and the affinity of a representative aptamer developed (B).

## B-420

### Routine-like testing of a cobas e 801 module* in an integrated cobas® 8000 configured as an IC/CC consolidator

K. Hegel[1], M. McGovern[2]. *[1]Labor Berlin - Charité Vivantes Services GmbH, Berlin, Germany, [2]Roche Diagnostics GmbH, Mannheim, Germany*

**Background:** The high throughput immunochemistry module from Roche Diagnostics, **cobas e** 801 is the newest member of the **cobas** 8000 modular analyzer series. It is designed to cover almost double the testing capacity of the predecessor **cobas e** modules and offers many features aimed to further enhance lab testing efficiency. During multicenter studies, the new module was tested in dedicated use as well as in combination with all other established **cobas** 8000 analyzer modules. Here we report on the outcome of the routine-like testing experiments processed at Lab Berlin on a platform including ISE, **cobas c** 701 and **cobas e** 801 covering a total of 75 assays, 38 immunochemistry assays and 37 general chemistry and protein assays.

**Methods:** All experiments were designed to stress the system under full workload conditions for up to eight hours, thereby testing the overall functionality and reliability using simulated routine testing patterns. In addition, while processing these high workloads with > 7000 results, numerous interactions (reagent loading, low sample volume, QC testing, STAT samples added, supplies run low, etc.) were initiated by the operators in order to provoke the system and to check for proper behavior. Aliquoted pooled QC materials were used as samples, the recovery of the respective analyte per aliquot was applied to check the performance throughout the experiment. The time to complete the experiment and the processing time per sample were also analyzed.

Throughout the study, QC's at two concentration levels per assay were measured in order to monitor the system performance and reliability.

**Results:** In total ~ 93,500 results were generated for 75 different methods over the six week study period. Stable system performance throughout the study was shown for all assays and modules with analyte recovery in QC material well within ± 2sd of the assigned target values.

Proper system functionality and interaction between the used analytical modules was shown during routine simulation precision experiments that included >30 immunochemistry assays from the indication areas thyroid, cardiac, fertility, oncology and infectious disease. Precision CVs calculated for results generated during random testing over >4 hours were mostly <2% and thus only marginally higher than those generated during batch-type testing. Analysis of workflow on our study system showed that **cobas e** 801 in a CC/IC consolidator configuration, can easily process typical workloads of two **cobas e** 602 modules without slowing down the high speed clinical chemistry **cobas c** 701 module (up to 2000 tests per hour). Typical hospital setting type peak workloads of ~900 samples were processed in less than 3 hours. Introduction of STAT samples or other provocations did not hinder the workflow nor lead to system malfunctions, the various workloads were handled efficiently.

**Conclusion:** The results of this study demonstrate that the **cobas e** 801analyzer met or exceeded laboratory requirements under simulated routine laboratory conditions. It showed excellent analytical performance, high reliability, speed of analysis and ease of use.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

## B-421

### Result consistency of cobas e 801* compared with Roche systems E170, cobas e 601 and cobas e 602 demonstrated in 6 European and US labs

I. Zahn[1], A. Leichtle[2], S. Wang[3], G. Soria*[4], P. Johnson[5], C. Bendavid[6], M. McGovern[7]. *[1]Laboratory Dr. Limbach and Colleagues, Heidelberg, Germany, [2]University Institute of Clinical Chemistry, Inselspital, University of Bern, Switzerland, [3]Department of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, [4]Laboratori de Referència de Catalunya, Barcelona, (*present address: Cerba International), Spain, [5]The Pathology Centre, NHS Foundation Trust, Queen Elizabeth Hospital, Gateshead, United Kingdom, [6]Laboratoire de Biochimie, CHU Rennes, France, [7]Roche Diagnostics GmbH, Mannheim, Germany*

**Background:** Manufacturers of in vitro diagnostic products design and develop new analytical systems to meet changing needs of different types of laboratories. When introducing a new technical solution for lab testing in order to, for example enhance testing efficiency, it is of ultimate importance to maintain consistency of analytical results. During a multicenter performance evaluation study in six labs, we compared the results generated on the new immunochemistry analyzer **cobas e** 801 with those generated on the three established Roche high throughput systems, MODULAR E170, **cobas e** 601 (**cobas** 6000) and **cobas e** 602 (**cobas** 8000). Here we report the outcome of our testing on all four analyzers for nine assays in the areas of cardiac, thyroid, oncology and fertility.

**Methods:** Testing on the **cobas e** 601 module was done using preselected left-over samples and conducted in parallel to that on a **cobas e** 801 module. For comparisons with the E170 and the **cobas e** 602 modules, results generated during routine testing on multiple routine analyzers were compared with those generated under simulated routine conditions on the **cobas e** 801 study systems. Left-over samples were used according to the attained site specific ethic commission or internal review board waiver.

**Results**

Overview comparisons **cobas e** 801 versus MODULAR E170, **cobas e** 601 and **cobas e** 602

| Assay | Unit | Predicate instrument | Type of compare results | Samples (n) | B/P Slope | B/P Intercept | Pearson r |
|---|---|---|---|---|---|---|---|
| TSH | mIU/L | E170 | routine | 3815 | 1.03 | 0.00 | 0.998 |
| | mIU/L | e601 | study | 201 | 0.96 | 0.01 | 0.999 |
| | mIU/L | e602 | routine | 843 | 1.05 | 0.00 | 0.999 |
| FT4 | pmol/L | E170 | routine | 3255 | 1.00 | 0.38 | 0.983 |
| | pmol/L | e601 | study | 123 | 0.98 | 0.38 | 0.993 |
| | pmol/L | e602 | routine | 371 | 1.01 | 0.10 | 0.994 |
| CEA | µg/L | E170 | routine | 932 | 1.06 | 0.01 | 0.998 |
| | µg/L | e601 | study | 201 | 0.95 | 0.09 | 0.999 |
| | µg/L | e602 | routine | 457 | 1.05 | 0.03 | 0.996 |
| CA 15-3 | U/mL | E170 | routine | 207 | 1.02 | 0.70 | 0.994 |
| | U/mL | e601 | study | 109 | 0.96 | 0.60 | 0.995 |
| | U/mL | e602 | routine | 207 | 1.01 | 0.40 | 0.972 |
| Estradiol | pmol/L | E170 | routine | 406 | 0.99 | 7.10 | 0.998 |
| | pmol/L | e601 | study | 204 | 0.99 | 0.60 | 0.999 |
| | pmol/L | e602 | routine | 82 | 0.96 | 3.40 | 0.998 |
| Testosterone | ng/mL | E170 | routine | 652 | 0.99 | 0.01 | 0.996 |
| | ng/mL | e601 | study | 140 | 1.01 | 0.00 | 0.999 |
| | ng/mL | e602 | routine | 138 | 1.02 | 0.02 | 0.998 |
| NT proBNP | pmol/L | E170 | routine | 91 | 1.02 | 0.39 | 0.999 |
| | pmol/L | e601 | study | 125 | 1.01 | 0.38 | 0.999 |
| | pmol/L | e602 | routine | 232 | 0.99 | 1.89 | 0.999 |

**Conclusion:** The study outcome demonstrates that results generated on the new immunochemistry analyzer **cobas e** 801 are consistent with those generated under

different conditions at six labs on other Roche immunochemistry analyzers. All systems use the same reagents based on the Elecsys technology; thereby supporting an easy transition between the systems.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

## B-422

### Abbott Alinity c Sigma Metrics and Precision Profiles for Clinical Chemistry Assays

S. Westgard[1], V. Petrides[2], S. Schneider[2], M. Berman[2], A. Orzechowski[2]. *[1]Westgard QC Inc, Madison, WI, [2]Abbott, Abbott Park, IL*

**Introduction:** Assay performance is dependent on the accuracy and precision of a given method. These attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 30 clinical chemistry assays tested on the Alinity c-series. Additionally, precision profile charts were created for a subset of assays to compare the precision performance of the assays tested using the Alinity c-series and the ARCHITECT *c* system.

**Methods:** A sigma value was estimated for each assay and was plotted on a method decision chart. The sigma value was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*. A precision study was conducted at Abbott on each assay using the Alinity c-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, 40-100 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity c-series and ARCHITECT c8000 systems. The mean concentration of the Alinity c-series results were regressed versus the mean ARCHITECT c8000 results and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated at a medical decision point. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity c-series and the ARCHITECT c8000 system, where the ARCHITECT c system within-laboratory %CV and mean concentration values were obtained from the assay package insert.

**Results:** The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near the medical decision point. The precision profile charts of the within-laboratory %CV results for the Alinity c-series overlaid with the ARCHITECT *c* system showed similar performance across the subset of assays evaluated.

**Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity c-series clinical chemistry assays had sigma values greater than 5. The precision performance on the Alinity c-series and ARCHITECT *c* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

## B-423

### Performance Evaluation of ZS050 in Measurement with SVACs and Wash in Alkaline Detergent

J. Yamaguchi. *Saitama Pref. Saiseikai Kawaguchi General Hospital, Saitama, Japan*

**Background:** JCA-ZS050 (ZS050) released from JEOL offers distinctive features of micro-volume measurement with the minimum 40µL reaction volume (SVACs: small volume analytical conditions) and wash in alkaline detergent to minimize sample-to-sample carryover. Performances are evaluated for these features.

**Methods:** SVACs were evaluated by comparing accuracy, precision and correlation with standard analytical conditions (SACs) specified by reagent manufacturers. 31 tests from chemistry, immunoassay and TDM tests were used. The measurement range and the matrix effects are assumed to be the same as the SVACs followed the same sample-reagent ratio with SACs. Accuracy was evaluated using MaCRM, the control serum from Japanese Committee for Clinical Laboratory Standards (JCCLS), for both conditions. Precision was evaluated using 3 levels of samples, 2 levels from controls and 1 level from pooled serum, to check within-run reproducibility. Correlations of test results by ZS050 with SVACs and SACs were evaluated using 100 patient samples to compare the results by AU5800 (from Beckman Coulters, Inc.). Correlation between SVACs and SACs was also evaluated. Sample-to-sample carryover with ZS050 was evaluated using HBs Antigen (HBsAg) which poses grave significance as a test result. The evaluation used concentrated HBsAg recombinant sample of approximately 1,500,000 IU/mL and patient sample with elevated HBsAg levels of 130,000 IU/mL, both measured by ARCHITECT (from Abbott Laboratories) with sensitivity of between 0.15 to 0.25 IU/mL. Carryover of HBsAg to next HBsAg-negative sample by a sample probe after probe wash in water or alkaline detergent was measured by ARCHITECT.

**Results:** Accuracy with SVACs was within the allowable limit defined by JCCLS in the certificate for MaCRM. Within-run reproducibility as precision demonstrated the equivalence between SACs (0.00 to 3.74%CV among 31 tests) and SVACs (0.00 to 3.92%CV among 29 tests). 2 out of 31 tests presented higher CV while some indicated better within-run reproducibility with SVACs. Correlation coefficient among the test results were calculated as follows: 0.959 to 0.999 with slope of 0.852 to 1.125 and intercept of -5.094 to 6.292 between AU5800 and SACs, 0.960 to 0.999 with slope of 0.876 to 1.134 and intercept of -6.514 to 3.764 between AU5800 and SVACs, and 0.965 to 0.999 with slope of 0.952 to 1.028 and intercept of -7.916 to 0.778 between SACs and SVACs. Sample-to-sample carryover was measured positive (0.68 IU/mL) after water wash and below the sensitivity after alkaline wash for 1,500,000 IU/mL sample, and below the sensitivity after water wash and alkaline wash for 130,000 IU/mL sample.

