SCIENTIFIC POSTER SESSION ABSTRACTS

2014 AACC Annual Meeting & Clinical Lab Expo
Chicago, IL

Below are the abstract topics and posters schedule times.

TUESDAY, JULY 29, POSTER SESSIONS

9:30am – 5:00pm

WEDNESDAY, JULY 30, 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'. These abstracts represent the supplement to the October issue of *Clinical Chemistry*.

Tuesday, July 29, 2014

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

Cancer/Tumor Markers

A-001

The expression of post-translational modification of Alpha-1-antichymotrypsin in the plasma of colorectal cancer

H. Lin[1], C. Liao[2], J. Lin[3], K. Lee[3], Y. Chen[3], H. Ning[4], C. Lin[3]. *[1]Chang Gung Memorial Hospital and School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [2]Proteomics Research Center, National Yang-Ming University, Taipei, Taiwan, [3]School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [4]Chang Gung Memorial Hospital, Tao-Yuan, Taiwan*

Colorectal cancer is the third major cause of cancer related death in the world according to the report of cancer statistics in 2013. At present, some screening biomarkers are applied for the detection of colorectal cancer but most of them do not have good specificity and sensitivity. Carcinoembryonic antigen (CEA) is the most use of tumor markers for colorectal cancer, but the specificity and sensitivity is poor. In this study, we used proteomic approaches to investigate the expression of post-translational modification (PTM) of alpha-1 antichymotrypsin (ACT) in the plasma of colorectal cancer. We used immunoprecipitation, western blot and nano-LC/MS/MS to analyze the plasma samples from normal and cancer groups. Then we compared some types of PTM between those samples in order to find out the useful PTM sites in ACT to be a diagnostic tool. In the result of immunoprecipitation, we identified the accurate site of ACT in the gel electrophoresis. The following result of western blot showed that there was no significance between the normal group and the early stage of colorectal caner group (p=0.010). However, it was significant between the normal and the late stage group (p<0.001). From the result of mass analysis, we identified four types of PTM in ACT, such as Hydroxylation (Asn-323), Methylation-2 (Glu-334), 4-Hydroxy-2-nonenal (Arg-298) and N-glucuronylation (Ser-415). The expression level of Hydroxylation was increased by 2-fold in colorectal samples when compared with normal samples (p<0.05). The sensitivity of that was 88.89% and the specificity was 77.78% (AUC=0.840). Our results suggest that using the expression level of PTMs in ACT would be applicable as biomarkers for the early detection of colorectal cancer.

A-002

Tumor Marker, Molecular, and Imaging Test Monitoring of a Non-Toxic Adjuvant Integrative Nutritional Therapy Option for Stage IV Brain, Lung, Prostate, and Breast Cancer Patients When Traditional Therapy Options Have Been Exhausted: Palladium Lipoic Acid Complex, Coenzyme Q10, and Vitamin D Impacting the Mitochondrial Reactive Oxygen Species (ROS) Production and Apoptosis

E. J. Neren. *Edward J. Neren, Biomedical Consultant, Suffern, NY*

Background: Metal compounds (Platinum, etc.) have been used as cancer therapies for years; however, patient toxicity usually results. Dr. Merrill Garnett synthesizing organo-metallic compounds (1960-1990) encapsulated the metal palladium in alpha lipoic acid (non-toxic and successful in treating mice with Ehrlich carcinoma). Cat/dog tumors were also successfully treated. Rudy Falk, MD (1992 University of Toronto) determined safety, improvement, and many remissions in gravely advanced cancer cases. Since then, 200+ U.S. physicians have used the palladium/lipoic acid complex (PdLAC), Coenzyme Q10, and Vitamin D as an adjuvant integrative nutritional therapy for late stage cancer patients.

Objective: To provide stage IV cancer patients/physicians with a documented nutritional therapy option that justifies physician calls to other physicians with clinical experience, regarding their latest clinical data and determine if the therapy monitored with tumor markers, molecular, and imaging testing is appropriate for the given patient.

Methods: After determining patient levels (tumor markers, Coenzyme Q10, and Vitamin D) PdLAC liquid/coenzyme Q10/Vitamin D is physician monitored and taken as nutritionals. PDLAC is water and fat soluble and impacts the mitochondrial ROS of both cancer and normal cells. Positive clinical response and improved quality of life results is expected within three months of intake. Eight to twelve PdLAC teaspoons is taken in juice 4 times a day (based on patient body weight - 1 teaspoon for each 30 pounds). This therapy seeks balance between therapy, nutrition, detoxification, and energy enhancement. Patient progress is monitored with traditional clinical chemistries, tumor markers, Coenzyme Q10, Vitamin D testing and imaging.

Mechanism: The PdLAC enters normal and cancer cells and the mitochondrial outer membrane by the voltage dependent anion channel, then through the inner membrane by the Complex 1. The oxidative phosphorylation (OXPHOS) channel produces low levels of ATP in the cancer cell. PdLAC (acting as an electrical shunt) in normal cells would ordinarily give off electrons to the OXPHOS producing more ATP. In the cancer cell (damaged OXPHOS) it donates electrons producing increased reactive oxygen species (ROS). The excessive ROS builds up between the outer and inner mitochondrial membranes. When the outer membrane ruptures, the ROS, Cytochrome C, and the Procapases 2, 3, and 9 enter the anaerobic cytoplasm of the cancer cell and death occurs.

Results: James Forsythe, MD, HMD conducted out-come based stage IV studies (500+ patents 2004-2012) He found improvement in quality of life issues directly proportional to improvement to overall response rate and found stable disease can be tolerated and transformed into chronic livable condition.

Conclusion:This clinical and scientific documentation/data, from several public/professional sources, provides a non-toxic adjuvant integrative nutritional therapy option for advanced diseased cancer patients/physicians, when traditional therapy options have been exhausted and non-traditional therapy options are under consideration. Physician calls to other physicians with PdLAC, Coenzyme Q10, and Vitamin D clinical experience, can determine if this therapy and monitoring of patient progress is appropriate for a given end stage patient. It is not meant to circumvent physician patient monitoring, good medical practice, medical ethics, and/or negatively impact the physician's license.

A-004

Epidermal Growth Factor Receptor (EGFR) gene mutations frequency in Brazilian lung adenocarcinoma samples by pyrosequencing.

V. D. T. Niewiadonski, P. Y. Nishimura, O. Fernandes, N. Gaburo Jr.. *DASA, Sao Paulo, Brazil*

Background: Lung cancer is the most prevalent life-threatening cancer worldwide with more than 80% being non-small cell lung cancer (NSCLC). Detection of mutations of EGFR gene is critical for predicting the response to therapy with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, in patients with NSCLC. Patients that are EGFR mutants have constitutive TK activity and, therefore, a greater sensitivity to anti-EGFR inhibition.

Objective: To describe the EGFR mutations frequency found in lung adenocarcinoma samples, using pyrosequencing method.

Method: Thirty samples of lung adenocarcinoma were analyzed from January 2013 to December 2013. The test was performed on formalin-fixed, paraffin-embedded tumor specimen, after the selection of the specimen region to be analyzed by a pathologist. The DNA was extracted using the Qiaamp FFPE Tissue kit (Qiagen, Hiden, Germany). Concentration of DNA sample was measured spectrophotometrically using a NanoDrop spectrophotometer (NanoDropTechnologies, Wilmington). Codons 719, 768, 790, 858, 861 and exon 19 were amplified by PCR using the EGFR Pyro kit (Qiagen, Hiden, Germany). Successful and specific amplification of the region of interest was verified by visualizing the PCR product on capillary electrophoresis using Qiaxel DNA Screening Kit (Qiagen, Hiden, Germany). Preparation of single-stranded DNA was done using PyroMark Q24 vacuum workstation (Qiagen, Hiden, Germany) according to the manufacturer instructions. The pyrosequencing reaction was analyzed on the Pyro Mark Q24 (Qiagen, Hiden, Germany)

Results: The frequency of EGFR mutations found is presented on Table 1. All mutations together represent only 27% of the samples.

Conclusion: The results are consistent with previous studies and reports. The single-point mutation L858R (CTG> CGG) on exon 21 and the frame deletions on exon 19 represents the majority mutations found in Brazilian lung adenocarcinoma samples, although most samples showed no mutation at the target regions.

Table 1. Frequency of EGFR mutations found in lung adenocarcinoma samples.

Results	Frequency
Wild type	73%
2235del15 (exon 19)	3,3%
2236del15 (exon 19)	3,3%
2237_2255>T (exon 19)	3,3%
2239_2248>C (exon 19)	3,3%
CTG>CAG (L861Q)	3,3%
CTG>CGG (L858R)	10%

A-005

Ovascreen Lateral Flow Device for Simultaneous Detection of CA125 and WFDC2 (HE4) in Ovarian Cancer

Y. Lebedin. *Xemabio LLC, Gainesville, FL*

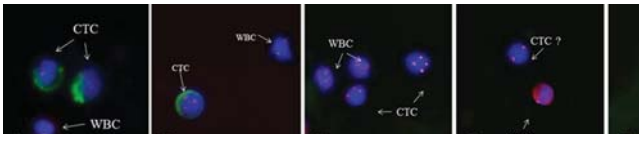
A lateral flow device named Ovascreen has been developed to simultaneously detect two tumor markers (CA125 and HE4) in ovarian cancer. Combined determination of serum HE4 (WFDC2 protein) and CA125 is demonstrated to increase the sensitivity and accuracy of early diagnosis and monitoring of ovarian carcinoma. However, a convenient and portable device for such a dual detection is not commercially available. In this study, a panel of monoclonal antibodies of HE4 (WFDC2) was obtained by immunization of human tumor-derived antigens at Xema Company. The HE4 antibodies were classified into 3 epitope groups for antibody matching based on their cross-inhibition and bindings to recombinant and natural antigens. On the other hand, the antibodies of MUC16 (antigen CA125) were also developed and the matched pairs (X306 and X325) were obtained by Xema. The selected pairs of both CA125 and HE4 were coated with colloidal gold particles, and combined onto a lateral flow device. Their assay performance was evaluated based their stability and their signal to background ratios. The best pair of CA125 and HE4 antibody-gold conjugates for lateral flow device were determined. The cut-off values for CA125 and HE4 on the device is set as 35 U/ml and 150 pmol/l, respectively. The resulting Ovascreen cassette device is validated with clinical samples of whole blood, serum, and plasma. 74 samples from primarily diagnosed but untreated serious adenocarcinoma of the ovary were evaluated. Ovascreen device showed excellent sensitivity and accuracy during the clinical evaluations. The combined positive results on Ovascreen device accounted for more than 50% cases in 69 patients which corresponding to 93% clinical sensitivity. 66 out of 69 Ovascreen positive results were further confirmed by commercial ELISA kits of CA125 (Xema Co.Ltd.) and HE4 (Fujirebio Inc.). The Ovascreen lateral flow device is suggested for use in POC based diagnosis and monitoring of ovarian cancer.

A-006

Characterizations of Circulating Tumor Cells Identified by Combination of Fluorescence in situ Hybridization and Immunostaining CK, CD45 in Pancreatic Cancer

Y. Zhang, N. Ning, Q. Chen, F. Wang, W. Cui. *Peking Union Medical College Hospital Chinese Academy of Medical Sciences, Beijing, China*

Background: To improve the identification for CTCs with weak or negative CK and diploid CTCs in pancreatic cancer, we combined the immune-staining of CK, CD45, DAPI and fluorescence *in situ* hybridization with the centromere of chromosome 8 (CEP8) probe method. **Methods**: CTCs in 3.75 mL of blood were negatively enriched with Epithelial Cell Adhesion Molecule independent magnetic beads coated with anti-CD45 antibodies and identified by combining CK, CD45, DAPI and CEP8 in 61 cases including 22 pancreatic cancers, 3 borderline pancreatic solid pseudopapillary tumors, 6 pancreatic benign tumors, and 30 healthy individuals. **Results:** Enriched cells could be classified into 5 patterns (Fig. A-E): CK+CD45-DAPI+CEP8=2 (2 hybridization signals), CK+CD45-DAPI+CEP8>2 (>2 hybridization signals), CK-CD45-DAPI+CEP8>2, CK-CD45-DAPI+CEP8=2, and CK+/-CD45+DAPI+CEP8=2or>2. Among 22 pancreatic cancers, the patterns of CK+CD45-DAPI+CEP8=2 and CK+CD45-DAPI+CEP8>2 were identified in 2 cases, the number of CTCs was 6, 12 cells/3.75mL and 2, 44 cells/3.75mL, respectively. The pattern of CK-CD45-CEP8>2 was identified in 16 cases with the range of 1-14 cells/3.75mL and the median of 3 cells/3.75mL. The pattern of CK-CD45-CEP8=2 and CK+/-CD45+CEP8=2 or >2 were detected in both pancreatic cancers and other control cases. Dynamically monitoring CTCs and platelet count prior to and after surgery in 7 pancreatic patients revealed that they were consistent both decreased or insignificantly decreased 3 days after surgery, whereas the count significantly increased 10 days after surgery. **Conclusion:** The patterns of CK+CD45-DAPI+CEP8 =2, CK+CD45-DAPI+CEP8>2 and CK-CD45-DAPI+CEP8>2 were considered as CTCs, and the patterns CK-CD45-CEP8=2 and CK+/-CD45+DAPI+CEP8=2 or >2 were considered as indeterminate cells. Postoperative increase in the platelet count might contribute to CTCs dissemination, and certain correlation might exist between those two events.