**Conclusion:** The results demonstrated high accuracy with SVACs. Good precision was obtained for 29 tests while parameters in SVACs require re-examination for 2 tests with higher CV. The correlation was found good for all three relationships. These results suggest that reagent volume reduction of 24 to 65% can be expected against SACs with 52.3 to 115.6µL reaction volume. Further discussion remains for optimal analytical conditions to maximize the performance of both ZS050 and reagents. For sample-to-sample carryover, alkaline wash is suggested from the result that carryover from 1,500,000 IU/mL sample was below the sensitivity.

## B-424

### Precision profiles for 20 assays on the cobas e 801* immunochemistry analyzer

P. Johnson[1], G. Soria**[2], S. Engelmann[3], S. Rutz[4], S. Potapov[4]. *[1]The Pathology Centre, NHS Foundation Trust, Queen Elizabeth Hospital Gateshead, United Kingdom, [2]Laboratori de Referència de Catalunya, **present address: Cerba International, Barcelona, Spain, [3]Roche Diagnostics GmbH, Mannheim, Germany, [4]Roche Diagnostics GmbH, Penzberg, Germany*

**Objectives:** When launching a new analytical system, there are various requirements on data generation to fulfill the regulatory needs for registration and launch around the globe.

Here we report on the outcome of a study performed at three sites to generate precision profiles according to CLSI EP05-A3 (5 days) using the 3x5x5 model for 20 assays on the **cobas e** 801 analyzer.

**Methods:** Aliquots of quality control material and multiple human specimen pools with analyte concentrations across the measuring range of the respective assay, were prepared and distributed to all study sites. Testing was done on five days in 5-fold determinations per sample pool at three sites. The 20 assays included in this study covered the indication areas Cardiac (NT proBNP II, Troponin T, CK-MB and Myoglobin), Oncology (CEA, CA 15-3 II, CA 19-9, CA 72-4, CYFRA, HE4, AFP and HCG+beta), and Infectious disease (Anti HBc IgM, HBsAg Quant, CMV IgG, CMV IgG Avidity, CMV IgM, Toxo IgG, Toxo IgG Avidity and Toxo IgM). CVs were calculated per site as within- lab precision and across the sites as reproducibility including the components repeatability, between-day and between-lab.

**Results:** The analyte concentrations ranges covered per assay are shown in the table below:

| Assay | Unit | Concentration range | Assay | Unit | Concentration range |
|---|---|---|---|---|---|
| NT proBNP II | pg/mL | ~13 - 30000 | AFP | IU/mL | ~2 - 900 |
| Troponin T | pg/mL | ~9 - 9500 | HCG+beta | IU/mL | ~5 - 8000 |
| CK-MB | ng/mL | ~1 - 300 | Anti HBc IgM | COI | ~0.1 - 2 |
| Myoglobin | ng/mL | ~30 - 2000 | HBsAg Quant | IU/mL | ~3 - 4500 |
| CEA | ng/mL | ~1- 950 | CMV IgG | U/mL | ~0.5 - 470 |
| CA 15-3 II | U/mL | ~4 - 290 | CMV IgG Avidity | % | ~20 - 100 |
| CA 19-9 | U/mL | ~10 - 900 | CMV IgM | COI | ~0.2 - 4 |
| CA 72-4 | U/mL | ~2 - 200 | Toxo IgG | IU/mL | ~0.5 - 600 |
| CYFRA | ng/mL | ~1 - 70 | Toxo IgG Avidity | % | ~40 - 90 |
| HE4 | pmol/L | ~15 - 1300 | Toxo IgM | COI | ~0.1 - 4 |

The calculated within-lab CVs for all 3 sites were mostly < 2 % over all assays across the tested concentrations. Similar repeatability, between-day and between-lab variation resulted in reproducibility CVs of < 3 % for most samples, indicating the stable and consistent performance over all assays and sites.

**Conclusion:** The precision CVs for the 20 assays tested on the **cobas e** 801 demonstrate the good result consistency of the new high-throughput immunochemistry analyzer within and across labs.

*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2017)

## B-425

### Fundamental evaluation of a novel reagent for Interleukin 2 receptor measurement using general clinical chemistry analyzers

E. Hamada, N. Taromaru, M. Maekawa. *Hamamatsu University School of Medicine, Hamamatsu, Japan*

**Background:** Soluble interleukin 2 receptor (IL-2R) is generally used for the diagnosis and disease activity monitoring for malignant lymphoma and any other lymphoproliferative disorders in routine laboratories. Recently, a novel reagent based on the latex enhanced immunoturbidimetric method for use on fully automated general clinical chemistry analyzers has been developed. Here we evaluated the analytical performance of this novel reagent, Nanopia IL-2R (Sekisui Medical Co., Japan). We also examined the distribution of serum IL-2R concentration in our routine laboratory. **Methods:** Serum samples collected from our inpatients/outpatients were used to evaluate the analytical performance of a novel reagent, Nanopia IL-2R on the 7180 Clinical Analyzer (Hitachi High-Technologies, Japan), and compared with IMMULITE 2000 XPi Immunoassay System and its dedicated reagents (Siemens Healthcare Diagnostics, USA) using correlation analysis. We also investigated the distribution of inpatient/outpatient serum IL-2R concentrations falling above the upper reference limit (582 U/mL) from December in 2003 to November in 2015 (12 years) using the clinical research database system. Moreover, patients with serum IL-2R concentration above 10000 U/mL were separately assessed for their pathological conditions. These studies have been approved by the ethical committee in Hamamatsu University School of Medicine. **Results:** Serum IL-2R was measured in 10646 specimens for 12 years and concentrations above the upper reference limit were observed in 2258 patients. Approximately half of the specimens were ordered by hematologists. Significant elevation above 10000 U/mL was observed in 1.6% of the total specimens. The highest value of 131000 U/mL was observed in a patient with malignant lymphoma. Other patients with significantly elevated levels of IL-2R carried malignant lymphoma, leukemia, malignant diseases, autoimmune diseases and infections. The within-run precision (CV) examined using control specimens was 2.14, 1.22 and 1.32% at approximately 500, 2000 and 5000 U/mL, respectively (n=20). The dynamic range was from 50 to 10000 U/mL. No significant interferences were observed with coexisting materials when analyzed with Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Japan). The relationship between Nanopia IL-2R and Immulite 2000 examined using patient specimen was 0.992 for the correlation efficient and y=0.979x - 5.40 for the regression line (n=168). **Discussion:** The basic performance of Nanopia IL-2R was acceptable. This latex enhanced immunoturbidimetric assay reagent can be applied on any general clinical chemistry analyzer and does not require specific immunoassay analyzers. The dynamic range is acceptably wide and reasonable relative to the distribution of serum IL-2R concentrations in our hospital. In conclusion, this reagent would be useful for the diagnosis and monitoring of lymphoma and other lymphoproliferative disorders.

## B-426

### Bio-Rad BioPlex® 2200 and DiaSorin LIAISON® XL Throughput Comparison Study

V. Kumar, D. Goode. *med fusion, Lewisville, TX*

**Background:** Multiplex technology that combines multiple assays into one test bead is assumed to have a higher throughput and faster TAT than other immunoassay instruments. As laboratories are expected to do more with less, it is important to ensure the automation selected meets the needs of the laboratory. This study was conducted to compare the throughput of Bio-Rad BioPlex® 2200 (multiplex technology) with DiaSorin LIAISON® XL (conventional technology) in a real life scenario at a reference laboratory. **Methods:** Three sample scripts were developed based on real-life laboratory ordering patterns for infectious disease assays that are common to the BioPlex 2200 and the LIAISON XL. Each script was comprised of 350 samples, each sample having from 1-7 different tests ordered. Samples were tested on the LIAISON XL first, followed by the BioPlex 2200 for the same length of time. Data was collected to capture the length of time to pipette all assays, the time to first result, and the number of assays completed within the LIAISON XL run time. **Results:** Table below shows the results of the study.

| | Script 1 | | Script 2 | | Script 3 | |
|---|---|---|---|---|---|---|
| Test Case Description | MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM, plus Vitamin D on LIAISON XL | | MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM, plus HAV IgM on XL, ANA on BioPlex 2200 | | MMRV, Treponema, EBV Panel, HSV-1,2, Toxo IgG, Rubella IgG & IgM, CMV IgG & IgM | |
| Measured Variable | LIAISON XL | BioPlex 2200 | LIAISON XL | BioPlex 2200 | LIAISON XL | BioPlex 2200 |
| Total Assays Completed | 729 | 631 | 900 | 895 | 839 | 818 |
| Total Samples Processed | 350 | 324 | 350 | 348 | 348 | 338 |
| Total Test Time | 6 h 23 min | 6 h 23 min | 8 h 05 min | 8 h 05 min | 7 h 29 min | 7 h 29 min |
| Time to First Result | 32 min | 44 min | 34 min | 44 min | 33 min | 44 min |
| Time to Pipette 100 Assays | 48 min | 63 min | 47 min | 48 min | 48 min | 60 min |
| Time to First 100 Results | 1 h 17 min | 1 h 47 min | 1 h 25 min | 1 h 33 min | 1 h 19 min | 1 h 32 min |
| Time to First 200 Results | 2 h 02 min | 2 h 37 min | 2 h 17 min | 2 h 29 min | 2 h 17 min | 2 h 36 min |

**Conclusion:** The LIAISON XL illustrated a faster time to first result and a higher throughput; regardless of the test menu it was presented with. Multiplex technology has the potential to have a very high throughput of tests, if tests ordered on patient samples utilize all of the tests on each bead. In a real life laboratory environment, tests are not ordered in the multiplex bead configuration and therefore, the BioPlex 2200 is not able to maximize the technology. The LIAISON XL is not inhibited by reagent configuration, as each assay has its own individual reagent; therefore, allowing for consistent time to pipette assays, time to first result, and time to complete testing.

## B-427

### Using Dried Tissue Homogenates to Preserve Solid Biospecimen for Molecular Analysis

R. T. Taylor, A. Fuentes, D. Shirvasti, R. Magesh, J. E. Hill, J. R. Hill. *Spot On Sciences, Austin, TX*

**Background:** Solid biospecimens from excised tissue represent a great source of molecular information that enables the discovery and development of personalized medicine and molecular diagnostics. Conventional methods for preservation of solid biospecimens such as FFPE or flash freezing have numerous limitations including, sample integrity, cost, infrastructure, and can be hazardous to the end user. We propose an innovative and universally accessible method to streamline biospecimen preservation, leveraging the advantages and successes from dried blood spot technology. The objective of this study is to assess the feasibility of preserving

solid biospecimen as dried homogenates for molecular analysis. The recovery and quality of molecular analytes, RNA and DNA, from dried tissue homogenates was investigated.

**Methods:** 30 - 50 mg of rat tissue (liver, kidney, lung, heart, and spleen) was excised from a frozen section and homogenized with PBS in a Dounce homogenizer. The homogenate was then added to a HemaSpot -HF device by pipette and allowed to dry at room temperature. Total RNA was extracted using the E.Z.N.A. mini column (Omega BioTek) and quantified by NanoVue and or a RiboGreen assay. RNA quality was determined by a Bioanalyzer. Reverse transcription was carried out by SuperScript IV (Applied Biosystems) and p53 expression was measured by Taqman (ABI). Genomic DNA was isolated from dried homogenates by a white blood cell lysis method. DNA recovery was measured by PicoGreen assay.

**Results:** : RNA and DNA was found to be stable in dried homogenates stored at ambient temperatures up to six months. The criteria used for acceptable RNA was an absorbance values (A260/A280) of ≥ 1.8. The p53 gene was detected by Taqman analysis in all tissue types studied (liver, kidney, and spleen) with greatest expression observed in rat liver tissue. The highest content of genomic DNA was observed in the dried liver samples.

**Conclusion:** The ability to detect molecular analytes from dried solid biospecimens demonstrates feasibility for preserving tissue biopsies as dried homogenate for molecular analysis. This method provides a simplified, low cost tissue specimen preparation and storage method with minimal processing and refrigeration while maintaining sample integrity for analysis of critical molecular analytes.

## B-428

### Basic performance of the new sepsis marker; presepsin immunoassay on STACIA®

T. Matsuo, S. Matsushita, S. Tashiro, T. Ono. *Research & Development Department, LSI Medience Corporation, Chiba, Japan*

***Background/Purpose:*** Presepsin is a useful sepsis biomarker found in Japan. It is known to be produced by cleavage of CD14 with some proteases in a phagocytosis process. Therefore, it is said that presepsin value is less susceptible to the effect of trauma, burns and surgery than PCT and also increases rapidly at an early stage of sepsis. As a result, presepsin is also said to reflect the clinical course and severity of sepsis patients well. Recently, presepsin received higher recommendation than PCT as an auxiliary diagnostic test in Japanese version of sepsis guideline 2016.

We evaluated the assay performance of a new presepsin immunoassay on STACIA® and examined its usefulness, reliability and continuity with a current PATHFAST® Presepsin data.

***Principles/Methods:*** STACIA® is an all-in-one instrument which consolidated major clinical assay principles such as chemiluminescence enzyme immunoassay (CLEIA), coagulation time, chromogenic substrate, latex agglutination, immunoturbidimetry, biochemistry. All results are available within 19 minutes and throughput is 270 tests per hour with fully random access.

The test method of presepsin assay on STACIA® is fully automated one-step sandwich immunoassay based upon CLEIA. Alkaline phosphatase (ALP)-labeled anti-presepsin monoclonal antibody reacts with presepsin in sample. After that, anti-presepsin monoclonal antibody-coated magnetic latexes (MG-LTX) specifically react with presepsin. Finally, after B/F separation, ALP on MG-LTX decomposes CDP-Star® substrate to an excited intermediate, which produces luminescent signal. Presepsin concentration is determined by comparison with the calibrator signal.

***Results:*** The presepsin assay has an analytical assay range from 50 to 20,000 pg/mL. Between day repeatability showed that a within-run and a total imprecision were less than 5%. Dilution recovery was excellent with mean recoveries within ±10% for all samples. Method comparison against PATHFAST® Presepsin showed a good correlation: y = 1.06x - 43.24, r = 0.994, n = 142 (y: STACIA® CLEIA Presepsin, x: PATHFAST® Presepsin). Further, it was shown a good correlation between plasma and serum samples: y = 0.99x + 31.07, r = 0.998, n = 98 (y: serum, x: EDTA plasma). The reference interval for normal donors was 59.0 -249.6 pg/mL, n = 198 (EDTA plasma).

***Conclusions:*** The newly developed presepsin immunoassay was rapid and precise assay. In addition, it was highly correlated with the current method and further it could test large numbers of specimens at central laboratories. These results suggest that STACIA® CLEIA Presepsin is useful for the daily monitoring of sepsis patients. Furthermore, that may lead to the rapid medical care, e. g. drug administration for treatment of sepsis.

## B-429

### Evaluation of the Roche immunochemistry platform cobas e 801 module

S. Wang[1], D. Payto[1], J. Daniel[1], M. Mitro[1], A. Ridgway[1], J. Hatheway[2], J. Layton[2]. *[1]Department of Laboratory Medicine Cleveland Clinic, Cleveland, OH, [2]Roche Diagnostics, Indianapolis, IN*

**Objectives**: The **cobas e** 801 module was evaluated by Cleveland Clinic Laboratories with selected applications of assays that represent the entire assay menu and challenge all functionalities of the **cobas e** 801 module. The **cobas e** 801 module is the newest member of the cobas 8000 modular analyzer series. The **cobas** 8000 modular analyzer series system is a fully automated, random-access, software controlled system for immunoassays and photometric analysis. The evaluation configuration consisted of **cobas** 8000 core and a **cobas e** 801 module.

**Methods**: The system and selected assays were evaluated for within-run-precision (1 run x 21 replicates on one measuring cell), repeatability CLSI (EP05-A3) (21 days x 2 runs/day x 2 replicates), performance of daily QC as a measure of calibration stability, routine simulation precision, in which a test CV based on randomized testing is compared to a CV based on within-run batch testing.

**Results:** Within-run-precision was well within acceptance criteria of CV ≤5% for all assays except folate, which had acceptance criteria of SD ≤0.35. Repeatability precision met the acceptance criteria of CV <5%.

| Test, Unit | Within-Run-Precision %CV | | | | Repeatability (21 day) %CV | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean | CV | mean | CV | n | mean | CV | mean | CV |
| A-HCV II, COI | 0.07 | 1.1 | 3.70 | 0.6 | 84[2] | 0.07 | 1.1 | 3.70 | 0.8 |
| CA 15-3, U/mL | 22.1 | 1.7 | 91.1 | 1.5 | 168 | 22.3 | 1.7 | 91.4 | 1.6 |
| CEA ng/mL | 4.86 | 1.1 | 47.1 | 0.8 | 168 | 4.87 | 1.4 | 47.4 | 1.2 |
| E2, pg/mL | 87.6 | 0.9 | 415 | 1.3 | 168 | 79.7 | 2.5 | 391 | 1.4 |
| FOL, ng/mL | 2.80 | 5.5[1] | 10.9 | 2.2 | 84[2] | 2.92 | 4.2 | 11.7 | 2.7 |
| FT4, ng/dL | 1.19 | 1.5 | 3.14 | 2.0 | 168 | 1.21 | 2.1 | 3.11 | 2.5 |
| Pro BNP(STAT) pg/mL | 148 | 1.1 | 4832 | 1.1 | 84[2] | 149 | 1.9 | 4874 | 1.3 |
| Pro BNP, pg/mL | 132 | 1.3 | 4304 | 0.9 | 168 | 135 | 2.0 | 4363 | 1.6 |
| TESTO, ng/dL | 551 | 1.4 | 235 | 1.5 | 168 | 534 | 1.7 | 226 | 1.5 |
| TSH, µU/mL | 1.49 | 0.8 | 8.74 | 0.6 | 168 | 1.48 | 1.4 | 8.60 | 1.3 |
| [1] SD is 0.15 (criteria SD ≤0.35) [2] Performed on only one measuring cell | | | | | | | | | |

Precision of daily QC was evaluated on 8 tests over a period of days (shortest 65 days to longest 95 days), with multiple runs and reagent e packs between calibrations. Good precision for two control levels demonstrated acceptable calibration stability (CV's 1.7% to 3.7%). Routine simulation precision testing with 627 requests analyzed in 2 hours 19 minutes had reference CV's of 0.7 to 2.0 and random CV's ranging 0.8 to 1.6.

**Conclusion:** Analytical performance on the **cobas e** 801 module met acceptance criteria for within-run-precision, repeatability, QC precision over many days, runs and reagent e packs demonstrating calibration stability, and routine simulation precision.

## B-430

### Automated sample preparation of user defined tests and subsequent analysis by flow cytometry in a single, integrated platform

R. Ravilisetty[1], L. Sheesley[1], B. Sahdala[1], A. Key[1], I. Perez[1], D. Flagler[1], N. Amata[1], A. M. Boehmler[2]. *[1]Beckman Coulter Inc., Miami, FL, [2]Beckman Coulter GmbH, Krefeld, Germany*

Many laboratories create their own reagent cocktails for routine applications in order to accommodate the marker combinations they require for their specific tests. However, with current technology, the tasks of sample preparation, analysis and data management can be major bottlenecks in the flow cytometry workflow. Even with semi-automated processes, laboratories may spend hundreds of additional hours per year on these tasks that don't add to their productivity.

To overcome the described issues, the AQUIOS Designer Software has been developed for use on the AQUIOS CL Flow Cytometry System. The software allows for the creation of customer-defined applications to be run on an instrument that combines sample preparation and flow cytometry analysis in one platform (AQUIOS Load & Go Technology). Until recently, this feature combination was only available for pre-defined applications like CD4 analysis and basic immunophenotyping.

Using the combination of AQUIOS CL and AQUIOS Designer Software, a panel of user-defined lymphocyte subset population and common activation marker assays

was established. For this study, assays were tested with peripheral blood from normal donors after informed consent, as well as with stabilized sample material from different vendors. All samples were analyzed from primary specimen tubes, using either the cassette autoloader or the single tube loader function of the AQUIOS CL system, with associated automated sample preparation functionality. The user-defined protocols, instrument settings and automated sample preparation steps created in the AQUIOS Designer Software resulted in excellent separation of all markers tested, including activation-related (and often dimly expressed) antigens.

While traditional flow cytometry workflows require a two-step approach to sample preparation and analysis on different instruments, the combination of the AQUIOS CL cytometer with the AQUIOS Designer Software offers a unique workflow concept that now combines sample preparation with data analysis in a single platform not only for pre-defined locked protocols, but for user-defined assays as well.

## B-431

### A Novel High Speed and High Performance Calprotectin PETIA for Serum and Plasma Samples

J. Yang, A. Huk, K. Sunde, T. Nilsen, M. Petersen. *Gentian Diagnostic AS, MOSS, Norway*

BACKGROUND

Human calprotectin (MRP8/MRP14) in serum and plasma has proven in several publications to be a promising inflammation bio-marker in several inflammatory conditions. Currently only ELISAs exist for the quantification of calprotectin in serum and plasma.

A fast, high performance Calprotectin PETIA (Particle Enhanced Tubidimetric Immunoassay) is under development by Gentian Diagnostic As. It is believed to be cheaper and faster (due to random access options) than Calprotectin ELISAs.

The assay uses polyclonal avian antibodies, raised to detect human MRP8/MRP14 complexes. Avian antibodies have the advantage of not reacting with rheumatoid factors, human anti-mouse IgG antibodies (HAMA) or the human complement system.

The assay calibrators contain highly pure Calprotectin antigen from human granulocytes, value assigned by $UV_{280}$ and Biuret.

The purpose of this study was to demonstrate high speed and high performance of the Gentian Calprotectin PETIA on Abbott Architect c4000 Clinical Chemistry Analyzer.

METHODS

Calprotectin was measured in human serum and plasma samples using the Gentian Calprotectin PETIA on Architect c4000. The assay consists of reaction buffer (R1) and immunoparticles (R2), calibrators (6 levels) and controls (2 levels).

The following studies were performed and assessed according to CLSI guidelines, where applicable.

- Precision (within run and total within lab)
- Detection capability (LoB, LoD and LoQ)
- Linearity
- Security zone
- Interference and cross-reactivity
- Between instrument variations and lot variations
- Method comparison (vs IDK® Calprotectin ELISA)
- Assay stability

The assay was designed to have a calibration range of approx. 0-20 mg/L, with QC controls of ~1.0 and ~10.0 mg/L.