A-007

Pca3 gene expression as biomarker to differential diagnosis of benign hyperplasia and prostatic cancer

F. F. Coelho[1], O. Romano[2], C. Corradi[1], L. Nogueira[1], W. Cabral[1], E. Mateo[3], K. Borges[1]. *[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Hermes Pardini Institute, Vespasiano, Brazil, [3]Hermes Pardini Institute, Belo Horizonte, Brazil*

Background: The Prostate Cancer (Pca) is the second most common type of cancer in men around the world. Because of the increasing numbers of cases, it is extremely important the development of a noninvasive test with high specificity and sensitivity to diagnosis cancer and other prostatic alterations. Studies showed that the gene 3 of Prostate Cancer (PCA3) presents high levels of expression in tumor tissue. High levels of PCA3 gene expression can be associated with an increased probability of positive biopsy and has arisen as a molecular marker in the diagnosis of PCa.

Objective: We proposed to evaluate the the expression of PCA3 gene in urine from patients with benign hyperplasia (BPH) or prostatic cancer.

Methods: The study included 33 men attended at the Clinical Hospital from Federal University of Minas Gerais (HC-UFMG) to performer a prostatic biopsy, being 13 patients with Pca, 8 with BPH and 12 patients with no alterations (controls). It was collected 30 mL of patient's urine after prostatic massage, which was immediately centrifuged. The pellet was added to RNA later® and stored for up to 24 hours, until the extraction of RNA. The samples were quantified in a spectrophotometer and submitted to treatment with DNase. After, this sample was quantified in a one-step RT-PCR for PCA3 gene and PSA/ ACTB genes for control or reaction normalization.

Results: The PCA3 gene expression was detected in 10 patients with PCa, 3 with BPH and 2 controls. For the remaining patients was not detected any gene expression. The test presented 77% of sensitivity for PCa screening and 38% for BPH. The specificity was 83% for both.

Conclusion: The PCA3 screening showed median sensitivity for PCa diagnosis; subsequently prostate biopsy is still considered the best standard procedure for detect prostatic alterations. Some patients with PCa no presented any expression of the PCA3 gene, which can be explained by the large number of interfering, as well as the prostate massage, the use of drugs and the high RNA degradation's rate in urine samples. It is required the standardization of these procedures and to analyze a larger number of samples in order to evaluate the importance of PCA3 gene expression in differential prostatic alterations and its use in the clinical practice.

A-010

Circulating tumor markers of benign and malignant disorders of breast in Libya.

J. R. Peela[1], S. Shakila[1], S. J. Dhoipode[2], A. R. Said[1], H. Beloch[2], S. Nang[2], L. T. Peela[3], A. M. Jarari[4], S. O. Alsaeoti[5], H. El Awamy[4], F. Elshaari[4], N. M. Jarari[6], M. J. Kadeer[4]. *[1]Department of Biochemistry, Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [2]Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [3]Great Eastern Medical School, Srikakulam, India, [4]Department of Biochemistry, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [5]Department of Surgery, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [6]Department of Pharmacology, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya*

BACKGROUND Breast cancer is most common malignant disorder in Libyan females. Breast cancer is the most dreadful disease in terms of quality of life, though heart disease is a more common cause of mortality here. The present study is a case control study of tumour markers CA 125, CA 15-3 and Carcino Embryonic Antigen (CEA) in serum of patients suffering from benign and malignant breast disorders in Libya.

Materials and methods: There are 12 cases of carcinoma of breast patients with age group ranging from 30 - 55 years of age, 10 cases of benign breast disorders i.e., Fibroadenosis with age group ranging from 18 to 50 years retrieved from department of surgery, 7th October Hospital, Benghazi, Libya and there are 12 cases of age matched controls free from both malignant and benign disorders of breast were included in this study. Venous sampling was done to the patients, all the patients and controls were measured serum CA125, CA15-3and CEA levels by authenticated methods by using Cobas E 411 analyser. Statistical analysis was done by using SPSS software by using Mann-Whitney and Wilcoxon tests.

Results: There is no significant rise of CA 125 in benign (p=0814) and malignant

(p= 0.676) disorders of breast when compared to controls. CA 15-3 was significantly high in patients suffering from breast cancer (p= 0.019) when compared to controls and also very significantly high when (p= 0.003) compared with patients with benign breast disorders. The level of CA 15-3 was not significantly high in patients with benign breast disorders (p=0.186) when compared to controls. The level of CEA was significantly high in patients of breast cancer when compared to patients of benign breast (p=0.009) disorders and (p=0.017) controls. The level of CEA is not significantly in high patients of benign breast disorders (p=0.634) when compared to controls.

Conclusion: The present study showing high levels CA 15-3 and CEA only in malignant disorders of breast may be useful as diagnostic and prognostic markers. CA 125 has not shown any significance in this study proving that it is not an important marker in malignant breast disorders.

A-011

Diagnostic value of Insulin-like growth factor-1, IGF-binding protein-3, Chromogranin-A in differentiation between benign prostatic hyperplasia and prostate cancer patients

S. A. K. Saleh. *Umm AlQura University, Makkah, Saudi Arabia*

Backgrounds: Prostate cancer (PCa) ranked the sixth most common cancer among males in Arab world. Elevated serum Insulin-like growth factor-1 (IGF-1) level appeared to be a possible risk factor for the development of PCa. Chromogranin A (CgA) is the most employed serum marker to detect neuroendocrine features. Many studies reported ***contradictory*** findings of association between IGF-binding protein-3 (IGFBP-3) and the risk of PCa. Although, the best and most sensitive screening test available for PCa is prostate specific antigen (PSA) there is a large overlap between PCa and benign prostatic hyperplasia (BPH) in patients with moderately increased PSA levels. **Objective:** This study aimed to explore the validity of IGF-1, IGFBP-3, CgA and thereof ratios with PSA in differentiation between BPH and PCa patients in Saudi Arabia. **Patients and Methods:** The study included 62 patients with PCa, 70 BPH patients and 56 healthy male subjects of matched age. Full history and clinical data were recorded for all subjects. PCa patients were undergo digital rectal examination (DRE), trans-rectal ultrasonography (TRUS) guided biopsy of the prostate, computed tomography (CT) scan of the pelvis, bone scan and histopathological examination, accordingly PCa stages and metastatic disease were confirmed. PCa patients were classified into localized (n = 48) and metastatic PCa (n = 14). Serum levels of IGF-1, IGFBP-3, CgA, PSA and free/total PSA were measured as well as possible association between parameters were assessed. The validity (sensitivity and specificity) were evaluated by ROC curve analysis. **Results**: Serum PSA levels were significantly higher in PCa than BPH and control groups ($p<0.05$) and attained sensitivity of 87% at 85% specificity with an accuracy of 86%. Although serum IGF-1, IGFBP-3 and CgA levels did not differentiate among PCa, BPH and control groups ($p>0.05$), IGF-1/PSA as well as IGFP-3/PSA ratios were found to differentiate significantly among metastatic, localized PCa, BPH and control groups ($p<0.05$). Combined use of IGF-1/PSA and IGFP-3/PSA ratios provide an overall value of sensitivity, specificity and diagnostic accuracy (92, 84 and 88% respectively) in the diagnosis of PCa. The addition of f/t PSA ratio to this combination seems to improve the overall value of sensitivity, specificity and diagnostic accuracy (94, 85 and 90% respectively). **Conclusion:** Although there is no association of PCa risk with serum IGF-I and IGFBP-3 levels; combination of these growth factors with PSA and f/tPSA may be useful and can improve the overall value of sensitivity, specificity and diagnostic accuracy of patients with PCa. Further studies are needed to elucidate the prognostic and predictive value of these growth factors as well as their association with PCa risk.

A-012

Stability of Serum Human Epididymis Protein 4 (HE4)

R. Radwan[1], C. Fermer[2], M. Lundin[2], S. Raju[1], S. Jones[1], C. Hall[2], D. Dickson[1], T. Kettlety[1], Z. Li[1]. *[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]Fujirebio Diagnostics AB, Gothenburg, Sweden*

Background: HE4 is encoded as a 13 kDa protein and belongs to the family of whey acidic four-disulfide core (WFDC) proteins (Israeli O et al, 2005; Bouchard D et al, 2006; Bingle L et al, 2002). HE4 was first identified in the epithelium of epididymis (Kirchhoff C et al, 1991; 1998). Secreted HE4 has become an important biomarker for the detection of ovarian cancer, a common cause of cancer-related death in women, with 67% sensitivity and 96% specificity (Hellstrom I et al, 2003). Furthermore, the combination of HE4 and CA 125 has been demonstrated to be a more accurate predictor of ovarian cancer with a sensitivity of 76% and a specificity of 95% (Moore RM et al, 2008). The purpose of this study was to determine the stability of the serum analyte HE4 under frozen conditions.

Methods: This is a retrospective study utilizing banked serum samples collected in an ovarian cancer clinical trial (NCT00315692). The patients were from women greater than or equal to 18 years of age, who selected to undergo laparotomy or laparoscopy based on finding of a pelvic mass. The samples were collected in the US under an IRB approved protocol. Samples were collected in red top tubes or serum separator tubes (SST); and undergone no more than five (5) Freeze/Thaw cycles prior to the date for stability testing. Samples were stored in a ≤-70°C freezer since the time of collection. Sample testing was performed using the manual HE4 EIA, a sandwich immunoassay. The initial HE4 EIA testing was carried out in July to August of 2007 and the results were retrieved from the clinical trial dataset. The stability testing with the HE4 EIA was carried out in June of 2013. A total of 100 available samples from this trial were tested. Among them, 84 from women with benign disease, 11 from women with border line/low malignant potential, 4 from women with ovarian cancer, and 1 from a woman with other gynecological cancer.

Results: The linear correlation coefficient between the two measurements was 0.986 (95% CI: 0.979 to 0.991). Weighted Deming regression gave an intercept of 2.76 (95% CI: -0.54 to 6.07); and a slope of 1.033 (95% CI: 0.970 to 1.095). The intercept and slope are not significantly different from 0 and 1 respectively. Passing-Bablok regression produced a similar intercept of 3.06 (95% CI: -1.26 to 6.35) and slope of 1.031 (95% CI: 0.965 to 1.108).

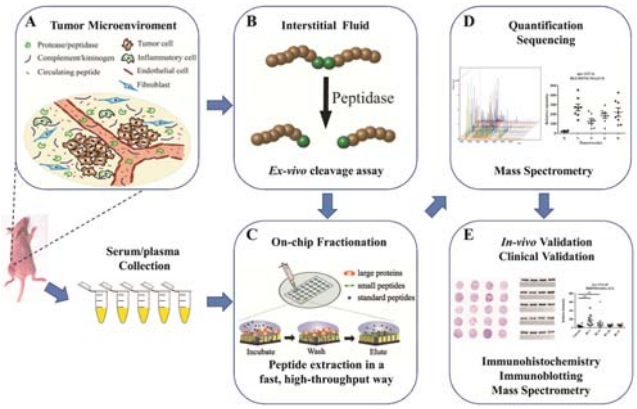
Conclusion: The human serum HE4 was demonstrated to be stable for at least five (5) years for the serum samples stored at ≤ -70°C and underwent no more than five (5) Freeze/Thaw cycles.

A-013

Circulating proteolytic products of tumor-resident enzyme as potential biomarkers for early detection of breast cancer

T. Hu. *Houston Methodist Research Institute, Houston, TX*

In theory, any or all of tumor-secreted proteins can serve as cancer biomarkers. However, the reality is challenging to monitor because of the large degree of fluctuation in abundance and localization of these tumor-secreted proteins, especially in the early stage of tumor development and/or metastasis. As such, it seems feasible that we might take advantage of the fact that secreted proteases/peptidases in the tumor microenvironment generate proteolytic products, also referred to as "circulating peptides", which are detectable in bloodstream and provide ample information about the body, "coded" in the patterns and quantity of these peptides. Herein we clearly link the catalytic activity of Carboxypeptidase N (CPN) to its proteolytic products during breast tumor progression in mouse model and clinical samples. CPN plays important roles in regulating vasoactive peptide hormones, growth factors, and cytokines by specifically cleaving their C-terminal basic residues. Circulating fragment profiling, by an approach combining nanopore fractionation and mass spectrometry, revealed the nature and extent of cleavage by CPN. These results correlated with the level of CPN-catalyzed peptides in blood taken from the tumor-bearing mice, healthy women and breast cancer patients. We showed that generation of C3f_R1310-L1319 specifically correlated with the CPN expression level. In both mouse and clinical patient samples, the amount of CPN was increased in tumor tissues compared to that seen in normal breast tissue, while its counterpart in blood remained constant. The amount of 6 CPN-catalyzed peptides predominantly increased in sera taken from both the mice at 2 weeks after orthotropic implantation and the patients' plasma as early as the first pathologic stage of breast cancer. In conclusion, the circulating level of the selected 6 CPN-catalyzed proteolytic products reflects the extent of this enzyme's activity in tumors, and our results clearly indicate their strong potential as biomarkers for non-invasive early diagnosis of breast cancer.



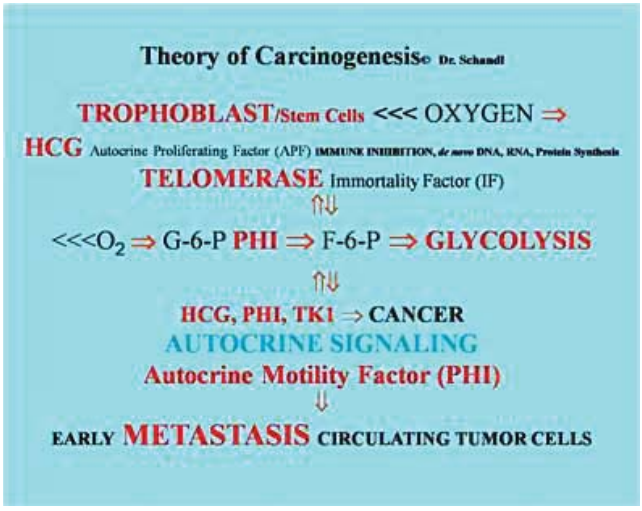
A-016

Clinical Utilization of the Cancer Profile and Longevity Profile and the Role of Thymidine Kinase (TK1) in Carcinogenesis

E. K. Schandl. *American Metabolic Laboratories, Hollywood, FL*

Cancer is usually detected by visualization. Clinical biochemical parameters, e.g. Cancer Profile (CA Profile) and Longevity Profile (expanded CA Profile), are capable to signal a developing malignancy much earlier. The methodologies used in this study are: Chemiluminescence (HCG, CEA, TSH, DHEA-S), IRMA (HCG) and enzyme kinetics (PHI, GGTP). *HCG*, the pregnancy/malignancy hormone is the *autocrine proliferative factor* (APF). It is responsible for *de novo* DNA, RNA, protein synthesis in pregnancy and may as well be so in malignancies. *PHI* is a neurokine. Amongst its many other functions it is the *autocrine motility factor* (AMF). PHI regulates anaerobic sugar metabolism by facilitating the Warburg effect. This enzyme is responsible for cytokines and as such, may be the facilitator of micrometastesis and circulating tumor cells by attaching to their membrane receptor (neurokine) and "jockeying" cells to a distant site. Thymidine Kinase (*TK1*) is a dynamic measure of tumor growth. It phophorilates deoxythymidine to deoxythymidine monophosphate, a prerequisite for DNA replication. All three factors, HCG, PHI, TK1, must be present for the development and sustenance of malignancies. Our data confirms the presence of PHI and HCG in most, if not all cancers. TK1 was found in cancer biopsies, but not in normal tissues. Reports showed the presence of the enzyme in embryonic, wound healing, and tumor cells.

Clinical laboratory results confirmed positive CA Profile markers in approximately 90% of hundreds of pathologically established malignancies. Breast cancer yielded 92% positives, lung 97%, and colon 93%. The clinical laboratory adaptation of the proposed profile may warn of developing, undiagnosed cases, and track progress monitoring. The Longevity Profile is a conglomerate of laboratory tests for cancer, coronary risk factors, sex hormones, bone health, adrenal stress, and generally an overall examination of most organs. It is a biochemical full body scan without radiation.



A-017

WHAT ARE THE TUMOURS MARKETS THAT BEST IDENTIFY MALIGNANT PLEURAL EFFUSIONS?

J. D. Santotoribio, A. García de la Torre, C. Cañavate-Solano, F. Arce-Matute, S. Pérez-Ramos. *Puerto Real University Hospital, Cádiz, Spain*

Introduction Malignant pleural effusions (MPE) are a common clinical problem in patients with neoplastic disease. The aim of this study was to determine the accuracy of carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9) and cancer antigen 125 (CA 125) measurement in pleural fluid for diagnosis of MPE.

Materials and methods We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion (PE). We measured CEA, CA 15.3, CA 19.9 and CA 125 in pleural fluid by electrochemiluminescence immunoassay in MODULAR E-170 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the aetiology of PE: benign PE (BPE) and MPE. PE was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. The accuracy for diagnosis of MPE was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

Results We studied 152 patients with ages between 1 and 89 years old (median = 61.5 years old). Fifty-one patients were MPE (22 lung cancer, 9 breast cancer, 7 mesotheliomas, 5 lymphomas, 4 kidney cancer, 2 colon cancer and 2 ovarian cancer) and 101 were BPE (46 transudates, 42 parapneumonic, 5 tuberculous, 5 pulmonary thromboembolism, 2 quilotórax and 1 rheumatoid arthritis). No statistically significant differences were found between MPE and BPE patients according to CA 125 ($p>0.05$). AUC values was 0.815 ($p=0.0024$) for CA 15.3, 0.682 ($p=0,0064$) for CEA and 0.639 ($p=0,0359$) for CA 19.9. Optimal cut off value were 16.2 U/mL (61.5% sensitivity and 86.3% specificity), 2.3 ng/mL (57.7% sensitivity and 76.6% specificity) and 2.4 U/mL (64.3 sensitivity and 62.3% specificity) for CA 15.3, CEA and CA 19.9 respectively.

Conclusions CA 15.3 levels improved accuracy for diagnosis of MPE compared with CEA, CA 19.9 or CA 125. CA 15.3 showed high diagnosis efficacy to predict whether a pleural effusion is benign or malignant.

A-018

Relation of total antioxidant capacity and CA 125 in patients with epithelial ovarian carcinoma

L. Kurti[1], I. Osmani[1], A. Kelmendi[1], L. Zeneli[2]. *[1]University of Prishtina, Faculty of Medicine, Institute of Biochemistry, Prishtina, Kosovo, Republic of, [2]University Clinical Centre of Kosova, Institute of Clinical Biochemistry, Prishtina, Kosovo, Republic of*

Background: We undertook the present study to investigate the possible relation between total antioxidant capacity (TAC) and CA 125 values in serum samples of patients with epithelial ovarian carcinoma.

Methods: Serum total antioxidant capacity was measured using trolox equivalent antioxidant capacity (TEAC) assay in 20 serum samples with elevated values of CA 125 and 20 age- and sex-matched healthy subjects (controls) with CA 125 values within reference ranges respectively.

Results: The measured level TAC was higher in serum samples from patients with elevated CA 125. TAC correllated significantly with CA 125 ($r: 0.516$, $P<0.05$) when the values of this tumor marker were pathological.

Conclusion: This study suggests that increased serum TAC of the patients with altered levels of CA 125 may be due to the response of increased reactive oxygen species and can be considered as a sign of oxidative stress of these patients. Therefore, the evaluation of serum antioxidant capacity in patients with epithelial ovarian carcinoma could contribute in diagnosis of these patients.

A-020

Experience with the use of the CellSearch system for enumeration of circulating tumor cells (CTCs) in Asian subjects

T. C. Aw, M. Goh, S. Swe. *Singapore Institute of Advanced Medicine, Singapore, Singapore*

Background: Circulating tumor cells (CTCs) are increasingly used as independent prognostic markers as well as predictors of response to anti-tumor treatment. Solid tumors are derived from the epithelium. Metastasis is initiated when tumor cells invade the blood stream. Normally absent in blood, these circulating epithelial-

derived cells can be captured using antibodies directed against the epithelial cell adhesion molecule (EpCAM). The CellSearch CTC system (Janssen Diagnostics) is the only FDA approved platform for CTC detection of metastatic cancers from the breast (since 2004), colon (2007), and prostate (2008). We acquired the CellSearch system recently and now describe our experience with its use in providing a clinical service for CTC analysis in the Asia-Pacific region.

Methods: The CellSearch system comprises an automated CellTracks AutoPrep instrument to capture and label the CTCs and a semi-automated immuno-fluorescent microscope (CellTracks Analyzer) for cell detection. Blood, collected in proprietary tubes, are stable for up to 96 hours prior to analysis. Buffer containing ferrofluid (nanoparticles with a magnetic core and an outer layer coated with anti-EpCAM antibodies) is added to the sample. Following immuno-magnetic capture and enrichment, CTCs are permeabilized before exposure to fluorescent antibodies directed against the cytoplasm (anti-CK-8,18,19), nucleus (DAPI) and leucocytes (anti-CD45). Thereafter the mixture is transferred into a plastic cartridge surrounded by a magnetic sheath. The CTCs are attracted to the surface of the cartridge by the magnet. The analyzer can accommodate up to 8 samples in each run of 2-3 hours. Each assay has a control sample comprising SK-BR3 breast cancer cell lines tagged with 2 different fluorescent labels one each for a population of low CTC count (approximately 40-50 cells) and another with high CTC count (approximately 1000 cells). Following a 30 min incubation, the samples are transferred singly to the Analyzer which scans the surface of the cartridge. Fluorescent objects are imaged and displayed in a gallery for classification. CTCs are defined as CK+,DAPI+,CD45- fluorescent objects of at least 4 microns and co-location of the cytoplasmic and nuclear images.

Results: Presumably healthy men (n=45: 27-85 years, mean age 55) had less than 3 CTCs (84% < 2 CTCs); on repeat testing CTCs declined to < 2. Healthy women (n=56: 25-79 years, mean age 48) had less than 2 CTCs (98%). We have served 102 patients with metastatic cancer (Breast: n=43, age 34-71; Prostate: n=29,age 50-79; Colon: n=30, age 28-80). We have also analyzed samples from other cancers (brain, nose, tongue, lung, stomach, kidney, ovary, endometrium, cervix). Inter-assay precision using the kit controls range from 10-27% for the low control and 3-13% for the high control (n=11 control lots). External quality assurance (EQA) comprises a gallery of 50 images sent out quarterly by the manufacturer. All users have attained a score of > 90%. We have become aware of a third party EQA program which we intend to subscribe.

Conclusion: CTC testing is gaining in utility. As oncologists gain more experience with using CTC, testing will become mainstream and impact the routine clinical laboratory.

A-023

New rapid urine test for the identification and quantitation of immunoglobulin free light chains (Bence Jones Proteins)

J. Campbell, J. Heaney, P. Patel, M. Goodall, M. Drayson. *University of Birmingham, Birmingham, United Kingdom*

Background. Monoclonal κ and λ immunoglobulin free light chains (FLC) in the urine are important biomarkers for the diagnosis and monitoring of a number of plasma cell dyscrasias including multiple myeloma. To date, laboratory FLC tests provide the only means of quantitating FLC and often have a slow turnaround time that prevents early diagnosis or prompt identification of changes in disease activity. Furthermore, the gold standards for identifying (immunofixation electrophoresis; IFE) and quantitating (densitometry) FLC in the urine have a number of limitations. IFE lacks analytical sensitivity (LOD >10-20 mg/L) and interpretation is often subjective. Densitometry has high inter-test variability that contributes to an inter-lab CV% of 50-95% in the UK National External Quality Assessment Service (NEQAS), and is poorly sensitive meaning that urines need to be concentrated before measurement, sometimes up to 150-fold. Further, clinical manifestations such as proteinuria may obscure monoclonal FLC bands and makes identification and quantitation of monoclonal protein bands inaccurate. Therefore, we have developed a rapid test (Seralite®) that identifies abnormal FLC levels in unconcentrated urine or blood in 10 minutes. Seralite® quantitates κ and λ FLC levels simultaneously using highly specific anti-κ and anti-λ FLC monoclonal antibodies. **Methods:** Seralite® validation was conducted by retrospective analysis of urine from patients presenting with multiple myeloma (n=100). All samples were also measured for FLC by electrophoresis immunofixation; densitometry on concentrated urines; and a recently validated new Luminex assay that incorporates the same mAbs as Seralite®. **Results:** Seralite® displayed excellent clinical concordance with Luminex. Analysis of IFE results revealed that Seralite® had no false negatives, and correlated excellently with densitometry. **Conclusion:** Seralite® detected all FLC in urine from 100 myeloma patients at diagnosis. Prospective use of Seralite® to diagnose and monitor plasma cell dyscrasias including multiple myeloma should now be investigated. The utility of Seralite® in the context of other FLC related disorders including AL amyloidosis should also be established.

A-024

Method Comparison to Quantify Free Light Chain Changes during Serial Monitoring of Multiple Myeloma Patients

O. Berlanga, L. Adie, H. Carr-Smith, S. Harding. *The Binding Site, Birmingham, United Kingdom*

Background: Serum free light chain (FLC) measurements utilising Freelite® immunoassays are an integral part of the international myeloma working group guidelines. The assays are available on both turbidimetric and nephelometric instruments. Here we compare responses in FLC measurements between instruments in serial samples from multiple myeloma (MM) patients.

Methods: Sequential sera from 6 MM patients (5 IgGκ, 1 IgGλ; total sample number=45; median (range) sample number: 8 (5-11); mean (range) follow-up: 289 (97-525) days)) treated with bortezomib were analysed retrospectively with Freelite (The Binding Site Group Ltd, UK) on a SPA_{PLUS}®, Cobas Integra 400® and Modular P® turbidimeters and the BNII® nephelometer. Percentage change in dFLC (involved-uninvolved FLC) relative to baseline was calculated for each instrument, and correlation and agreement assessed using Pearson's linear regression, the coefficient of determination (R^2) and Bland-Altman test. Responses were assigned for each sequential sample following IMWG criteria. Agreement between instruments for response assignment was assessed using weighted kappa analysis; values >0.81 indicate near perfect agreement.