RESULTS

Gentian Calprotectin PETIA demonstrated

- Detection capability: LoB (0.05 mg/L); LoD (0.07 mg/L); LoQ (0.30 mg/L)
- Security zone up to 100 mg/L
- Within run precision: CV from 0.24 % to 3.45 % for samples in the range (0.90-16.0 mg/L); total within lab precision (20 days, 2 runs per day, 2 replicates per run): CV < 6 % for samples in the range (0.90-16.0 mg/L)
- Linear range: 0.39-18.19 mg/L
- Method comparison (vs IDK® Calprotectin ELISA): correlation ($R^2 > 0.98$) when measuring serum samples (n >100; n: number of samples) spanning from approx. 0.50-18.0 mg/L
- Lot variations: average % bias (Bland Altman) between two lots was 4.20 % when measuring serum samples (n >100) spanning from approx. 0.50-18.0 mg/L- Instrument variations (Architect c4000 vs Mindray BS400): average Passing Bablok slope 1.02 and average intercept 0.03 mg/L when measuring serum samples (n >100) spanning from approx. 0.50-18.0 mg/L
- Interference: no significant interference was detected by testing 8.0 g/L hemoglobin, 600 mg/L bilirubin and 10.0 g/L intralipid.
- Cross reactivity: no significant cross reactivity was observed with monomer MRP8, MRP9 and MRP6
- Assay stability: on board stability of reagents (> 8 weeks); calibration curve stability (2 weeks)
- Total test time: approx.10 minutes

CONCLUSIONS

The Gentian Calprotectin PETIA demonstrates high speed and high performance in these evaluation studies. The assay could be used as tool for professional lab users in order to measure calprotectin in serum and plasma precisely, accurately and rapidly.

## B-432

### Nanoparticle plasma emission based digital optical encoding of liquid biochip for molecular analysis

Y. He[1], Q. He[1], D. Li[1], Y. Liu[1], M. Xin[2]. *[1]Tsinghua University, Shenzhen, China, [2]Foshan University, Foshan, China*

**Background:** Encoded beads based suspension array attracts more and more attentions in clinical diagnosis and biological detection. It is of great essential to expand the encoding numbers and optimize the decoding performance.

**Methods:** In this work, we provided a digital encoding method for liquid biochip based on nanoparticle plasma emission. we proposed a novel synthesized method to prepare digital encoded liquid biochip with better performance, and applied the prepared liquid biochip in analysis of both biomolecules and small molecules. With chosed encoding material, including Zinc oxide, Silver, Magnesium oxide and Cuprous oxide nanoparticles, we prepared encoded microbeads and accomplished their surface modifications and bioprobe grafting to synthesize digital encoded liquid biochip.

The liquid biochip was utilized in biomolecular analysis and decoded in our home built laser induced breakdown spectroscopy system. The result showed that the digital encoding method can highly promote the accuracy and stability of decoding. The plasma emission based encoding signal can avoid the cross talk with label signals in fluoroimmunoassay, which can improve the performance of whole analysis process. On the basis of digital encoding, we finished one step assembling of nanoparticles and quantum dots with self-healing method to achieve the complex utilization of plasma emission encoding and fluorescence encoding.

**Results:** In our experiments, we coupled digital encoding channel and analog encoding channel together, greatly expanded the encoding numbers and improved the decoding stability of liquid biochip. We proposed a digital encoded microbeads preparation method with reversed-phase micromulsion and ultraviolet light curing. With this synthesis method, we prepared digital encoded microbeads with better hydrophility in high productivity and simple process. The molecular examination showed that the liquid biochip based the prepared encoded microbeads owns well biocompatibility and high resistence to nonspecific adsorption, which is essential to promote the detection sensitivity of molecular analysis.

Furthermore, we firstly focused on utilizing the prepared digital encoded liquid biochip in biomolecular detection, mostly working in double-antibody sandwich fluoroimmunoassay mode. Then, the combination of digital encoding method and molecular imprinting technology had expanded the application field from biomolecular analysis to small molecular analysis. By creating the specific adsorption micro-cavity, which has the ability to capture target small molecules, on the surface of nanoparticles assembled microbeads, we prepared the bionic liquid biochip to accomplish multi-channel analysis to small molecules. Meanwhile, to build a detection mode for biomolecules which are not suitable for antigen-antibody bindingor base pairing based analysis method, we chose the target biomolecule to play the role of the template molecule in molecular imprinting technology to prepare the digital encoded biomolecular imprinting microbeads, the generalized liquid biochip.

**Conclusion:** With the nanoparticle plasma emission detected using laser induced breakdown spectroscopy, the effective application of digital encoded liquid biochip can cover the most types of molecules, which might induce a motivation in multiplexing of molecular analysis.

## B-433

### Development of a Novel Assay for the Simultaneous Identification of Deinococcus radiodurans and Determination of Susceptibility to a Selected Antibiotic

J. Faro, G. Riddle, S. Faro. *The Woman's Hospital of Texas, Houston, TX*

**Objective:** To examine the ability of a novel diagnostic ELISA to identify *Deinococcus radiodurans* in a reduced time when compared to culture. *D. radiodurans* is a relatively new bacteria, first discovered in spoiled food which had first been irradiated. Although slow growing, the bacterium has been shown to be very resistant to radiation, extremes in temperature, and dehydration. As culture of this organism may take up to two weeks, food and beverage handlers, as well as medical device manufactures, would benefit greatly from an assay that allows for more rapid detection of *D. radiodurans*.

**Methods:** Microtiter wells were coated with polyclonal IgG rabbit antibody directed against *D. radiodurans* at a dilution of 1:50 in coating buffer. After 120 minutes, wells were emptied and then blocked with StartingBock™. After blocking for 30 min, wells were emptied, and then bacterial isolates were added. For the 30-minute test, a series of dilutions of *D. radiodurans* were prepared in PBS, starting at $10^7$ bacteria/ml, and diluting out ten-fold to $10^0$ bacteria/ml. Samples were either run with *D. radiodurans* alone, or in the presence of other selected bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, Gordonia, *Streptococcus agalactieae*, Kocuria, Roseamonas) all at $10^7$ bacteria/ml. In order to increase the limit of detection, *D. radiodurans* dilutions were prepared in FastidiousBroth™, and cultured for predetermined lengths of time. After this culture period, samples were added to microtiter wells diluted 1:1 in phosphate buffered saline (PBS), and allowed to stand at room temperature for 30 minutes. Following this incubation step, wells were washed, and then bound bacteria was detected with HRP-conjugated anti-*D. radiodurans* antibody at 1:50 dilution for 20 minutes at room temperature. Wells were then washed, and signal was generated with TMB solution. Optical density was read at 450 nm.

In order to determine antibiotic susceptibility, *D. radiodurans* dilutions were incubated for 72 hours in increasing concentrations of Cefazolin, starting at 0.08 ʋg/ml, and increasing up to 32 µg/ml. Cefazolin was selected from a panel of antibiotics which were shown to promote inhibition of growth by in-house turbidity assays.

**Results:** Following a 30-minute incubation in PBS, *D. radiodurans* was detected at a limit of $10^6$ bacteria/ml. No interference was observed with any of the other bacteria tested. By increasing the incubation time in broth, the limit of detection increased to $10^5$ bacteria/ml at 24 hours, $10^4$ bacteria/ml at 48 hours, and $10^1$ bacteria/ml following 72 hours. When dilutions of *D. radiodurans* were prepared in the presence of Cefazolin, the limit of detection (LoD) decreased as the antibiotic concentration increased: with 0.08 µg/ml Cefazolin, the LoD was $10^2$ bacteria/ml; with 0.8 µg/ml Cefazolin, the LoD was $10^4$ bacteria/ml, and with 8 µg/ml Cefazolin, the LoD was $10^6$ bacteria/ml; following 72-hour incubation.

**Conclusion:** This novel assay allows for the simultaneous identification *D. radiodurans* and determination of antibiotic susceptibility in as little as 72 hours.

## B-434

### High-throughput, multiplex genotyping directly from saliva and buccal swabs without DNA purification

y. zhao, Y. zhao, Z. Zheng. *Chinese Academy of Medical Science and School of Basic Medicine, Peking Union Medical College, beijing, China*

Population screening of clinically significant SNP markers calls for multiplexed genotyping of SNPs on a large scale. Current SNP genotyping tools, despite many advantages, invariably require DNA extraction, which remains a key throughput-limiting step for population screening. We have previously developed a high-throughput genotyping approach for blood samples, MELPA, which has multiplex SNP genotyping capability, eliminates DNA extraction, and achieves uniform PCR amplification using a single pair of universal primers. Here we describe MELPA for saliva and buccal swab samples. MELPA lysed saliva/ buccal swabs and captured the target DNA directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic extension and ligation of the probes, a single-stranded template for each target SNP site was formed, and all templates were PCR-amplified using universal primers targeting the tail sequences. Multiplexed genotyping by single-base primer extensions were analyzed with a MALDI-TOF mass spectrometry platform. We tested the feasibility of the assay for saliva and buccal swabs, and evaluated the accuracy by comparing MELPA with commercial multiplex SNP assay (iPLEX), for the detection of 20 G6PD gene variants known to be at risk for primaquine-induced hemolysis in antimalarial therapy. We successfully developed a 20-plex panel for G6PD genotyping. A typical 50 ul saliva or one buccal swab sample is sufficient for running 2 assays. Six 384-samples can be processed from sample to result in a 24-hour workflow, with a hands-on time of 2 hours. Results were consistent with iPLEX, and 100% concordant with sequencing. Saliva and swab samples can be stored at room temperature for at least 24h without affecting the performance. MELPA represents an efficient and cost-effective approach to multiplex SNP genotyping at population level.

## B-435

### Evaluation of the Abbott Alinity Clinical Chemistry and Immunoassay Systems

S. Busse[1], J. Perl[1], J. Lotz[1], K. J. Lackner[1], J. L. Rogers[2], G. J. Gomez[2]. *[1]University Medical Center Mainz, Mainz, Germany, [2]Abbott Diagnostics, Abbott Park, IL*

Background. To evaluate the performance of the newly developed Alinity clinical chemistry (CC) and immunoassay (IA) systems in an independent laboratory and compare it to the performance of the respective Architect systems.

Methods. For CC performance AST, ALT, calcium, and total protein were analyzed. For IA qualitative and quantitative HBsAg-Assays, anti-HCV, combined HIV antigen and antibody (HIV-Combo), HTLV-I/II, and syphilis were analyzed. For all linearity and precision tests control material was used.

Results. For all four CC assays linearity of the Alinity results was excellent with coefficients > 0.999 over a broad concentration range. Slopes of the regression lines were between 1.00 and 1.04. Within day, between day, and total %CV was always <1.8 % with the exception of the low level of ALT which had a between day %CV of 2.6% and a total %CV of 3.56%. Analytical precision of the IA are listed in the table.

Throughput of the Alinity CC system was compared to Architect c8000 module and was approx. 10% higher. The IA system performed approx. 160 tests per hour. Finally, correlations of the Alinity results with the Architect system were analyzed with serum or plasma samples. For CC all correlation coefficients were > 0.99 over a broad concentration range. Agreement between Alinity and Architect IA was between 95 and 100% for all assays.