Results: Median FLCκ, FLCλ and dFLC concentrations were not significantly different between instruments (Mann-Whitney test), except for slightly higher FLCλ measurements on the Modular P. Regression analysis between analysers displayed dFLC % change slopes between 0.95 and 1.01 and intercepts between -0.06 and 0.03, with $R^2 \geq 0.96$ for all comparisons (Table 1). Bland-Altman test revealed a bias between 0.2 and 2.7% between instruments, with 95% limits of agreement no greater than ±16%. Weighted kappa analysis for response assignment was ≥0.89 for all instrument comparisons (Table 1).

Conclusion: There is good correlation and agreement for FLC changes and response assignment by Freelite on different analysers, indicating that routine monitoring of the disease would not be affected by instrument selection. Small between instrument differences in the measured FLC levels suggest not changing analysers during monitoring patients is preferable.

Table 1. Performance of Freelite immunoassays for monitoring dFLC changes on different instruments

		Correlations and agreement for dFLC % change			
		SPA_{PLUS}	BNII	Integra	Modular P
Weighted kappa for response assignment	SPA_{PLUS}	-	y = 0.96x - 0.05 R^2 = 0.97 1.7% (-14.7 to 11.4)	y = 0.99x - 0.004 R^2 = 0.98 1.1% (-10.1 to 12.3)	y = 0.95x - 0.05 R^2 = 0.98 1.6% (-12.0 to 8.8)
	BNII	0.96	-	y = 1.01x + 0.03 R^2 = 0.96 2.7% (-11.0 to 16)	y = 0.97x - 0.02 R^2 = 0.97 0.2% (-11.5 to 11.9)
	Integra	0.89	0.93	-	y = 0.95x - 0.06 R^2 = 0.97 2.5% (-14.5 to 9.4)
	Modular P	0.92	0.96	0.91	-

Red cells: Pearson's equation with slope and intercept, coefficient of determination (R^2) and % bias (95% limits of agreement) by Bland-Altman test.
Blue cells: weighted kappa with quadratic weighting; values >0.81 indicate almost perfect agreement.

A-025

Differential diagnosis the property of ascites by a novel logistic regression model

C. Lin, W. Feng, L. Leilei, S. Yuanyuan, W. Yueping, S. Jianguo. *Hospital, Nantong, China*

Background: Differential diagnosis of malignant from benign ascites has a great clinical significance for the treatment and prognosis of the disease. However, complete discrimination between malignant ascites and nonmalignant ascites has not yet been substantially improved in recent years. Herein, we established a logistic regression model on the basis of multiple ascitic indices in differential diagnosis both benign and malignant ascites. Moreover, the further assessment of its diagnostic value is presented.

Methods: A total of 103 consecutive ascitic patients were enrolled in this study. Nine biomarkers including telomerase, DNA ploidy, adenosine deaminase (ADA), lactate dehydrogenase (LDH), CEA, CA125, CA19-9, Golgi Protein 73 (GP73) and serum-ascites albumin gradient (SAAG) were measured. The data were further analyzed

by using receiver operating characteristic (ROC) curve, univariate and multivariate logistic regression to evaluate the value of differential diagnosis the property of ascites.

Results: The median values of the ascitic telomerase, LDH, CEA, CA125, CA19-9, GP73 in the malignant ascites group were 0.314, 235 U/L, 20.64 ng/mL, 306 U/mL, 45.21 U/mL and 185.1 μg/L, as they were compared with those of benign group (0.046, 109 U/L, 4.84 ng/mL, 62.13 U/mL, 19.5 U/mL, 69.8 μg/L), respectively, P<0.001. However, the concentration of SAAG in the malignant ascites group were obviously lower than that of benign ascites group (median, 7.09 g/L *vs* 19.20 g/L), P<0.001, Moreover, there was no significant difference in the concentration of ADA between the two groups (median, 8.5 U/L *vs* 8.0 U/L), P>0.05. In addition, DNA aneuploidy rate in the malignant group was significantly higher than that in the benign group (76.0% *vs* 9.4%), P<0.001. By using ROC curve, univariate and multivariate logistic regression analysis, ascitic telomerase (X_1), CEA (X_2), GP73 (X_3), SAAG (X_4) were rolled into logistic regression model:$P=1/[1+e^{(-6.320+2.351X1+2.338X2+4.246X3+3.459X4)}]$, (P: probability predictive value, e: natural logarithm). The area under the curve of the P value of the predictive probability was 0.986, the cut-off point was 0.469, the sensitivity was 96% and the specificity was 98.1%. When P≥0.469, it was predictively diagnosed as malignant ascites; vice versa, when P<0.469, it was predictively diagnosed as benign ascites.

Conclusion: Our study highlights that the novel logistic regression model is an attractive strategy in differentiating property of the ascites and justifies its value in the studies of diagnosis and therapy in malignant ascites patients.

A-026

Performance Evaluation of the Free PSA Immunoassay on the LUMIPULSE™ G1200 System

Y. Ishii, H. Murakami, H. Karasawa, C. Okamura. *Fujirebio Inc, Hachioji-shi, Tokyo, Japan*

Background: PSA, a glycoprotein with a molecular weight of 30 kDa localized in prostate glandular epithelial cells, is known to be released into blood when prostate epithelial cells are damaged by malignancies. It has been demonstrated that the measurement of PSA in blood is useful in the diagnosis of prostate cancer, follow-up, and evaluation of therapeutic effect as well as in screening for early detection of prostate cancer. Percentage of free PSA has been shown to improve diagnostic sensitivity and specificity around gray zone (4 to 10 ng/mL total PSA). Total PSA immunoassay is already available on LUMIPULSE G1200 system[1]. A method has been developed to measure free PSA and result of performance evaluation is presented.

Methods: Lumipulse G Free PSA is a chemiluminescent enzyme immunoassay (CLEIA) that uses a two-step method for analysis. In the first step, anti-free PSA monoclonal antibody coated magnetic particles are incubated with a patient sample. Following a wash, the alkaline phosphatase-conjugated anti PSA mAb are added to the mixture and incubated in the second step. Following another wash, the instrument adds substrate solution to initiate chemiluminescence reactions. The resulting reaction signals are proportional to the amount of free PSA in the sample and allow a quantitative determination of free PSA in serum and plasma.

Results: The imprecision of the free PSA assay measured over 20 days using two controls and three panels (ranging from 0.5 to 23 ng/mL) was total imprecision of < 3.3%.The calibration range of the Lumipulse G Free PSA was 0 - 30 ng/mL and showed a linear dose-response relationship within the calibration range. The Lumipulse G Free PSA correlated linearly with ARCHITECT Free PSA (Slope = 0.96; r = 0.99), Access Hybritech free PSA (Slope = 1.04; r = 0.99) and ELECSYS free PSA (Slope = 1.02; r = 0.99) within the range of 0.058 to 27.86 ng/mL via testing 120 serum samples. No hook effect was observed at 3,000 ng/mL, and no cross-reactivity was observed to PAP (1000 ng/mL). No interference was observed with unconjugated (18.3 mg/dL) or conjugated bilirubin (20.6 mg/dL), hemoglobin (487 mg/dL), triglycerides (2000 mg/dL), RF (rheumatoid factor, 1000 IU/mL) and HAMA (1000 ng/mL). The LOB, LOD, and LOQ were 0.001, 0.002 and 0.009 ng/mL with LUMIPULSE G1200, respectively. Correlation between serum tube and plasma tube (EDTA-2K, Na heparin) was tested (Slope = 0.99). Serum specimens (60 prostate cancer, 97 non cancer) whose PSA measurement values were in the gray zone (4-10 ng/mL) were tested using the Lumipulse G Free PSA and the mean percentage of free PSA (prostate cancer; 14.8%) showed significantly lower than that of non cancer (20.2%).

Conclusion: The Lumipulse G Free PSA assay appears to be an accurate and precise assay for the automated measurement of free PSA in human serum and plasma.

[1]Not available in the US

A-027

QUANTITATIVE DETERMINATION OF FREE LIGHT CHAINS (FLC) - KAPPA AND LAMBDA IN GROUP OF PATIENTS WITH ABNORMAL SERUM ELECTROPHORESIS PATTERN.

Y. S. Medeiros[1], R. Freitas[2], M. Debiasi[3]. *[1]UFSC, Florianopolis SC, Brazil, [2]Santa Luzia Laboratório Médico, Florianopolis SC, Brazil, [3]Santa Luzia Laboratório Medico, Florianopolis SC, Brazil*

OBJECTIVE : The dosage of free light chains (kappa and lambda - FLC) have been incorporated into guidelines for some hematological malignant diseases such as multiple myeloma (MM) and other monoclonal gammopathies, in order to aid diagnosis and monitoring. In physiological and pathological conditions such chains are likely to be measured in the bloodstream along with the other intact molecules. This study intends to investigate the concentration of FLC in a group of patients when compared with the reference values.

PATIENTS AND METHODS: 56 samples of patients with ages within 19 and 88 years old, which 22 were men and 34 were women were evaluated. Serum electrophoresis was performed on CAPILLARYS 2 (Sebia ®) and FLC dosage was performed by nephelometry on BN - ProSpec (Siemens®) using Siemens N Latex FLC kappa and N Latex FLC lambda. From these patients, based on renal function verification by MDRD (Modification of Diet in renal Disease Study- NKDEP) and serum protein electrophoresis results, 12 samples were excluded due to indication of potential renal dysfunction; whereas 17 with a record of monoclonal gammopathy and 27 with polyclonal gammopathy result were included.

RESULTS AND CONCLUSION: Only the results of FLC in patients with normal renal function (n = 44) were analyzed. For patients with monoclonal gammopathy (n = 17), eight presented kappa/ lambda index with approximately six times greater than the reference value, nine with normal values. Regarding patients with polyclonal gammopathy (n = 27), about 50 % of FLC results were high. Based on these data, we conclude that the inclusion of FLC assays will contribute to the clinical evaluation of monoclonal gammopathy. Regarding to polyclonal gammopathy, overproduction of free light chains as described in infectious processes, and autoimmune liver diseases, among others, the clinical value of this dosage is still under study.

A-028

Best practices in incidental clinical findings associated with Multiple Myeloma in patients attending the Emergency Service

J. L. García de Veas Silva[1], R. Rios Tamayo[2], C. Bermudo Guitarte[1], P. Navarro Alvarez[2], V. Sánchez Margalet[1], C. González Rodriguez[1]. *[1]Hospital Universitario Virgen Macarena, Sevilla, Spain, [2]Hospital Universitario Virgen de las Nieves, Granada, Spain*

Background: The presence of incidental clinical findings (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia, acute kidney injury) related to Multiple Myeloma (MM) in Emergency Service and Primary Care should be studied for screening the existence of a possible MM. A quick panel based on serum protein electrophoresis (SPE) and quantification of serum free light chains (sFLC) enables sensitive quantification of monoclonal component in the study of MM. The application of this screening panel in patients with these incidental clinical finding without other diagnosis can help us to efficiently detect a possible MM in much sorter times.

Methods: we studied 5 patients where we found incidental clinical finding characteristic of MM (anemia, hyperproteinemia, intense bone pain). Sera of the five patients were sent to the Immunology Lab for the screening of a monoclonal protein. SPE were performed on CAPILLARYS 2 (Sebia) and the sFLC were measured with Freelite (The Binding Site) turbidimetric assay. Positive results of the screening panel, remit the patient to the Hematology Service to complete the study

Results: The results are shown in the table.

Conclusions: In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that alerts to a possible MM case in patients without obvious clinical diagnosis, we found the application of this protocol (SPE+sFLC) to be efficient and advisable. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies which in the context of the emergency service is of particular importance.

Case	Sex	Age (years)	Cause of Emergency	Clinical Finding at Emergency Service	SPE	sFLC	Diagnosis
1	Female	67	Severe abdominal pain	Hyperproteinemia (12 g/dl)	Large peak (4.18 g/dl)	KL= 10.47 mg/l LL=99.59 mg/l Ratio=0.11	Multiple Myeloma IgG Lambda Stage 2 ISS
2	Female	65	Infection and bone pain	Hyperproteinemia, hyperviscosity and thrombocytopenia	Large peak (3.28 g/dl)	KL=617 mg/l LL=11.1 mg/l Ratio=55.59	Multiple Myeloma IgG Kappa Stage 2 ISS
3	Female	64	Intense lower back pain	Intense back pain	Large peak (3.22 g/dl)	KL=3.15 mg/l LL=102 mg/l Ratio=0.031	Multiple Myeloma IgA Lambda Stage 3 ISS
4	Female	55	Lower back pain and she had a fall	Pathological fracture at D12	Two weak peaks (0.15 g/dl)	KL=28600 mg/l LL= 5.36 mg/l Ratio=5335.82	Light Chain Kappa Multiple Myeloma Stage 3 ISS
5	Male	12	Lower back pain and he had a fall in the school	Hypercalcemia (16.6 mg/dl)	Very large peak (4.34 g/dl)	KL=219 mg/l LL=1.01 mg/l Ratio=216.83	Multiple Myeloma IgA Kappa Stage 3 ISS

A-029

Performance Evaluation of Tumor Marker CA15-3 on Roche Cobas e601 Immunoassay Analyzer

S. St. Romain, I. Bermudez, B. Handy, E. Wagar, Q. H. Meng. *University of Texas MD Anderson Cancer Center, Houston, TX*

Backgrounds: Breast cancer is the most prevalent form of cancer diagnosed in women and is the leading cause of cancer death in women worldwide. Cancer antigen 15-3 (CA15-3) and CA27.29 are different epitopes on the same protein antigen product of the breast cancer-associated MUC1 gene. Substantial evidence has shown that elevated cancer antigen 15-3 (CA15-3) levels are associated with advanced breast cancer and metastasis. Thus, serum CA15-3 is used to monitor the therapeutic response and recurrence of breast cancer. This study was to assess the analytical performance of CA15-3 on Roche Cobas e601.

Methods: The Roche CA15-3 method is a sandwich electrochemiluminescence immunoassay that employs a biotinylated monoclonal CA15-3-specific antibody and a monoclonal CA15-3-specific antibody labeled with a ruthenium complex, forming a sandwich complex. The evaluation was performed following CLSI guidelines. The performance was evaluated for precision, lower limit of detection, linearity, and accuracy. The within-run and between-run precisions were assessed by analyzing QC material at low and high level of concentrations. The correlation between CA15-3 results on Roche Cobas e601 and CA27.29 results on Siemens Centaur was assessed.

Results: The within-run CVs for CA15-3 were 1.6% and 1.4% at the levels of 22 U/ml and 102 U/ml, respectively. The between-run CVs at low and high levels were 2.64% and 2.59%, respectively. The measuring range was determined to be linear between 1.00 - 300 U/ml. The lower limit of detection was 0.1 U/ml using measurable value obtained from zero standard + 2SD (n=20). Comparison of CA15-3 on Roche Cobas e601 with CA27.29 on Siemens Centaur showed that the slope was 1.0 (95% CI = 0.912 to 1.087) with intercept of -12.61 and correlation coefficient r = 0.967 (Deming). The mean bias was -12.65.

Conclusion: Our data demonstrates that the CA15-3 assay on Roche Cobas e601 analyzer has an excellent precision of performance with good linearity. There is a good correlation between serum CA15-3 and CA27.29. Serum CA15-3 can be precisely and accurately measured on Roche Cobas e601 in monitoring response to therapy and recurrence in breast cancer patients.

A-030

Urinary Free Light Chains in Patients With Polyclonal Hypergammaglobulinemia And/Or Renal Impairment

V. Brunel[1], B. Legallicier[2], J. Wils[3], M. Quillard[1], M. Godin[2], S. Claeyssens[3]. *[1]Medical Biochemistry, University Hospital, Rouen, France, [2]Nephrology, University Hospital - Inserm UMR1073 - IRIB, Rouen, France, [3]Medical Biochemistry, University Hospital - Inserm UMR1073 - IRIB, Rouen, France*

Background: In plasma cell dyscrasias, monoclonal free light chains (FLC) are involved in the pathogenesis of renal failure, a major cause of morbi-mortality. Besides, urinary excretions of polyclonal FLC are known to increase in patients with RI and, according to rare data, in those with hypergammaglobulinemia (H). However, evidence for polyclonal FLC-mediated injury is limited. In this study, we assessed the effect of H and/or RI on urinary FLC excretions.

Methods: Fresh paired samples of serum and 24h urine were analyzed in 270 patients exempt of monoclonal gammopathy. Patients with H (n=87) had sum of serum Ig G, A and M (Σ Ig) concentrations ≥ 20 g/L. All patients were classified in 6 groups according to their renal function and the presence, or not, of H; Control patients (C, HC) had physiologic proteinuria (<150 mg/24h) and serum creatinine concentration in reference ranges. Additionally, control patients C had serum K and L concentrations and rFLC values in the 100% reference interval. Patients with predominant tubular (T, HT) or glomerular (G, HG) proteinuria were determined by SDS-AGE profil (Hydragel Proteinuria®, Sebia) and by albuminuria ≤ or ≥ 50% of total proteinuria, respectively. FLC renal clearance (Clr) was calculated as the ratio of 24h urinary excretion of K or L FLC (mg/24h) to their respective serum concentration (mg/L).

Results: median (ranges); Mann-Withney test (significance: P<0.05), Spearman correlations (significance: P<0.05)

Results: Both in patients with H and in those without, K and L FLC urinary excretions were significantly greater in T and HT and in G and HG patients than in their respective control patients C and HC; these values were up to 151 (7-574) and 35 (2-261) mg/24h, respectively in G patients, 421 (93-8640) and 96 (34-3270) mg/24h, respectively in HG patients. Moreover, FLC excretions significantly correlated with both serum FLC and creatinine concentrations; in addition, in patients with H, they significantly correlated with Σ Ig concentrations. Ratios of K Clr to L Clr (K Clr /L Clr) decreased significantly through different states of renal function from C to T and to G patients and were negatively correlated with serum creatinine and Σ Ig concentrations. Patients with H, as compared to those without at the same stage of renal function, had significant and similar increases in K and L FLC excretions (3.5 and 4.0 fold for K and L FLC, respectively). While K Clr/L Clr values were similar in C and HC patients, they were significantly lower in HT than in T patients and in HG than in G patients.

Conclusion: This study determined the appropriate reference intervals for patients with H and/or RI. In all these patients, urinary polyclonal FLC excretions varied according to renal function. Besides, H was associated with an increase in FLC excretions values that was independent of renal function. RI progression was associated with a decrease in K Clr/L Clr values showing that the renal capacity to clear K faster than L is progressively lost. In addition, this effect worsened in patients with H suggesting a polyclonal FLC-mediated injury.

A-031

Serum Free Light Chains in Patients With Polyclonal Hypergammaglobulinemia And/Or Renal Impairment

J. Wils[1], B. Legallicier[2], V. Brunel[1], M. Quillard[1], M. Godin[2], S. Claeyssens[3]. *[1]Medical Biochemistry, University Hospital, Rouen, France, [2]Nephrology, University Hospital, Rouen, France, [3]Medical Biochemistry, University Hospital - Inserm UMR107 - IRIB, Rouen, France*

Background: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the identification and management of patients with plasma cell dyscrasias. However, since these patients frequently present renal impairment (RI), it should be interpreted with caution owing to the dependence of polyclonal FLC values on renal function. Besides, recent data reported that serum FLC concentrations increased in patients with polyclonal hypergammaglobulinemia (H); however, data were rare and renal function was not always assessed. In this study, we assessed the effect of H and/or RI on serum FLC concentrations.

Methods: Fresh paired samples of serum and 24h urine were analyzed in 270 patients exempt of monoclonal gammopathy. Patients with H (n=87) had sum of serum Ig G, A and M (Σ Ig) concentrations ≥ 20 g/L. All patients were classified in 6 groups according to their renal function and the presence, or not, of H. Control patients (C, HC) had physiologic proteinuria (<150 mg/24h) and serum creatinine concentration in reference ranges. Additionally, control patients C had serum K and L concentrations and rFLC values in the 100% reference interval. Patients with predominant tubular (T, HT) or glomerular (G, HG) proteinuria were determined by SDS-AGE profil (Hydragel Proteinuria®, Sebia) and by albuminuria ≤ or ≥ 50% of total proteinuria, respectively. Serum FLC (Freelite®, The Binding Site) were analysed on a BNII nephelometer (Siemens Healthcare). Results: median (ranges); Mann-Withney test (significance: P<0.05), Spearman correlations (significance: P<0.05).

Results: Both in patients with H and in those without, serum K and L FLC concentrations rose significantly through different states of renal function from C to T and to G patients; in those latter, these values reached 36 (9-115) and 36 (9-106) mg/l, respectively in G patients, 139 (40-945) and 109 (30-691) mg/l, respectively in HG patients. Moreover, FLC concentrations and rFLC values were significantly correlated with creatinine concentrations; in addition, in patients with H, FLC concentrations significantly correlated with Σ Ig concentrations. Patients with H, as compared to those without at the same state of renal function, had significant and similar increases in FLC concentrations (3.5 and 3.0 fold for K and L FLC, respectively, for comparisons

of C vs HC patients, T vs HT and G vs HG patients). Alike, patients with H had a significantly and similar increase in rFLC values than those without (1.2 fold for comparisons of C vs HC patients, T vs HT and G vs HG patients).

Conclusion: This study determined the appropriate reference intervals for patients with H and/or RI. In all these patients, serum polyclonal FLC concentrations and rFLC values shifted to higher values with RI progression and varied according to renal function. Besides, H was associated with an increase in FLC concentrations and in rFLC values that were independent of renal function. Therefore, rFLC values should be interpreted with caution, not only in case of RI, but also in case of H: we showed that rFLC values between 0.24 to 0.74 should provoke a thorough search for plasma cell dyscrasias and lymphoproliferative disease.

A-034

Huaier suppresses proliferation and induces apoptosis in human lung adenocarcinoma cells via promotion of miR-26b-5p

Z. Lu, T. Wu, W. Chen, S. Liu, H. Lu, H. Wang, X. Huang, Q. Kong. *Central Hospital of Wuhan, Wuhan, China*

Background: Aqueous extract of Trametes robiniophila murr(Huaier)has been applied for cancer complementary therapy in recent decades. Varies studies have reported that Huaier possess the anti-tumor effects. However, the mechanisms are not completely elucidated. MicroRNAs (miRNAs) are small (18-25 nucleotides), noncoding RNAs whose dysregulation have been discovered to involve in tumorigenesis and development.

Methods and Results: In this study, we found miRNA expression profiles were altered in Huaier-treated human pulmonary adenocarcinoma A549 cells. MiR-26b-5p, which is upregulated in the expression profiles and simultaneously downregulated in both several lung cancer cell lines and patients, was selected for further study. We then used miRNA mimics or inhibitors to perform gain- and loss-of-function studies to demonstrate the roles of miR-26b-5p in pulmonary cancer. Moreover, EZH2 was identified as a target of miR-26b-5p by luciferase reporter assay and by EZH2 knock-down we observed a decrease in cell proliferation and an increase in apoptosis rates of A549 cells, which was corresponding to the effects of both Huaier treatment and the transfection of miR-26b-5p mimic. Additionally,β-catenin and bcl-2, as the indirect downstream effectors of EZH2, was found attenuated after Huaier treatment and miR-26b-5p overexpression.

Conclusion: Taken together,our findings shed light on the mechanisms that Huaier might suppress proliferation and induce apoptosis in lung cancer by miR-26b-5p-EZH2-mediated approach in lung cancer cells, which provides a new idea for understanding the anti-tumor effects of Huaier.

A-035

Blood Test for Early Detection of Lung Cancer

M. Stengelin[1], H. Pass[2], W. Rom[2], S. Kumar[1], S. Vaithlingam[1], A. Aghvanyan[1], D. Roy[1], G. Dobrescu[1], R. Sivakamasundari[1], E. N. Glezer[1], J. N. Wohlstadter[1]. *[1]Meso Scale Diagnostics, LLC, Rockville, MD, [2]New York University, New York, NY*

Background: Lung cancer is the largest single cause of death from cancer worldwide. Even though lung cancer often can be treated successfully when detected early, approximately 90% of patients diagnosed with lung cancer die of the disease. Screening with low dose CT can reduce mortality, but the positive predictive value of this test is low, leading to a large number of suspicious but ultimately non-malignant results that nevertheless require follow-up. Our objective was to develop a simple blood test to risk-stratify patients at high risk of lung cancer.**Methods:** We developed multiplexed, serum/plasma immunoassay panels to measure more than 40 lung cancer-related biomarkers using a 96-well, 7-spot format and electrochemiluminescence detection. Due to the high sensitivity of MSD's MULTI-ARRAY® technology, these panels were run with diluted serum or plasma, bringing the total sample volume required to run all 40 assays down to approximately 40 µL per replicate. This enabled us to measure all markers simultaneously in precious, high-quality serum and EDTA plasma samples. We used samples from early-stage lung cancer patients (drawn before lung cancer surgery) and from a lung-cancer screening cohort of age-matched heavy smokers who did not have lung cancer at the time of the blood draw.**Results:** In a training set of 300 samples, 12 serum and 6 plasma markers had areas under an ROC curve (ROC areas) of 0.7 or higher. We used a logistic regression model with 100x cross-validation to develop a multi-marker panel. One serum panel (Flt-3L, EGFR, MMP-3, and NME-2) and one plasma panel (Flt-3L, cytokeratin-19, MMP-3, Flt-1, KGF, and PlGF) were selected and tested using approximately 250 additional samples from the same cohort. For the serum panel, the ROC area dropped to 0.85 (vs. 0.95 for the training set); for the plasma panel, the ROC area dropped to 0.81 (vs. 0.93). Nevertheless, even the ROC area of 0.85 for the serum panel with clinical sensitivity and specificity of 81% and 84%, respectively, and the ROC area of 0.81 for the plasma panel with clinical sensitivity and specificity of 76% and 78%, respectively, should be clinically useful. Analysis of the combined training and test sets with 100x cross-validation resulted in a 4-marker serum panel (Flt-3L, EGFR, MMP-3, and NME-2) with an ROC area of 0.91 and clinical sensitivity and specificity of 88% and 82%, respectively, and a 5-marker plasma panel (Flt-3L, cytokeratin-19, Flt-1, KGF, and HGF) with an ROC area of 0.91 and clinical sensitivity and specificity of 84% and 83%, respectively. **Conclusion:**Using MULTI-ARRAY technology and high quality clinical samples, we were able to identify promising biomarker panels for early detection of lung cancer in high-risk individuals.