Conclusions. Several CC and IA tests for the novel Alinity systems have been evaluated under conditions of routine laboratory testing. Linearity, precision, and correlation to the current Architect systems have been fully satisfying. Sample throughput of the Alinity systems is moderately higher than with the Architect systems, but with a much smaller footprint.

| Immunoassay precision | | | |
|---|---|---|---|
| **Assay** | **Level** | **Within day %CV** | **Total %CV (95% CI)** |
| HBsAg (qual) | neg | 8.32 | 10.56 (7.48-17.95) |
| | pos | 1.84 | 1.98 (1.51-2.89) |
| HBsAg (qual – confirm) | pos | 2.40 | 2.68 (2.04-3.93) |
| HBsAg (quant) | neg | 0.00 | 0.00 |
| | pos* | 2.69 | 2.77 (2.15-3.88) |
| Anti-HCV | neg | 5.35 | 5.92 (4.52-8.62) |
| | pos | 3.04 | 3.71 (2.73-5.78) |
| HIV-Combo | neg | 9.70 | 11.19 (8.41-16.70) |
| | pos* | 2.55 | 2.85 (2.17-4.18) |
| rHTLV I/II | neg | 8.02 | 8.12 (6.33-11.34) |
| | pos | 7.53 | 8.24 (6.31-11.90) |
| Syphilis | neg | 0.00 | 0.00 |
| | pos | 1.25 | 1.42 (1.07-2.10) |

*) If more than one positive level was tested, the lowest positive level is shown

## B-436

### Biologic drug monitoring assays

J. Fecteau, L. Ruff, D. Uzri, B. Messmer. *Abreos Biosciences, Inc., San Diego, CA*

Background: Monoclonal antibodies (mAbs) are among the most rapidly growing class of pharmaceuticals and the most expensive. Pharmacokinetic (PK) variability among patients treated with these drugs is significant, typically varying by over two orders of magnitude, and blood drug levels correlate with clinical efficacy in most

cases. This variation can be compounded in combination therapies or by formation of anti-drug antibodies during prolonged treatment. Nonetheless, therapeutic dose monitoring is not routine practice partly to due to the lack of robust clinical laboratory or point-of-care (POC) assay solutions.

Methods: Peptide mimetic ligands are an attractive option for immunoassay reagents because they are stable, highly selective, and easier to develop and manufacture than natural ligands or anti-idiotype antibodies. We have developed mimetope peptides, termed Veritopes™, against a broad range of therapeutic mAbs, and these peptides are ideal for capture and quantification of free and active mAbs in biological samples such as human serum. We have implemented these peptides in ELISA format, where they are used as a surrogate ligand to capture the drug. We have developed Veritopes for several widely used mAb drugs, including natalizumab, vedolizumab, rituximab, trastuzumab, ipilimumab, and pembrolizumab. Veritope ELISAs can be commercialized as laboratory developed tests (LDTs). Alternatively, Veritopes can be integrated into lateral flow assays for POC dose monitoring applications such as patient stratification during clinical trials or personalized dosing of marketed drugs.

Validation: When used as a capture reagent in ELISA, these mimetope peptides display sufficient sensitivity, specificity, and linearity across the requisite concentration ranges relevant for most mAb PK studies. In all cases, the selected peptides effectively bind only the intended target in the presence of circulating human IgG and do not crossreact with other mAbs. The natalizumab ELISA was analytically validated in a CLIA setting in preparation for future marketing as an LDT. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for natalizumab in undiluted serum were determined to be 2.0ug/mL and 16.0ug/mL, respectively. These data were obtained from 5 independent runs, where each sample was run in triplicate. The Limit of Blank (LoB) and Limit of Detection (LoD) were 0.6ug/mL and 0.8ug/mL, respectively. Intra- and inter-assay accuracy and precision were determined using spike and recovery experiments with three concentrations of natalizumab covering the dynamic range and analyzed in five independent runs either in triplicate or quintuplicate. Analyte recovery was calculated for each concentration as a measure of accuracy and was consistently between 80% and 120% of nominal concentrations (Calibrated value/Nominal Value*100). Intra- and inter-assay precision were calculated using the same samples, and the coefficients of variation (%CV) were below 15% for all concentrations tested (SD/mean*100).

Results and Conclusion: Veritopes are robust reagents adaptable to a variety of immunoassay formats suitable for both laboratory and POC measurements of mAb levels in biological samples such as human serum. Direct monitoring of mAb drug levels in patients will enable precise, personalized dosing that can improve outcomes, minimize side effects, and reduce treatment costs.

## B-437

### Report on a European and two Korean population clinical trials for multiplex detection of HIV and HCV

S. Kim, C. C. Oste, Y. Lee, Y. Ki. *PCL Inc., Seoul, Korea, Republic of*

Background:

A novel, multiplex detection system for infectious diseases is based upon standard ELISA protocols, with the main difference that the process is conducted in an actual 3-D environment. The sol-gel nanoporous capturing technology represents a powerful approach where the sol-gel matrix constrains the motion of the encapsulated biomolecules (proteins, peptides, chemicals, antibodies, oligonucleotides, etc.) without physical adsorption or any modification. This technology can be applied to multiplex immunoassay platform because several disease biomarkers can be immobilized and tested concomitantly in a single well.

Methods:

The Hi3-1 Multiplex HIV1/2 and HCV antibody detection kit, a two-step fluorescence-based immunoassay, is designed to detect antibodies against HCV protein (Core, NS3, NS4, NS5) and HIV 1/2/O type protein, respectively, in human serum or plasma. Sol-gel spots are arrayed on the bottom of a microtiter plate wells, and antigens from HIV1/2/O and HCV are encapsulated within two sets of spots in each well. HIV and HCV antibodies in serum or plasma bind to antigens in the sol-gel spots and form antigen-antibody-fluorescently labeled secondary antibody complexes. Following a wash cycle, fluorophore-labeled secondary antibodies against human IgG and IgM are added to the wells. After washing to remove samples and unbound fluorescently labeled antibodies, the plate is scanned in a fluorescence scanner. In the absence of HIV and HCV antibodies, no fluorescence is detected. The clinical trial for this system is performed according to CTS guideline (Guidance on the In Vitro Diagnostic Medical Devices Directive 98/79/EC; Commission Decision of 3 February 2009) and KFDA guidelines (Release No.B1-2012-5-005).

Results:

In the Clinical trial at the Korea University Guro Hospital in Korea, the results suggest that the sensitivity of both the HIV-Ab and HCV-Ab assays using Hi3-1 kit was 100.00% [100%, n=353: including 102 HIV Korean positive specimens, 150 HIV 1, 100 HIV 2 and 1 HIV 1 O subtypes, and n=431 HCV Korean positive] and the concordance of the corresponding HIV Ab and HCV Ab assays between the Hi3-1 system and the Architect systems for negative specimens was 99.96% (n=4,479 negative specimens for HIV) and 99.76% (n=4,150 negative specimens for HCV), respectively.

In the Clinical trial at the Seoul St. Mary's Hospital in Korea, the result showed a highest sensitivity (100%, n = 500 HIV-positive specimens and n = 400 HCV-positive) and specificity (100% for HIV 1/2 and 99.84% for HCV, n = 4,306 negative specimens) by using the kit, which simultaneously screens for the presence of HIV1/2 and HCV antibodies.

In the clinical trial at CERBA in France, a total of 3400 clinical negative samples (collected from the CERBA Specimen Service of France) were tested for HIV1/2 and HCV using the Hi3-1 kit. Concordance of the corresponding HIV Ab and HCV-Ab assays between the Hi3-1 and Architect systems for panel 1 was 99.97% and 99.82%, respectively.

Conclusion:

Given that the new technology has sensitivity and specificity equivalent to the commercially available CLIA tests, the sol-gel based microarray has the potential to be used as a high-throughput screening tool for simultaneous detection of HIV and HCV in blood banks.

## B-438

### Process Qualification for Production and Purification of OC125 Antibody in Cell Culture

J. Angstadt, C. Chessnoe, C. Blamey, J. Meyers, J. Klein, Z. Li. *Fujirebio Disgnostics, Inc., Malvern, PA*

**Background:** Production of quality monoclonal antibody (mAb)suitable for serological diagnosis and compliant with ISO standards and Regulatory Regulations, is key to *IVD* industry. Ascites generation and bioreactor systems represent *in vivo* and *in vitro* approaches for mAb production, respectively. Bioreactor systems have some advantages over ascites generation in preventing the introduction of endogenous contaminating protein and reducing variability in antibody generation (Marx 1995; Jackson 1996; Bruce 2002). This study was to qualify the production and purification of OC125 antibody, a mAb against cancer biomarker OC125 defined antigen, produced in a bioreactor system. **Methods:** Three lots of an OC125 proprietary mouse hybridoma cell line were cultured in the Applikon EZ-Control bioreactor containing serum-free media for at least 70 days, with at least 14 harvests. The supernatants were purified on a mAb SelectSuRe column, concentrated with a Pellicon unit and polished with SP Sepharose HP column. Samples from each lot were assessed for functionality by incorporating the antibody on a mainstay *IVD* immunoassay platform, and characterized with the HPLC, isoelectric focusing electrophoresis (IEF), Immunoelectrophoresis (IEP) and SDS-PAGE electrophoresis. Moreover, the hybridoma cells were collected prior to culture and at the end of 90 day culture to undergo cDNA sequencing of the antibody. **Results:** The collected total volumes of three lots of culture supernatants were 533, 893 and 1301 L, and the final yields of purified antibodies were 6.3, 45.7 and 59.3 g, respectively. The purified OC125 antibody samples were coated on the solid phase of a mainstay *IVD* automated immunoassay platform. Functional testing of a reagent set with the OC125 antibody-coated on solid phase led to an acceptable Calibrator B/A ratio >7; Calibrator B, C, D, E, and F within the limit of 12.4 - 25.5, 40.7 - 108.2, 119.3 - 322.1, 272.3 - 718.2 and 624.9 - 1363.6 U/mL, respectively; and Control L, M and H within the limit of 32.0 - 48.0, 240.0 - 360.0 and 520.0 - 780.0 U/mL, respectively. SDS-PAGE of the antibody showed a single primary band of ~200 kDa in non-reduced gels, and two primary bands of ~50 kDa and ~30 kDa, respectively. IEF gel running of the antibody showed three bands at pI of 6.5 - 7.0 consistently. IEP gel running denoted the OC125 mAb as IgG1 type. HPLC-SEC analysis demonstrated the purity of all three lots of the purified mAb at 100% with a clear uniform peak at the retention time of ~7.5 minutes consistently. All of the gels were comparable to or better than the reference. The sequencing results denoted 100% alignment match between the cells prior to culture and that at the end of the 90-day culture, indicating the cell line was stable during the 90-day production cycle. **Conclusion:** The functionality and characterization of three lots of OC125 antibodies derived and purified from the bioreactor-based cell culture system met all of the acceptance criteria for release and bulk functional testing. The methods and techniques utilized to produce and purify the antibody are validated to be a robust process for production and purification of OC125 Antibody.