A-036

Serum MicroRNA Panel as Novel Non-Invasive Biomarker for Early Diagnosis of Cervical Cancer

Y. Zhang[1], N. Ning[1], Q. Li[2], W. Cui[1]. *[1]Peking Union Medical College Hospital Chinese Academy of Medical Sciences, Beijing, China, [2]Duke University Medical Center, Durham, NC*

Background: Currently, pathologic evidence of malignant cells, which typically requires an invasive strategy such as vaginoscopy and cervical biopsy, or loop electrosurgical excisional procedure, is referenced as the gold standard in diagnosing human cervical cancer. And serum tumor biomarkers, such as CEA, AFP, CA125, SCCAg, have provided some predictive information to tumor diagnosis, but poor sensitivity and specificity has also limited their clinical applications. We aimed to identify serum miRNAs for diagnosing cervical cancer.

Methods: Serum miRNA expression was investigated from 348 participants by using qRT-PCR, including 111 patients with cervical cancer, 115 cervical intraepithelial neoplasia (CIN) individuals and 122 healthy controls, recruited between July 2012 and November 2013 from Peking Union Medical College Hospital in China. First, we fully screened the differently expressed 425 miRNAs in 9 serum samples for diagnosing cervical cancer. A logistic regression model was constructed using a training cohort (n=72) and then validated using an independent cohort (n=269). Hela cells stably expressing miR-497 were established to analyze their roles in vivo and vitro.

Results: We identified a miRNAs panel (miR-124-3p, miR-195, miR-2861, miR-497) that provided high accuracy in discriminating cervical cancer from healthy controls (AUC=0.907 and 0.793 for training and validation groups, respectively), from CIN individuals (AUC=0.960 and 0.963 for training and validation groups, respectively). This 4 miRNAs can also differentiate CIN from healthy controls (AUC=0.903 and 0.87 for training and validation groups, respectively). Among the 4 up-regulated miRNAs, miR-497 levels in serum were the most specific one for cervical cancer that had no significance between ovarian or breast cancer patients and healthy controls. Forced expression of miR-497 suppressed proliferation and induced apoptosis of Hela cells ($p<0.05$) in vitro. Further investigation showed that Hela xenograft mouse treated miR-497 overexpression was significantly smaller in weight than control ($p<0.05$). MiR-497 could exert the effect of tumor growth inhibition in vivo.

Conclusion: Our results have identified a miRNAs panel (miR-124-3p, miR-195, miR-2861, miR-497) that has considerable clinical value in diagnosing cervical cancer as a novel noninvasive approach.

Keywords: miRNA panel; cervical cancer; serum

A-038

Can the incomplete serum separation on gel tube vacutainers lead to the diagnosis of Multiple Myeloma ?

S. Sen[1], S. Chakraborty[2]. *[1]Calcutta Medical Research Institute (CMRI), Kolkata, India, [2]Peerless Hospital & B K Roy Research Centre, Kolkata, India*

BACKGROUND: Gel tubes have become common in clinical labs and have made analysis and storage of samples easier; eliminating the need for transferring of serum into secondary tubes The basic principle is gradient density centrifugation using the thixotropic property of the gel, where it forms a barrier separating serum from the cells. Occasionally incomplete separation is seen in some samples where the gel packed cells fail to go below the gel. There are very few studies conducted on this phenomenon and some studies have suggested that incomplete separation of serum occur in patients with paraproteinemia particularly multiple myeloma (MM).

METHODS: Hence we conducted a prospective study on the relationship between incomplete serum separation on gel tubes and paraproteinemia. This was done to identify whether incomplete gel separation was associated with increased total protein. The gel tubes used in our study was BD- SST.

RESULTS: This study was done for a period of two years. Incomplete gel separation was seen in a total 14 gel tube samples out of a total of 99,850 patient samples (0.010 %).In 4 samples the incomplete separation was corrected on repeat sample collection. In 10 patient samples we observed incomplete separation even on repeat sampling. Raised total protein with altered albumin to globulin ratio was seen in those samples. Serum Protein Electrophoresis (SPEP) confirmed the presence of M bands in all the 10 cases and subsequently multiple myeloma was confirmed with bone marrow aspiration. The immunoglobulin subtypes with immunofixation were: Ig A (5/10), Ig M (4/10) and biclonal type with Ig M and Ig G (1/10).

CONCLUSIONS: Our study shows that incomplete separation on gel tubes is very commonly associated with paraproteinemia like multiple myeloma. The increase in paraprotein component possibly increases the viscosity of the sample leading to inhibition in separation. Clinical laboratorians need to be aware of this and should estimate the total protein and albumin reflectively in those patients showing incomplete separation. Patients having increased total protein and altered albumin globulin ratio should be followed up with SPEP. Clinical correlations and interaction with treating physicians might lead to early diagnosis in such patients

A-039

Clinical comparisons of two free light chain assays to immunofixation electrophoresis for detecting monoclonal gammopathy

M. Park, H. Kim, H. Kim, K. Shin, W. Song, H. Kim, H. Kim. *Hallym University College of Medicine, Seoul, Korea, Republic of*

Background: Free light chains (FLC) are useful biomarkers for the diagnosis and monitoring of various plasma cell dyscrasias. Recently, several monoclonal antibody-based assays for serum FLC have become available.

Methods: One hundred fifty seven samples from 120 patients for screening or monitoring of monoclonal gammopathy (MG) were included in this study. The new N Latex FLC assays (Siemens Healthcare Diagnostics GmbH, Germany) were compared with the Freelite FLC assays (The Binding Site Ltd, UK) and immunofixation electrophoresis (IFE).

Results: The Freelite FLC assay showed significantly wider assay ranges than the N Latex FLC assay. The correlation coefficients of the two FLC kappa (κ) assays, lambda (λ) assays, and the κ/λ ratio were 0.9792, 0.8264, and 0.9064, respectively. The concordance rate was 84.7% for the FLC κ assays, 79.6% for FLC λ, and 89.2% for the κ/λ ratio. Compared to the results for IFE, the clinical sensitivity, specificity, and percent agreement of the κ/λ ratios were as follows: 72.2%, 93.6%, and 82.8%, respectively, for the Freelite assay and 64.6%, 100%, and 82.2%, respectively, for the N Latex FLC assay. **Conclusion:**

Several differences in dynamic assay ranges were observed between the two FLC assays. The N Latex FLC assay showed good correlations and concordance with the Freelite FLC assay. The clinical sensitivity of the κ/λ ratio was higher in the Freelite FLC assays; however, clinical specificity was higher in the N Latex FLC assay.

A-040

Contribution of FokI polymorphism to disease development and risk prediction values in bladder cancer cases

O. Baykan[1], M. Akgul[1], N. Uren[2], F. Gerin[1], I. Tınay[1], E. Ergul[1], A. Sazci[2], L. Turkeri[1], G. Haklar[1]. *[1]Marmara University School of Medicine, Istanbul, Turkey, [2]University of Kocaeli, Kocaeli, Turkey*

Bladder cancer is the fourth most common cancer in men. Although smoking is known to be the most important etiological factor in bladder cancer, 40% of cases remained to be unknown. In our study we aimed to investigate the contribution of a common single nucleotide polymorphisms rs2228570 (FokI) in the vitamin D receptor gene to the formation of urothelial bladder cancer.

Age and gender matched 101 patients diagnosed as urothelial bladder cancer and 109 healthy individuals who has no history of cancer in their first degree relative were included in the study. Polymerase chain reaction, and restriction fragment lenght polymorphism techniques were used to determine the polymorphisms. The frequencies of FokI polymorphism FF, Ff and ff genotypes were 60.4%, 31.7%, 7.9% in bladder cancer and 44%, 47.7% and 8.3% in controls, respectively (p=0.048). FF genotype frequences were higher (p=0.018) in patients, while Ff frequences were lower (p=0.018) compared to controls (Table 1).

Table 1. Genotypic distribution of FokI polymorphism

VDR gene	Patient (n=101)	Control (n=109)	OR (%95 GA)	p	OR_{adj}* (%95 GA)	p
FF	61 (60.4%)	48 (44.0%)	1.94 (1.12–3.36)	0.018[a]	1.64 (0.89–3.02)	0.114[a]
Ff	32 (31.7%)	52 (47.7%)	0.51 (0.29–0.89)	0.018[b]	0.63 (0.34–1,19)	0.154[b]
ff	8 (7.9%)	9 (8.3%)	0.96 (0.35–2.58)	0.929[c]	0.82 (0.27–2.48)	0.727[c]

[a]FF vs.Ff+ff; [b]Ff vs. FF+ff; [c]ff vs. FF+Ff

*OR (95% CI) adjusted according to smoking history

Associations between risk factors and cancer were estimated by calculating OR_{adj} using logistic regression analyses. When smoking status and FokI polymorphism analysed together, the effect of genotype and allel frequencies on cancer risk prediction were not statistically significant, however smoking increased bladder cancer risk 7.27 times (OR_{adj}= 7.27; 95% CI= 3.8-13.9; p<0.001) The genotype distributions of the polymorphisms were in agreement with Hardy-Weinberg equilibrium among the cases and controls.

Studies investigating the contribution of VDR gene polymorphism and urothelial cancers were limited, although there were many publications for other cancer types. Our study is the first for inverstigating this relation in Turkish population. We demonstrated significant polymorphism in the patient group when compared to the control, however there was no effect of genotype on cancer occurence. Further studies which will be planned to reveal the effect of this difference may be beneficial in the etiopathogenesis of urothelial cancers.

A-041

Enrichment of heterogenous circulating tumor cells by multiplexed immunomagnetic micro particles

K. Bourcy[1], K. Goudy[1], L. M. Millner[2], M. W. Linder[1], R. Valdes[1]. *[1]PGXL Technologies, Louisville, KY, [2]University of Louisville, Louisville, KY*

Background: Circulating tumor cells (CTCs) are low abundance cells that have detached from the primary tumor and may produce metastatic lesions. They represent the greatest threat to a cancer patient. Paradoxically, they may also be an invaluable source of prognostic information by predicting metastases and chemotherapy resistance. Significant obstacles and limitations exist in current methods utilized for the detection and isolation of CTCs. Currently, the only FDA-cleared system for the detection of CTCs has a number of drawbacks including: i) the requirement that buffy coat be used, resulting in cell loss; ii) the cells must be fixed, which severely limits the ability to perform downstream analysis; and, iii) this system uses a single antigen for the recovery of CTCs, which can miss cells that do not express the target antigen. In this study we describe the application of a magnetic bead technology for enrichment of low abundance CTC where we evaluated this technology for enrichment of low abundance cells from whole blood, potential benefits in enrichment using a multi-antigen approach, and viability of CTCs following the enrichment procedure. *The long-term objective of this study is to develop a multi-plex immunomagnetic method for the isolation and recovery of circulating tumor cells from whole blood, with the ultimate goal of characterizing circulating tumor cells in breast cancer patients*.

Methods: We pre-labeled SKBR cells with carboxyfluorescein succinimidyl ester and spiked 10 or fewer cells into 1 mL of whole blood. The spiked blood was incubated with anti-EpCAM or a combination of anti-EpCAM and anti-HER2 conjugated magnetic microbeads. Blood was applied to a magnetized separation column and washed 4x with buffer. Magnetically labeled cells are retained in the column while unlabeled cells pass through. The column was removed from the magnet and labeled cells were eluted with buffer, sedimented by centrifugation and resuspended in 10 µL of elution buffer. We visualized and quantified cells using a fluorescent microscope and cell viability was determined based on a Trypan blue exclusion. All procedures were performed at room temperature.

Results: The average percent recovery from 1 mL of blood using anti-EpCAM-conjugated beads was 70.95% ± 5.27 (mean ± SEM), interassay CV of 40.68% (N=30). The average percent recovery from 1 mL of blood using a combination of anti-EpCAM and anti-HER2 beads improved to 81.32% ± 3.959 and interassay CV of 24.82% (N=26), p = 0.1308. The data was analyzed using an unpaired t-test. For each method of cell recovery, 100% of CTCs remained viable.

Conclusion: Based on these data, we concluded that this technology can be adapted for the purpose of enriching low abundance cells directly from whole blood. Use

of a multiplex strategy has the potential to achieve recovery efficiency consistent with the current state of the art, and improves % recovery over a single antigen approach. This method consistently yielded recovery of viable cells which will make this approach uniquely useful for single cell phenotyping studies such as for testing chemotherapeutic sensitivity.

A-042

Decoding miRNA Expression of Breast Carcinoma Behavior using Next Generation Sequencing of LCM Procured Cells

J. L. Wittliff[1], K. Bramlett[2], S. A. Andres[1], J. Schageman[2], C. Hinahon[2], J. Cienfuegos[2], R. Setterquist[2]. *[1]University of Louisville, Louisville, KY, [2]Thermo Fisher Scientific, Austin, TX*

Refinements in selecting biomarkers for breast carcinoma management require identification of clinically relevant parameters complementing patient endocrine status and tissue biopsy content of estrogen receptors (ER), progestin receptors (PR) and HER-2/neu oncoprotein, which correlate with prognosis and therapy response. Our objective is to compare miRNA expression profiles of intact tissue sections from breast cancers with those of laser capture microdissection (LCM)-procured carcinoma cells. The hypothesis is that miRNA signatures, discerned from LCM acquired populations of specific cell types, more accurately reflect the molecular basis of cancer clinical behavior than provided by protein biomarkers of intact tissue biopsies. miRNAs are 19-24 nucleotide non-coding RNA species regulating gene expression by inhibiting translation or by triggering degradation of specific mRNA targets. De-identified frozen tissue biopsies were selected from our IRB-approved Biorepository using criteria in the comprehensive de-identified Database to standardize the study population (e.g., invasive ductal carcinomas of known grade and biomarker status). Serial tissue sections containing 55 +/- 23% cancer were prepared and stained with H & E using established protocols, and carcinoma cells (~ 14000 LCM pulses) were procured non-destructively from an adjacent section. RNA was extracted from intact tissue sections and LCM-isolated cells using PureLink RNA Mini KitsTM (Invitrogen), evaluated for integrity (Agilent Bioanalyzer) and analyzed for miRNA expression using the Ion TorrentTM Next Generation Sequencing System (Thermo Fisher Scientific). Total RNAs were enriched for small RNA species using mirVana miRNA isolation kits (Thermo Fisher Scientific) and RNA libraries were constructed from 5 ng of enriched RNA using Ion Total RNA-Seq LibraryTM kits. Barcodes were utilized to multiplex libraries for template preparation and sequencing on two Proton PI chips as two twelve-plex library pools. Resulting sequences were aligned to mirBASE precursors, and expression levels were calculated by tallying the number of reads mapping to each individual miRNA precursor. Counts/1M reads values for each miRNA were normalized to a housekeeping gene to determine relative miRNA expression. Reads mapping to mirBase were assessed for each carcinoma preparation. Comparison of the top 20 expressed miRNAs in the intact tissue sections with those of cognate carcinoma cells procured by LCM, in general, revealed that smaller defined miRNA gene sets were expressed in isolated populations of carcinoma cells. Furthermore, miRNA expression patterns of experimental pairs (intact section vs LCM-procured cells) were highly variable in carcinomas with different grades, suggesting relationships to disease status. Strikingly, when miRNA gene frequency plots (transcript abundance vs fold-change) were developed, comparing expression from intact tissue sections to that of LCM-procured cell population, subsets of miRNA genes were revealed. Although the limited number of samples analyzed precluded identification of particular miRNA gene signatures associated with a specific breast pathology or biomarker status (e.g., ER, PR, HER-2/neu), application of Next Generation Sequencing of miRNAs using LCM-procured carcinoma cells provides an innovative approach for decoding miRNAs involved in breast cancer behavior. Supported in part by a grant from the Phi Beta Psi Charity Trust (JLW & SAA) and a CTSP Award from the Commonwealth of Kentucky (JLW).

A-044

Novel Approach to Diagnose High Grade of Cervical Lesion: Combination of HPV E6/E7 and hTERT mRNA Real-Time RT-PCR Assay

S. Park[1], H. Wang[2], S. Kim[3], G. Kim[1], D. Lee[4], Y. Kim[1], H. Kim[1], J. Kim[1], S. Ahn[1], H. Jin[5], K. Park[6], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [3]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [4]Department of Clinical Laboratory Science, Hyejeon College, Hongseoung, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Pathology, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of*

Background: Human Papillomavirus (HPV) is a major causative factor of cervical cancer, which is the third of the most common cancer in women. The Real-HPV-E6/E7 mRNA® multiplex RT-qPCR assay (M&D, Wonju, Republic of Korea) has been developed and evaluated because E6 oncoprotein inhibits apoptosis by degradation of cellular tumor suppressor protein p53 and E7 oncoprotein prevents cell cycle arrest of damaged cells. HPV high-risk types (HPV genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 69) are regard to be detectable and significant marker in high grades of cervical lesion. Human telomerase reverse transcriptase (hTERT) is also considered as complementary marker that may provide a criteria in high grades of cervical lesions. Aberrant telomerase activity has been suggested to be critical for human tumor genesis and related to following mechanism of E6 oncoprotein.

Methods: In this study, HPV E6/E7 oncogene and hTERT are detected by Real-HPV-E6/E7 mRNA® multiplex RT-qPCR and Real-hTERT mRNA® RT-qPCR assay (M&D), respectively. A total of 545 patients including 18 squamous cell carcinoma (SCC), 21 high grade squamous intraepithelial lesion (HSIL), 17 atypical squamous cells-cannot exclude HSIL (ASC-H), 101 low grade squamous intraepithelial lesion (LSIL), 100 atypical squamous cells of undetermined significance (ASC-US), and 288 normal cytology samples, were enrolled and analyzed by cytological diagnostic grades, respectively. 39 samples in high grades of cytological diagnosis (≥ HSIL) were confirmed as high grades in histological diagnosis.

Results: The positive rates of HPV E6/E7 mRNA RT-qPCR assay were 94.4% (17/18), 95.2% (20/21), 82.4% (14/17), 46.5% (47/101), 25.0% (25/100), and 1.1% (3/286) in SCC, HSIL, ASC-H, ASC-US, LSIL, and normal samples, respectively. Relative hTERT mRNA expression levels were able to distinguish high grade and low grade of cervical lesion significantly (p <0.001). Relative hTERT mRNA expression levels in low grades of cervical lesion were dramatically lower than in high grade of cervical lesion. Notably, 5 high grades of cervical samples (≥ HSIL) were not detected by HPV E6/E7 mRNA real-time RT-PCR assay, but those samples were high relative expression levels with hTERT mRNA real-time RT-PCR assay.

Conclusion: For predicting the outcomes of cervical intraepithelial neoplasia (CIN) 1 or CIN 2 patients, the combined use of HPV E6/E7 and hTERT mRNA RT-qPCR assay could be a significantly complementary approach for diagnosing high grade cervical lesions because of the hTERT mRNA expression levels highly increased in cervical cancer and which was very low in low grade cervical lesions and normal tissues. Therefore the combined detection of HPV and human factors as a predictive marker might be a very useful for monitoring of patients who have low grade of cervical lesions.

A-045

SENTIFIT®-FOB Gold® latex Fecal Immunoassay Test (FIT) evaluation on SENTiFIT®270 analyzer in CoreLab at the AUSL Modena-Nuovo S.Agostino Estense hospital in Emilia Romagna Region.

M. C. Anelli[1], A. R. Soliera[2], N. Conti[1], A. Cugini[1], T. Trenti[2], F. Torricelli[2], R. Corradini[2], M. Gramegna[1]. *[1]Sentinel CH SpA, Milano, Italy, [2]CoreLab, Clinical Pathology Department, NOCSAE-AUSL Modena, Baggiovara, Italy*

Background: the fecal immunochemical test (FIT) for hemoglobin is considered to be superior guaiac fecal occult blood test for colorectal cancer (CRC) screening and is becoming central in international CRC screening programs development. Identify the most appropriate FIT is now a priority. The focus of this study is two-fold: to assess the FOBGold® Latex reagent FIT on a dedicated instrument with pierceable device; to compare two quantitative FITs.

Methodology: analytical evaluation: Limit of Blank (LoB) and of Quantitation (LoQ),

(CLSI EP17-A); total, between days and runs imprecision (EP5-A2) on 3 quality control levels (QC Low, 1, 2) and one hemoglobin spiked pool in buffer (target 50, 77, 309 and around 150 ng/mL respectively); linearity (EP6-A), prozone, on-board reagent and calibration stability (EP25-A).

Methods: 120 selected samples from frozen anonymous routine (not from CRC screening program population) residuals, with high positive prevalence, sampled with Eiken and Sentinel devices (OC-Auto Sampling Bottle3 and SENTiFIT®pierceTube) and run with OC-Sensor Diana and SENTiFIT®270 respectively; statistical analysis: concordance table. Analyzer evaluation: one week familiarization; piercing, sample barcode reading, timing evaluation. Training and rating questionnaire (from 1 to 5 where 1=very poor, 3=neither good nor poor, 5=very good) to 18 technicians/degrees.

Results: LoB 4.1 ng/mL; LoQ 15.4 ng/mL. QC Low total imprecision: CV=6.2%, SD=3.0; between days CV=3.4%, SD=1.7; between runs CV=0.8%, SD=0.4. QC 1 total imprecision: CV=4.0%, SD=3.4; between days CV=0.2%, SD=0.2; between runs CV=1.5%, SD=1.3. QC 2 total imprecision: CV=2.1%, SD=7.5; between days CV=0.9%, SD=3.1; between runs CV:0.8%, SD=2.8. Pool total imprecision: CV=2.7%, SD=4.2; between days CV=1.2%, SD=1.8; between runs CV=0.4%, SD=0.7. Accuracy: Recovery QC Low 98%, QC1 111.7%, QC2 113.7%, Pool 102.7%. Linearity up to 871 ng/mL; prozone checked-up to 50,000 ng/mL; on-board reagent and calibration stability 33 days. Method comparison: Hamza et al. published a cut-off value of 117 ng/mL for FOBGold corresponding to 100 ng/mL for OC-Sensor. On this basis we evaluate the concordance: 99 results were negative and 16 positive with both methods; 5 results were positive with FOBGold only. Positive rate in the evaluated samples: OC-Sensor=13.3% and FOB Gold=17.5%. FOB Gold had higher positive rate than OC-Sensor (24%). SENTiFIT270: Familiarization was done successfully and the instrument is user friendly and reliable. Piercing and barcode reading on 200 tubes were always correct; time to first result: 14 minutes. Questionnaire rating mean values for software, sample management, calibration, quality control and maintenance are 4.1/4.2/4.0/4.1/4.8 respectively.

Conclusion: FOBGold Latex reagent FIT on SENTiFIT270 analyzer shows some important features: very low LoB, a Low QC at 50 ng/mL, 1250 on-bard test autonomy and automated maintenance; on-board samples capability should be improved. A regression study is not appropriate, due to sampling bias (different devices, different buffers) and different signal origin (different calibration materials and wavelenghts between OC-Sensor and SENTiFIT270) therefore a concordance table is the only possible statistical analysis. The discrepant samples cannot be investigated in this study with anonymous samples selected from routine with high positive prevalence, as it is clear that a final clinical validation needs colonoscopy check.

A-046

Prostate Specific mRNAs as Potential Specific Markers of Circulating Tumor Cells and for Detection of Prostate Cancer

W. Zhang, Z. Wang, E. Klein, M. K. Gupta. *Cleveland Clinic Foundation, Cleveland, OH*

Background: Currently, prostate cancer (PCa) screening relies on prostate specific antigen for early-detection of PCa. However, this test suffers with poor specificity with high rate of negative biopsy stressing the necessity of more specific biomarkers. Prostate-specific RNA markers can be used to detect circulating cancer cells in peripheral blood to provide a sensitive and specific alternative to detect PCa. We performed a pilot study to explore the role of a combined RNA markers including prostate specific membrane antigen (PSMA), prostate cancer antigen 3 (PCA3) and TMPRSS2-ERG fusion gene (TM-ERG) in screening for PCa.

Methods: Total RNA was extracted from peripheral blood mononuclear cells (PBMC) and was analyzed for PSMA, PCA3 and TM-ERG transcripts using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The assays were calibrated using either purified RNA from PCa cell line (VCap, CRL-2876) or synthesized complemented RNA. Analytic sensitivity was tested on RNA extracted from spiked female blood with VCap. Total of 33 patients with PCa before treatment, and 19 age-matched male patients with no PCa were analyzed for all three markers.

Results: Based on experiments using VCap spiked female blood PSMA and TM-ERG mRNA were reliably detected at 1-10 cell/ml respectively. The median (95percentile) mRNA levels in patients with PCa were significantly higher for all three markers than benign controls ($p \leq 0.001$). Furthermore, PCa patients with surgical margin(+) had significantly higher levels than margin(-) patients. Combination of all three markers significantly increased the clinical sensitivity and specificity for PCa as evidenced by logistic regression analysis. Results are summarized in Table 1.

Conclusion: Measurement of PSMA, PCA3 and TM-ERG transcripts extracted from PBMC increases the detection rate of PCa, especially invasive PCa with high specificity. Further studies with large cohort are needed for clinical validation and to assess pathologic stage prior to radical prostatectomy.

Table 1: Quantitation of mRNA for PSMA, PCA3,TM-ERG in Blood

	PSMA	PCA3[a]	TM-ERG	LR[c]
Benign				
Median (95% CI)	0.8 (0.2-1.5)	256 (128-1020)	9.7 (7.0-12)	NA
PCa				
Median (95% CI)	2.8 (1.6-9.2)	3049 (1947-4988)	51 (15-81)	NA
p-value[b]	0.0001	<0.0001	0.0001	NA
Margin(+)(N=13)	17 (1.3-24)	4355 (2839-14631)	81 (61-111)	NA
Margin(-)(N=20)	2.2 (1.5-4.0)	2016 (832-5003)	17 (8.0-52)	NA
p-value	0.021	0.019	0.003	NA
ROC Analysis				
AUC (%)	83	88	78	96
p-value[d]	*0.004*	*0.090*	*0.001*	NA
Sensitivity (%)	76	76	68	85
Specificity (%)	79	90	100	95
[a]PSMA, TM-ERG: ng/PCR, PCA3: copies/PCR	[b]Mann-Whitney test	[c]Logistic regression analysis	[d]LR compare to individual marker (McNemar)	

A-047

Utility of Stringent Complete Response in routine treatment of Multiple Myeloma patients with novel agents

J. L. García de Veas Silva, C. Bermudo Guitarte, R. Duro Millán, V. Sánchez Margalet, C. González Rodriguez. *Hospital Universitario Virgen Macarena, Sevilla, Spain*

Background: Normalization of serum free light chains (sFLC) ratio in patients with Multiple Myeloma (MM) achieving complete response (CR) may define a deeper degree of response after therapy than that defined by the CR criteria. The stringent CR (sCR) requires normalization of sFLC ratio and absence of clonal plasma cells in bone marrow in addition to the criteria for CR (Negative immunofixation of serum and urine, disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow). The aim of this study is to evaluate the prognostic utility of sCR in patients newly diagnosed with MM treated with novel agents in the routine practice.