## B-439

### Advanced Centrifugal Microfluidic Platform for the Automation of Clinical Assays

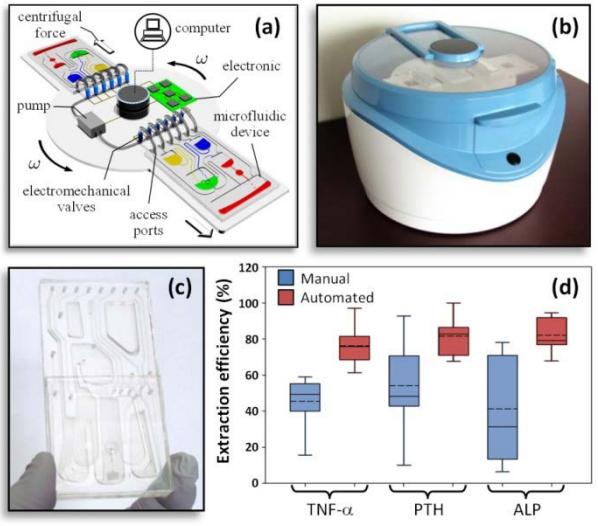
D. Brassard[1], L. Clime[1], J. Daoud[1], M. Geissler[1], L. Malic[1], D. Charlebois[2], T. Veres[1]. *[1]National Research Council, Boucherville, QC, Canada, [2]Canadian Space Agency, Saint-Hubert, QC, Canada*

**Background:** Centrifugal microfluidics offers the interesting prospect to automate the liquid handling steps required by clinical assays. Unfortunately, traditional centrifugal microfluidics offers only limited liquid control capabilities, which is problematic for the integration of complex assays. Herein, we report the development of an automated protein extraction assay from whole blood using a novel centrifugal microfluidic platform where advanced liquid control is achieved through a combination of centrifugal forces and active pneumatic pumping.

**Methods:** We fabricated a centrifugal microfluidic platform capable of applying air pressure pulses (0-5 psi) to the ports of microfluidic devices while the platform is rotating at high speed (1000 rpm), providing precise control to automate on-chip liquid handling steps (Fig. 1a and b). Microfluidic devices were fabricated from low-cost thermoplastic materials and contained no active components such as valves or electrodes (Fig 1c). Capture of target proteins is performed on 100 µm silica beads functionalized through carbodiimide chemistry and conjugated with antibodies for TNF-α, PTH or ALP.

**Results:** Extraction of target proteins from whole blood is shown here as an example of an assay that can be automated with the developed technology. For this assay, the following steps were successfully automated: metering and transfer of a density gradient medium, transfer and metering of a blood sample from an external tube (600 µl), blood fractionation, plasma extraction, active back-and-forth displacement of plasma through a bead-bed containing functionalized silica beads, three washes, elution, and transfer of eluted sample to an external vial. The automated assay demonstrated significantly higher protein extraction efficiency (about 80%) and lower variance compared with assays performed manually using standard protocols (Fig 1d).

**Conclusion:** Using the developed centrifugal microfluidics platform, a multistep protein extraction assay was successfully automated using passive thermoplastic microfluidic devices, which highlights the potential of the technology for clinical applications.




## B-440

### A Comparison of the New Beckman Coulter DxC 700 AU Clinical Chemistry System to the UniCel DxC 800 Synchron Clinical System

B. Godber, K. Breasley, D. FitzGerald. *Beckman Coulter, Co. Clare, Ireland*

**Background:** The Beckman Coulter DxC 700 AU analyser is the latest clinical chemistry system from Beckman Coulter. It is a fully automated, random access analyzer, designed for medium to high throughput laboratories, with a throughput of 1200 tests/hour including ion selective electrodes. The purpose of this study was to compare the recovery of patient samples on the new DxC 700 AU with the UniCel DxC 800 Synchron Clinical Systems for a selection of routine assays.

**Methods:** To compare the Beckman Coulter DxC 700 AU and the UniCel DxC 800 Synchron Clinical Systems, several Beckman Coulter assays were selected for evaluation that covered serum and urine sample types and a range of assay methodologies. These systems were compared using patient serum or urine samples. Samples were run in duplicate and the sample means compared using Deming regression.

**Results:** All DxC 700 AU assays showed excellent correlation with the UniCel DxC 800 Synchron Clinical System. The Deming regression statistics parameters for selected assays are summarised in the table below.

| Assay | Units | N | Slope (95% CI) | Intercept (95% CI) | R | Range |
|---|---|---|---|---|---|---|
| Glucose | mg/dL | 130 | 1.030 (1.028 to 1.033) | -2.7 (-3.2 to -2.3) | 1.000 | 12.5 to 760.4 |
| Creatinine | mg/dL | 122 | 0.966 (0.962 to 0.969) | 0.05 (0.04 to 0.07) | 1.000 | 0.38 to 23.54 |
| BUN | mg/dL | 98 | 0.990 (0.987 to 0.993) | -0.4 (-0.5 to -0.3) | 1.000 | 11.0 to 123.8 |
| Albumin | g/dL | 121 | 0.935 (0.916 to 0.954) | 0.36 (0.28 to 0.44) | 0.988 | 1.56 to 5.22 |
| Total Protein | g/dL | 134 | 1.022 (1.010 to 1.033) | 0.1 (0.0 to 0.1) | 0.996 | 3.4 to 10.2 |
| ALP | U/L | 110 | 1.065 (1.059 to 1.071) | 0.50 (-0.2 to 1.2) | 0.999 | 24.8 to 718.6 |
| AST | U/L | 118 | 0.913 (0.908 to 0.918) | -3.0 (-3.2 to -2.8) | 0.999 | 8.2 to 360.2 |
| GGT | U/L | 73 | 1.181 (1.175 to 1.187) | -1.2 (-1.6 to -0.8) | 1.000 | 6.2 to 459.9 |
| IgG | mg/dL | 120 | 0.895 (0.886 to 0.904) | 30.4 (20.1 to 40.7) | 0.997 | 174.9 to 2845.3 |
| Urinary Albumin | mg/dL | 77 | 1.023 (1.010 to 1.036) | -0.2 (-0.4 to 0.1) | 0.997 | 1.6 to 29.4 |

**Conclusion:** The results of the study demonstrate that the new Beckman Coulter DxC 700 AU analyzer has comparable performance to the UniCel DxC 800 Synchron Clinical System.



## B-441

### Quality Control Algorithm for Protein Determination Using Coomassie Brilliant Blue

F. Vitzthum[1], H. Schwarz[2]. *[1]Siemens Healthineers, Tarrytown, NY, [2]Siemens Healthineers, Marburg, Germany*

**Background:** Accurate protein determination is essential in biochemical laboratories and for the manufacture of immunoassays. Interfering substances can lead to erroneous results. Quality control (QC) testing helps to identify issues. When using dyes such as Coomassie brilliant blue (CBB), bromocresol green, and bromocresol purple, spectral analysis represents an elaborate, but laborious QC. Reference measurements at single wavelengths, typically at wavelengths far higher than the actual measurement, are more practical. However, subtle changes caused by interfering substances can be missed. We set out to assess the use of reference measurements closer to the actual measurements as a QC.

**Methods:** Using the CBB assay in a microplate format as a model, we determined the spectra of various proteins at different concentrations ranging from 10 to 100 µg/mL using the SpectraMax Plus Microplate Reader (Molecular Devices). Focusing on the determination of bovine serum albumin with and without interfering substances, the utility of applying absorption values at various reference wavelengths around the actual measurement wavelengths was assessed.

**Results:** The use of the isosbestic point of the CBB protein reaction at ~530 nm proved to be a convenient reference measurement. The interference of substances such as glycine, SDS, and HEPES could be determined by an upper and lower limit independent of the absorption at the actual measurement wavelength. When using other reference wavelengths, e.g., 440 nm, a function between the absorption at the actual and reference measurement wavelengths had to be applied. Adequate upper and lower confidence limits of polynomial or linear regression could be used as thresholds to identify the interference.

**Conclusion:** Reference measurements and respective algorithms can aid in the identification of erroneous results of protein determination by CBB and potentially other assays for protein determination using respective dyes.

## B-442

### Evaluation of Immunoglobulin G_2 and Immunoglobulin M_2 Assays* on the Atellica CH Analyzer**

C. Briggs, L. Bantum, T. Johnson, T. Nyamwange, R. Prusisz, J. Snyder. *Siemens Healthineers, Newark, DE*

**Background:** This study evaluated the performance of the Immunoglobulin G_2 (IgG_2) and Immunoglobulin M_2 (IgM_2) assays,* clinical chemistry PEG-enhanced immunoturbidimetric assays*, being developed for use on the Atellica™ Chemistry (CH) Analyzer** (Siemens Healthcare Diagnostics Inc.).

**Methods:** Performance testing was conducted using Clinical and Laboratory Standards Institute (CLSI) guidelines and included precision (CLSI EP05-A3); method comparison (CLSI EP09-A3); limit of blank (LoB), detection (LoD), and quantitation (LoQ) (CLSI EP17-A2); interference (CLSI EP07-A2); and serum/plasma equivalence (CLSI EP09-A3) studies.

**Results:** Assay range for the IgG_2 assay was 140–3400 mg/dL (1.40–34.00 g/L) and for the IgM_2 assay was 21.0–330.0 mg/dL (0.21–3.30 g/L). Observed agreement in patient sample method comparison studies using Deming regression: Atellica CH IgG_2 assay = 1.00 × ADVIA® Chemistry IgG_2 assay + 6 mg/dL (+ 0.06 g/L) (r = 0.999, n = 113, range: 148–3593 mg/dL [1.48–35.93 g/L]); Atellica CH IgM_2 assay = 1.01 × ADVIA Chemistry IgM_2 + 2.5 mg/dL (+ 0.02 g/L) (r = 0.999, n = 105, range: 21.7–324.7 mg/dL (0.22–3.25 g/L). Precision was evaluated across the assay range using serum/plasma pools and commercial quality control materials. Each sample was assayed in duplicate twice a day for 20 days. IgG_2 assay repeatability and within-lab precision were ≤1.6% CV and ≤1.8% CV, and IgM_2 assay repeatability and within-lab precision were ≤1.1% CV and ≤2.5% CV, respectively. LoB and LoD were observed to be 7 mg/dL (0.07 g/L) and 21 mg/dL (0.21 g/L) for the IgG_2 assay and 1.9 mg/dL (0.02 g/L) and 3.9 mg/dL (0.04 g/L) for the IgM_2 assay, respectively. Interference ≤10% was observed for the IgG_2 assay with hemolysate (1000 mg/dL), bilirubin (50 mg/dL), and lipemia (1000 mg/dL) and for the IgM_2 assay with hemolysate (1000 mg/dL), bilirubin (60 mg/dL), and lipemia (1000 mg/dL). Agreement of serum and plasma in the IgG_2 assay is represented by lithium heparin plasma = 1.01 × serum − 26 mg/dL (− 0.26 g/L) (r = 0.998, n = 54, range: 252–2498 mg/dL [2.52–24.98 g/L]) and in the IgM_2 assay is represented by lithium heparin plasma = 1.01 × serum − 2.1 mg/dL (− 0.02 g/L) (r = 0.999, n = 54, range: 25.8–319.5 mg/dL [0.26 – 3.20 g/L]) and K EDTA plasma = 0.99 × serum − 0.1 mg/dL (− 0.00 g/L) (r = 0.999, n = 54, range: 25.8–319.5 mg/dL [0.26–3.20 g/L]).

**Conclusions:** The Immunoglobulin G_2 and Immunoglobulin M_2 assays tested on the Atellica CH Analyzer demonstrated acceptable performance in all tested areas.

*All assays under development and not available for sale. Future availability cannot be guaranteed.

** Not available for sale. Not CE marked. Future availability cannot be guaranteed.

## B-443

### Evaluation of Cell-Free DNA Recovery During Extraction Using A Next-generation Sequencing Assay

C. Christopherson, P. Sankey, L. Eubank, T. Harrison, W. Woods, L. Cabiling, L. Nguyen, M. Nelles, S. Scott. *CareDx, Brisbane, CA*

Background: In solid organ transplant recipients, circulating cell-free DNA from the donor in the background of the recipient's DNA measured as percent donor-derived cfDNA (dd-cfDNA) is an important marker for allograft rejection. We have developed a non-invasive next-generation sequencing (NGS) assay that utilizes SNPs located throughout the genome to quantify the dd-cfDNA (AlloSure®). Prior knowledge of donor and recipient genotypes are not required to measure the proportion of cfDNA in the recipient's plasma that is released from the donated organ. The levels of cfDNA found in plasma are low which challenges the recovery of cfDNA during sample extraction. In order to accurately quantify the low amounts of cfDNA, the extraction method must be robust and reproducible. Extraction methods must be tested to identify the best method for each specific assay employing cfDNA.

Objectives: The objective of this study was to determine the reproducibility and linearity of cfDNA extraction in our lab. Reproducibility was analyzed using multiple operators, replicate extractions and extractions on multiple days. Linearity was assessed by performing dilutions of plasma made before extracting.

Methods: Blood was collected from normal healthy volunteers into Streck Cell-Free DNA BCT® collection tubes. Panels were created by mixing plasma from one individual (donor) into the plasma of another (recipient) in proportions that are consistent to those found in transplant patients. Three panels were created to test reproducibility representing 3 different spike-in levels. To assess linearity, dilutions were made of spike-in samples using the plasma from the "recipient" to dilute the proportion of cfDNA present from the "donor". Plasma was extracted using Qiagen's Circulating Nucleic Acid kit. After extraction, samples were quantified for total cfDNA using a qPCR method while dd-cfDNA was calculated using the AlloSure workflow.

Results: Total recovery of cfDNA as measured by qPCR ranged from 11 to 34ng per 5ml of plasma which is consistent with ranges expected from healthy volunteers. Intra-operator variability for total cfDNA ranged from 1.5% to 11% CV. The inter-operator variability ranged from 9% to 17%. When dd-cfDNA was measured, the intra-operator variability ranged from 5.6% to 7.8% and the Inter-operator CVs ranged from 1.7% to 12.8%, both within the established variability of the assay. Linearity tests showed the assay performed as expected; the measured dd-cfDNA was consistent with expected values based on dilution.

Conclusions: The recovery of cfDNA was reproducible within one operator and between operators. The cfDNA from the donor is consistently recovered at different spike-in amounts indicating that the extraction method is appropriate for use with AlloSure where low levels of donor cfDNA require reproducible and robust recovery.

## B-444

### Standardized Reagent Formulation and Data-driven QC Criteria Ensure Efficient and Consistent Delivery of Plasma Cell-free DNA Results for Organ Transplant Rejection

P. Sankey, C. Christopherson, L. Eubank, C. Chan, K. Sterling, F. Lao, M. Velez, C. Garcia, R. Woodward, M. Nelles, S. Scott. *CareDx, Brisbane, CA*

Background: Workflows for complex molecular diagnostic testing often require multiple steps and formulation of specialized reagents. High quality standardized reagents are critical to ensure accurate and consistent test results. AlloSure® is a recently released Laboratory Developed Test that quantifies the amount of donor-derived cell-free DNA (dd-cfDNA) in the plasma of kidney transplant recipients by targeted next generation sequencing of 266 SNPs. We identified key reagents in the AlloSure workflow that would benefit from transfer to our reagent manufacturing group for formulation. Formulation of these reagents under a GMP compliant lab ensures standardized and validated processes, QC prior to release for use in patient testing, and reduces variabilty introduced when reagents are prepared at the time of testing. The transfer of these key reagents to our manufacturing group required establishing independent QC processes and acceptance criteria in addition to training staff and validating the successful transfer of the formulation procedures.

Objectives: The purpose of this study was to establish formulation procedures and defined QC criteria for key reagents used in a cell-free DNA next generation sequencing assay.

Methods: The preparation of 266 AlloSure targeted amplification primers was identified as a key component that warrants transfer to our manufacturing group. Transfer of the primer preparation, both as a pool used in the pre-amp step and the 48 different multiplexes used in the secondary amplification, included creating a training plan, SOPs, batch records, standardized lot labeling, and production of 3 independent lots before the official transfer was complete. QC criteria were developed by testing the NIST NA12878 reference standard in the AlloSure NGS workflow over the course of 1 year. The criteria used for passing lots of primers were set using number of SNPs that pass QC as determined by the same AlloSure QC algorithm. Statistical analysis of the number of AlloSure SNPs successfully sequenced was performed using binomial quantile estimation. The data from this analysis were used to define the QC testing criteria for the formulated primer pools. Additional QC criteria which requires obtaining the expected dd-cfDNA results from spike-in controls is used for CLIA lab acceptance of the materials.

Results: The historical data were used to estimate the binomial probability of an individual SNP passing QC using the mean as calculated from all of the runs. The resulting minimum 1% quantile is 230 SNPs passing QC. Based on this cut-off, 99% of primer sets used succesfully in AlloSure testing to date passed this QC.

Conclusions: Performance and the QC metric for primer lots was defined by the specific requirements needed using data from multiple runs over long periods of time. The metrics can be used to reject lots of primers that do not meet specifications at two levels, primer performance against a reference standard (NIST) and obtaining the expected dd-cfDNA results using well-characterized spike-in controls. This ensures high quality lots of primers are used in AlloSure testing and accurate and consistent results are delivered for managing organ transplant patients.

## B-445

### Improving the Sensitivity of the Coomassie Brilliant Blue (CBB) Test

F. Vitzthum[1], H. Schwarz[2]. *[1]Siemens Healthineers, Tarrytown, NY, [2]Siemens Healthineers, Marburg, Germany*

**Background:** Sensitive and accurate protein quantitation is important for the manufacture of immunoassays. The CBB test is widely used as a sensitive and rapid method.(1) After its introduction, several improvements of the test have been reported, including the use of the ratio of the absorption values at 590 nm and 450 nm(2) instead of measuring at ~590 nm only. Increasing the linearity of a test improves accuracy when linear regression is used for value assignment. Further increasing the sensitivity allows for broader and more flexible application. Since measurement at different wavelengths captures differently protonated forms of the dye, we set out to determine whether altering the pH-value of the reagent could further improve linearity or sensitivity of the assay.

**Methods:** We determined the impact of different pH-values of Bradford reagent (Bio-Rad) on absorption values and combinations at different wavelengths. Slopes and correlation coefficients ($r^2$) of bovine serum albumin (Sigma) standard curves ranging from 10 to 100 µg/mL were used as indicators for sensitivity and linearity. 60 µL of sample was placed in a well of PS standard F-bottom microplates (Greiner Bio-One) followed by the addition of 240 µL of reagent that had been pH-adjusted. The absorption measurements were performed using the Spectra Max Plus Microplate Reader (Molecular Devices).

**Results:** A pH-value of ~0.8 for the original reagent was confirmed to be the optimum for the measurement at 595 nm. However, the highest slope was observed at a pH-value of ~1.0 when using the ratio 595/470. The slope was ~4.7-fold higher compared to the absorption measurement at 595 nm at a pH-value of ~0.8, and the slope was ~1.6-fold higher compared to the absorption ratio 595/470 at the pH-value of ~0.8. $r^2$ values in the pH-range from 0.5 to 1.0 were comparable (0.9824 and 0.9693 for the 595/470 ratio and 595 nm, respectively).

**Conclusion:** When using the 595/470 ratio, the slope of the CBB assay can be significantly increased by using a pH-value of ~1 for the reagent. Linearity is not markedly changed, moving the original pH-value of the reagent from about 0.8 to 1. Thus, a reagent pH-value of ~1 represents a simple means to improve the sensitivity of CBB testing, maintaining linearity and consequently accuracy of testing throughout the measuring range.

**References:**

1. Anal Biochem. 1976;72:248.

2. Anal Biochem. 1996;236:302.

## B-446

### Multiplex Immunomagnetic Biosensing Platform for Detection of Small Molecules in Clinical Samples

P. I. Nikitin[1], N. V. Guteneva[2], S. L. Znoyko[1], A. V. Orlov[1], V. A. Bragina[1], B. G. Gorshkov[1], T. I. Ksenevich[2], V. R. Cherkasov[2], M. P. Nikitin[2]. *[1]General Physics Institute, Moscow, Russian Federation, [2]Moscow Institute of Physics and Technology, Dolgoprudniy, Moscow region, Russian Federation*

**Background:** Magnetic nanoparticles (MP) are very attractive robust labels for advanced immunoassays (Orlov et al. Biosens. Bioelectron. 2016, 79, 423-429). In this work, we present a quantitative multiplex Dry-Reagent ImmunoMagnetic (DRIM) platform for simultaneous on-site detection of small molecules such as B-type vitamins and thyroid-associated disease markers: free and total T3, free and total T4. The developed multiplex DRIM platform is innovative by virtually no sacrifice in performance compared to single-plex assays and by characteristics on the level of laboratory quantitative methods as shown recently for botulinum neurotoxin types A, B and E in complex matrices (Orlov et al. Anal. Chem. 2016, 88, 10419-10426).

**Methods:** We present a multiplex quantitative lateral flow (LF) assay for on-site simultaneous detection of several small molecules in clinical samples. The novel approach to easy multiplexing is realized via joining an on-demand set of single-plex LF strips based on magnetic nanolabels, into a miniature cylinder cartridge that mimics an LF strip during all assay stages. The cartridge is readout by an original portable multi-channel reader based on the magnetic particle quantification (MPQ) technique using non-linear magnetization of MP (Nikitin & Vetoshko, EP 1262766, 2001). The sensitivity of this electronic detection method is on the level of gamma-radioactive technique for counting MP based on 59-Fe isotope (Nikitin et al. J. Appl. Phys. 2008, 103, 07A30). The developed new generation of multi-channel MPQ reader offers the unmatched 60-zmol detection limit and 7-order linear dynamic range for volumetric registration of magnetic labels inside a cartridge of several millimeters in diameter regardless of its optical transparency. Each of the test strips, developed here as building blocks for the multiplex assay, can be used "as is" for autonomous quantitative single-plex detection with the same measuring setup.

**Results:** The developed biosensing platform has demonstrated attractive limits of detection (LOD) for B-type vitamins and thyroid-associated disease markers: free and total T3, free and total T4. The LOD for T4 hormone in human serum was as low as 0.03 pM or 0.023 pg/mL using blood samples of 40 patients. The experiments were done using clinical samples of known concentration measured by reference electrochemiluminescent Abbott apparatus. Some of the samples were also diluted by purified serum with zero concentration of T4. The achieved LOD is much better than the required clinical range for human in vitro diagnostics. It demonstrates the technology potential, which can be used to measure concentrations of other small molecules such as toxins, carcinogens, pharmaceuticals, natural and synthetic poisons, etc.

**Conclusion:** The multiplex DRIM platform based on highly sensitive detection of magnetic nanoparticles in 3D solid phase has demonstrated LOD of T4 hormone in clinical samples as low as 0.03 pM or 0.023 pg/mL and wide 3-order dynamic range of hormone concentration. The developed multiplex biosensing platform can be used for rapid multi-analyte tests for point-of-care in vitro diagnostics, food analysis, biosafety and environmental monitoring, forensics and security, etc.

## B-447

### Alphafetoprotein: correlation between the analyzers Centaur XP and Dimension Vista 500

R. Escobar Conesa, D. González Benito, A. Cienfuegos González, B. Laborda Gonzalez, S. Garcia Castañon, M. Martinez Gago. *Hospital de Cabueñes, Gijon, Spain*

**Background:** Alpha-fetoprotein (AFP) is one of the largest glycoproteins in fetal plasma, though its main usefulness in clinical practice is as a marker of non-seminomatous hepatocellular and germ cells carcinoma. The analytical method used is chemiluminescence; recently, due to a problem in the supply of reagent, we were forced to use a different autoanalyzer, passing from Centaur XP ® to Dimension Vista 500 ®, being necessary to carry out a correlation before applying the change.
**Methods:** A total amount of 57 samples were analyzed consecutively by both analyzers. Previously, aliquots which were freezed at -20º until the moment of being processed, had been made. Levels 1 and 3 of Tumor Marker Immunology ® by Biorad were used as a control.

**Results:** The statistical analysis was made with MedCalc ® software, and it consisted on the calculation of the equation of the regression line by the nonparametric method of Passing-Bablok, in order to assess the degree of substitutability between both samples.

The correlation has the following equation: **y= 0.2 + 1 x** where Y corresponds to the instrument DIMENSION VISTA 500 and X to the ADVIA CENTAUR XP. The correlation coefficient was r = 1, with an IC95% for the slope of 0.9333 to 1.0254, and for the intercept, of 0.0466 to 0.4067. The values of AFP were expressed in both cases in ng/mL. **Conclusion:** The calculated parameters show that there exists a good correlation between both methods (r = 1). The intersection includes the 0 and the slope includes the 1, so it's not necessary to apply the correction given by the above-mentioned formula, therefore, the methods are interchangeable and their results are transferable. The change of analyzer led to the modification of the reference values of AFP (from < 8.1 ng/ml with ADVIA CENTAUR XP to < 8.00 ng/ml with DIMENSION VISTA 500), being notified to the ordering doctors, and registered through a cutline in the informatic system, in order to take account of it in the patient follow up. Furthermore, at the operational level, the necessary time for the analysis has been reduced to 10 minutes, being specially important for those samples requiring dilution.

## B-448

### Application of extracellular flux analysis in the differential diagnosis of mitochondrial diseases

B. K. Chacko, G. A. Benavides, A. C. Hurst, D. Leon, E. E. Ubogu, R. Hardy, V. Darley-Usmar. *University of Alabama at Birmingham, Birmingham, AL*

**Background:** Mitochondrial diseases, a heterogeneous group of genetic diseases arising from mutations in the mitochondrial or nuclear genome, are presented with

a wide spectrum of neurological, muscular and cardiac symptoms. The multi-system involvement and the heterogeneous features of these defects mimic neurological and systemic diseases that make diagnosis often challenging. The influence of genetic, environmental, lifestyle factors and age on mitochondrial activity further complicate disease presentation. Current methods of diagnosis include genomic analysis and invasive muscle biopsies which will not provide any functional assessment of the mitochondria. In addition, there are no standardized set of guidelines for the biochemical and molecular evaluation of the suspected mitochondrial disease patient currently exist. A common feature of the mitochondrial disease is the impaired energy metabolism due to defective cellular oxidative phosphorylation and metabolism. Objective: The major goals of this study are to develop and validate cellular bioenergetic and mitochondrial assays for the diagnosis of mitochondrial diseases using peripheral blood leukocytes and platelets. This test utilises the concept that circulating leukocytes and platelets can act as sensors or biomarkers of bioenergetic dysfunction that occurs in mitochondrial diseases. **Methods:** Using the extracellular flux analyzer the oxygen consumption rates of mitochondria in intact monocytes and platelets are measured employing two different protocols (1) the mitochondrial stress test and (2) mitochondrial respiratory complex activities. The mitochondrial stress test will determine the bioenergetic parameters in intact cells (basal, ATP-linked, proton-leak, maximal, reserve capacity and non-mitochondrial respiration) which are used to calculate the health of the mitochondria termed as the bioenergetic health index (BHI). For mitochondrial complex activity assays, the plasma membrane is selectively permeabilized without altering the mitochondrial membrane and the maximal activity of mitochondrial respiratory enzyme complexes (Complex I, Complex II and Complex IV) are determined in the presence of specific metabolic substrates. The development of all the mitochondrial tests included determination of linearity, precision, accuracy and measurement ranges of oxygen consumption rates using multiple leukocyte cell types and platelets. **Results:** Results and Conclusions: The results show that there is a significant loss of specific mitochondrial complexes (Complex I, Complex II or Complex IV) exist in mitochondrial disease patients. However, the nature and the extent of the defect are highly varied among the patients and also presented differently in different leukocyte populations. The bioenergetic health remained largely unaffected owing to the cellular compensatory mechanisms. **Conclusion:** This suggests that the integration of cellular bioenergetics and mitochondrial assays have the potential for the differential determination of mitochondrial defects and thereby the diagnosis of mitochondrial diseases.

## B-449

### Performance characteristics of new UIBC reagent on ARCHITECT cSystems

Y. Gore[1], T. Alligrant[1], F. Vespasiani[2], M. Valdambrini[2], M. La Motta[2], R. Lucini[2], J. De Giorgio[2], J. Ulloor[1], K. Sikder[1], M. Beischer[1]. *[1]Abbott Laboratories, Irving, TX, [2]Sentinel CH, Milan, Italy*

**OBJECTIVE:** To report the performance characteristics of the new Abbott liquid UIBC assay on the ARCHITECT *c*System instruments.

**RELEVANCE:** The new UIBC assay is a ready to use liquid reagent with an improved measuring interval, calibration interval and precision profile. Previous UIBC assay (LN 4P79) was lyophilized kit configuration.

**METHODOLOGY:** This UIBC assay (LN 04R29) uses the Ferene methodology, that is the same as the predicate assay (LN 04P79). Serum or plasma sample is added to the cuvette followed by reagent 1 containing a known concentration of iron to saturate the free binding sites on transferrin. Later, reagent 2 containing *3-(2-pyridyl)-5,6-bis-[2-(5-furylsulfonic acid)]-1,2,4-triazine* (Ferene-S) and a reducing agent is added to the reaction mixture. Residual iron after transferrin saturation, in a reduced, ferrous state, forms a stable complex with Ferene-S. The color intensity of this complex, measurable at 604 nm, is directly proportional to the unbound excess iron-binding capacity.

**VALIDATION:** The table below displays the critical performance characteristics of the new UIBC assay (LN 04R29).

**Table 1.** Critical performance characteristics of the new UIBC assay (LN 04R29).

| **Table 1.** Critical performance characteristics of the new UIBC assay (LN 04R29). | | | | |
|---|---|---|---|---|
| **Characteristic** | **LN 04R29** | | | |
| Sample Type | Serum and plasma | | | |
| Limit of Quantitation (µg/dL) | ≤ 25 | | | |
| Linearity and Measuring Interval (µg/dL) | 25 - 500 | | | |
| **Interferents:** | **[Interferent]** | **[UIBC] (µg/dL)** | **Difference (µg/dL)** | **% Diff.** |
| Hemoglobin | 62 mg/dL | 168 | -13.67 | -8.1 |
| | 125 mg/dL | 323 | -30.34 | -9.4 |
| Intralipid | 1000 mg/dL | 142 | -9.7 | -6.78 |
| | 1000 mg/dL | 295 | -4.73 | -1.6 |
| Bilirubin (conjugated) | 59 mg/dL | 141 | -10.6 | -7.5 |
| | 62 mg/dL | 298 | -11.8 | -4.0 |
| Bilirubin (unconjugated) | 53 mg/dL | 156 | -2.8 | -1.8 |
| | 56 mg/dL | 284 | -7.0 | -2.5 |
| Triglycerides | 901 mg/dL | 147 | -4.09 | -2.8 |
| | 1157 mg/dL | 269 | -18.05 | -6.7 |
| Total Protein | 13.2 g/dL | 152 | 7.9 | 5.2 |
| | 13.4 g/dL | 252 | 25.2 | 10.0 |
| Rheumatoid Factor | 100 IU/mL | 145 | 3.5 | 2.4 |
| | 100 IU/mL | 300 | -0.7 | -0.2 |
| Precision | ≤ 7% or ≤ 10µg/dL | | | |
| Calibration Interval (hours) | 168 | | | |
| On-Board Stability (days) | 28 | | | |
| Method Comparison | **LN 04R29 vs. LN 4P79** | | | |
| | N | 109 | | |
| | R | 0.995 | | |
| | Equation | Y = 8.03 + 0.9736X | | |
| | Range (µg/dL) | 31.5 to 458.8 | | |

**CONCLUSIONS:** The new UIBC assay (LN 04R29) demonstrates improved measuring range, calibration interval and precision profile when compared to the predicate method (LN 4P79) and is packaged in a ready to use assay configuration.

## B-450

### Evaluation of endogenous amino acids as preanalytical controls for blood samples

J. Hill, R. Magesh, R. Taylor, J. Hill. *Spot On Sciences, Austin, TX*

Background: Comparison of analyte concentrations between blood sample types including wet and dried plasma/serum and capillary and venous whole blood is difficult. A pre-analytical, endogenous standard would allow normalization between blood sample types to control for sample quality and volume and would allow more precise quantitation and quality analysis. The objective of this study was to determine the feasibility for use of amino acid (AA) levels as a suitable pre-analytical standard (PS) for dried and wet blood samples.

Methods: The free AA concentrations in wet and dried plasma and whole blood for 12 AA was measured for nine healthy donors (ages 29 to 60) by LC-MS/MS. Fasting (early morning) and fed (one hour post lunch) AA levels were determined for three donors on three separate days, using whole blood collected with HemaSpot™-HF devices by finger stick. AA stability over time (1, 7, 30, 60 and 90 days) and temperature (-20, 22, 37 and 45 ° C) was determined for dried whole blood from three separate donors.

Results: Levels of five AA (Val, Thr, Ile, Leu, Phe) showed strong correlation (<11% CV) between nine donors for four sample types: wet and dried plasma, and wet and dried whole blood. Minimal differences in AA levels were observed between fasting and fed state. Levels of Phe, Ile, Pro, Val, Leu, Tyr and Trp were stable (<10% loss) as dried blood up to 90 days at temperatures of -20 to 45 ° C, while Gly, Ser and Thr were not stable over time.

Conclusions: Several AA including Val, Ile, Leu and Phe show promise for use as a pre-analytical standard for dried and wet plasma and whole blood samples.

# AUTHOR INDEX TO ABSTRACTS OF SCIENTIFIC POSTERS, AACC MEETING, 2017

(Numbers refer to Poster Numbers; see pages S2 to SXXX)

## C

## D

## E



## F



## G

## H

## I



## J



## K

## L



## M

## N

## O



## P

## Q



## R



## S

## T

## U



## V



## W

## X



## Y



## Z

# KEYWORD INDEX TO ABSTRACTS OF SCIENTIFIC POSTERS, AACC MEETING, 2017

(Numbers refer to Poster Numbers; see pages S2 to SXXX)



## A



## B

## C

## D



## E

## F



## G



## H

## I



## J



## K



## L

## M



## N

## O



## P

## Q



## R



## S

## T



## U



## V



## W

## X



## Y



## Z