Methods: Twenty three patients with MM (10 IgG MM, 5 IgA MM, 2 IgD MM and 6 Bence Jones MM) achieving CR after therapy with Bortezomib/Dexametasone were included in this study. Disease Free Survival (DFS or time after treatment where disease remains stable) was estimated by Kaplan-Meier method and compared by log-rank tests. Cox proportional hazard analysis was performed for multivariate analysis. Serum free light chains were measured by turbidimetry (Freelite) in a SPA PLUS analyzer (The Binding Site Group Ltd, Birmingham, UK) and immunofixation was performed in a HYDRASYS (Sebia, FR) analyzer.

Results: The median follow-up of the patients was 18 months (range 14-31 months). Eleven patients achieved CR and 12 patients achieved sCR. During the period of study there were 8 relapses, six in patients achieving CR and two in patients achieving sCR. The median DFS for patients achieving CR was 18 months and not reached for those achieving sCR. Patients achieving CR had a DFS rate of 24% compared with 75% for sCR (p=0.022). Results showed that achieving a sCR was an independent prognostic factor for survival (HR = 6.57; 95% CI, 1.09-39.80; vs CR; p = 0.039).

Conclusion: The presence of an altered sFLC ratio suggests the existence of a persistent clonal population that is secreting small amounts of monoclonal protein. Our results indicate that sCR represents a deeper response state compared with conventional CR which translates into a longer DFS. Despite the small cohort, analysis of sFLC ratio was able to identify a group of patients with more favorable prognosis and support its inclusion in the response criteria for MM patients treated with novel agents.

A-048

Determination of ROMA Score Performance Using the Roche Elecsys HE4 and CA 125 Immunoassays

M. A. Lasho, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Ovarian cancer is the fifth-leading cause of death in women. Ovarian cancer symptoms are related to the presence of an adnexal mass and are often vague and unspecific. Treatment of women presenting with an adnexal mass and at high risk for ovarian cancer by specialized gynecologic oncologists has been shown to improve patient outcomes. The risk of ovarian malignancy algorithm (ROMA) is a calculation that incorporates the patient's serum concentrations of cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) in conjunction with the menopausal status to calculate a predictive probability of finding epithelial ovarian cancer on surgery in women presenting with an adnexal mass. Women are classified as high-risk or low-risk for ovarian cancer. ROMA has only been validated using HE4 by enzyme immunoassay (EIA) in conjunction with Abbott ARCHITECT CA125 assay

or CA125 by EIA. This study evaluates the use of the Roche Elecsys HE4 and CA125 electrochemiluminescence immunoassay (ECLIA) for the calculation of the ROMA score.

Methods: Serum samples from 114 premenopausal females (75 benign gynecological conditions, 39 epithelial ovarian cancer [EOC]) and 93 postmenopausal females (56 benign gynecological conditions, 37 EOC) were included in the study. Benign gynecological conditions included cysts, cystadenomas, leiomyomas, myomas, or fibromas. Epithelial ovarian cancers included stages I through IV (stage I N=19, II N= 3, III N=45, IV N= 9). HE4 and CA125 were measured using the Roche Elecsys ECLIA on a Roche Cobas e601 instrument. Serum HE4 and CA125 concentrations and menopausal status were used to calculate the ROMA score. Equations used to calculate the ROMA score were as follow: Premenopausal Predictive Index (PI) = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125]; Postmenopausal PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125]; and ROMA score = exp(PI) / [1 + exp(PI)] * 10. Receiver Operating Characteristic (ROC) curve analysis was used to determine optimal clinical cut-points and clinical specificity and sensitivity.

Results: In premenopausal women, the ROMA ROC curve area under the curve (AUC) was 0.95. A ROMA score equal or greater than 1.00 yielded 75% specificity and 95% sensitivity. Using the manufacturer suggested cut-point of equal or greater than 1.14 yielded 84% specificity and 95% sensitivity. In postmenopausal women, the ROMA ROC AUC was 0.94. A ROMA score equal or greater than 2.44 yielded 75% specificity and 95% sensitivity. Using the manufacturer suggested cut-point of equal or greater than 2.99 yielded 86% specificity and 92% sensitivity.

Conclusion: This study established the performance of ROMA score cut-points using the Roche Elecsys HE4 and CA125 immunoassays. This information could serve as guidance for laboratories implementing the ROMA score in clinical practice.

A-049

Comparing the Performance of Newly Developed Heavy Chain/ Light Chain Immunoassays with Serum Protein Electrophoresis and Nephelometric Measurements of Total Immunoglobulin for Monitoring Multiple Myeloma Patients

L. Adie, O. Berlanga, H. Carr-Smith, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Background: Both serum protein electrophoresis (SPEP) and total immunoglobulin (tIg) measurements have been recommended for quantification of monoclonal Ig (M-Ig). However, SPEP is inaccurate at low (<10 g/L) and due to dye saturations at high (>20-30 g/L) concentrations of M-Ig. By contrast tIg measurement is an accurate method but is unable to distinguish between monoclonal and polyclonal Ig. Newly developed Heavy chain/ light chain immunoassays may provide an alternative method of quantifying M-Ig concentrations. Here, we compare the performance of these assays with traditional methods for monitoring MM patients. **Methods:** HLC Ig'κ and Ig'λ were quantified in 127 IgG (87 IgGκ, 40 IgGλ) and 61 IgA (37 IgAκ, 24 IgAλ) MM patient sera. The results were compared to published normal ranges (IgGκ: 4.03-9.78 g/L, IgGλ: 1.97-5.71 g/L, IgGκ/ IgGλ: 0.98-2.75; IgAκ: 0.48-2.82 g/L, IgAλ: 0.36-1.98 g/L, IgAκ/ IgAλ: 0.80-2.04), historic SPEP, immunofixation and tIg concentrations. Weighted Kappa and Pearson correlation were used to analyse results. **Results:** At presentation all 127 IgG and 61 IgA patients had an abnormal HLC ratio and involved HLC (iHLC) concentrations (median (range) IgGκ: ratio 56 (6- 1275), iHLC 32 g/L (14-102); IgGλ: ratio 0.024 (0.001- 0.329), iHLC 34 g/L (9- 90); IgAκ: ratio 233 (10- 6226), iHLC 34 g/L (6- 79); IgAλ: ratio 0.0119 (0.0003- 0.1181), iHLC 29 g/L (6- 72)). Whilst M-Ig concentrations were measurable by SPEP in all IgG patients, only 66% (40/61) IgA patients were quantifiable. In all samples, iHLC and dHLC (involved HLC-uninvolved HLC) concentrations showed a good correlation with SPEP for IgG (iHLC y=0.83x+1.8, R^2=0.87; dHLC y=0.84x+0.48, R^2=0.88), IgA (iHLC y=0.88x+0.88, R^2=0.87; dHLC y=0.89x+0.44, R^2=0.88) and with tIgA measurement in IgA patients (iHLC y=0.87x-0.68, R^2=0.90; dHLC y=0.88x-1.33; R^2=0.90). During the course of the patients disease, changes in iHLC and dHLC concentrations reflected the changes in M-Ig measured by SPEP (IgG: iHLC y=0.87x-0.05, R^2=0.83; dHLC y=0.91x-0.06, R^2=0.86; IgA: iHLC y=1.31x+0.25, R^2=0.87; dHLC y=1.36x+0.28, R^2=0.88) and with tIgA changes in IgA patients (iHLC y=0.94x-0.03, R^2=0.90; dHLC y=0.96x-0.03, R^2=0.90). Responses assigned based on reductions in M-Ig measured by either iHLC, dHLC or traditional methods showed substantial agreement for IgG and near perfect agreement for IgA patients using Weighted Kappa analysis (IgG: iHLC vs. SPEP 81% agreement, Weighted Kappa (95% CI): 0.78 (0.56-1.00); dHLC vs. SPEP 80% agreement, Weighted Kappa (95%CI): 0.77 (0.55-1.00); IgA: iHLC vs. SPEP/tIgA 89% agreement, Weighted Kappa (95% CI): 0.92 (0.84-1.00); dHLC vs. SPEP/tIgA 89% agreement, Weighted Kappa 0.92 (0.84-1.00)). Changes in HLC ratio similarly showed a good comparison to the assigned responses (IgG: 71% agreement, Weighted Kappa (95% CI): 0.74 (0.56-0.92); IgA: 73% agreement, Weighted Kappa (95% CI): 0.86 (0.81-0.91)). **Conclusion:** Responses assigned using reductions in iHLC, dHLC, HLCr or SPEP showed a good agreement. Furthermore, iHLC, dHLC and HLC ratio were able to assign responses in 34% of IgA patients that were not quantifiable by SPEP. The HLC immunoassays provide an alternative method of quantifying M-Ig in patients with MM.

A-050

Serum MicroRNA Panel as Biomarkers for Early Diagnosis of Colorectal Adenocarcinoma

C. Wang, G. Zheng, L. Du, L. Wang, X. Zhang, Y. Yang, J. Li, Y. Wang, Y. Liang. *Qilu Hospital, Shandong University, Jinan, China*

Background: Due to the high mortality of colorectal adenocarcinoma (CAC), there is an urgent need to identify new biomarkers with high sensitivity and specificity. The recent discovery of serum microRNA (miRNA) profile in human cancer has provided a new auxiliary approach for tumor diagnosis. Our study is the first global analysis of serum miRNAs based on the normal-colorectal adenoma (CA)-CAC sequence.

Methods: Serum samples were collected from 307 CAC patients, 164 CA patients and 226 healthy controls. We firstly profiled pooled serum of CAC, CA and healthy controls by Miseq sequencing. The differentially expressed serum miRNAs were chosen as candidate biomarkers for CAC. Both the candidate reference genes and candidate biomarkers were validated by the reverse-transcription polymerase chain reaction (RT-qPCR). The miRNA panel was developed with a logistic regression model and then validated using an independent cohort. Receiver operating characteristic (ROC) curves were constructed, and area under the ROC curve (AUC) was used to evaluate the diagnostic accuracy of the panel.

Results: The Miseq sequencing results revealed 15 differentially expressed miRNAs in CAC patients compared with controls. Using the selected reference gene of miR-191-5p and U6, we identified a 4-miRNA panel (miR-19a-3p, miR-223-3p, miR-92a-3p and miR-422a) with a high diagnostic accuracy of CAC. Even in the low carcinoembryonic antigen (CEA) level group, the diagnostic accuracy of this miRNA panel was still acceptable (AUC = 0.810). Surprisingly, our results indicated that the miRNA panel could differentiate stage I/II CAC patients from controls. In addition, this panel could also differentiate CAC from CA (AUC=0.886).

Conclusions: In the present study, we established a serum miRNA panel with considerable clinical value in the early-stage diagnosis of CAC.

A-051

Total, free, and complexed prostate-specific antigen concentrations among U.S. men, 2007-2010

D. A. Lacher, J. P. Hughes. *National Center for Health Statistics, Hyattsville, MD*

Background: Screening for prostate cancer using prostate-specific antigen (PSA) is common but remains controversial. Prostate cancer has been associated with higher total PSA (tPSA), lower free PSA (fPSA), and lower percent free PSA (fPSA/tPSA x 100%). More recently, higher complexed PSA (cPSA), bound primarilywith α-1-antichymotrpsin, has been associated with prostate cancer. The distributions of total, free and complexed PSA concentrations, percent free PSA and percent complexed PSA (cPSA/tPSA x 100%), and the free/complexed PSA ratio in men were examined in the 2007-2010 National Health and Nutrition Examination

Survey (NHANES).

Methods: Total, free and complexed PSA were performed on 3251 men aged 40 years and older who were examined in the 2007-2010 National Health and Nutrition Examination Survey. NHANES is a cross-sectional, nationally representative, area probability survey of U.S. non-institutionalized participants. Distributions of the PSA tests were examined by age, race and ethnicity, and body mass index (BMI) groups. In addition, percentages of men at total and percent free PSA cut-points were examined. All PSA tests were log-normal in distribution except percent complexed PSA, which was normally distributed. The geometric mean (GM) or arithmetic mean (for percent complexed PSA), standard error (SE) of the mean, and selected percentiles were determined. Age-adjusted means were used for analysis of PSA tests for race and ethnicity and BMI groups.

Results: The geometric mean (SE) for tPSA was 0.96 (0.02) µg/L with 5.2% of men ≥ 4.0 µg/L and 1.1% ≥ 10.0 µg/L. Free PSA had a GM of 0.27 (0.01) µg/L. The GM of percent fPSA was 28.1 (0.3) % and 8.6% of men had ≤ 15% percent fPSA.

Complexed PSA had a GM of 0.53 (0.01) µg/L. The arithmetic mean of percent cPSA was 56.0 (0.5) % and the free/complexed PSA ratio GM was 0.52 (0.01). Total, free, and complexed PSA increased with age. Total PSA GM increased from 0.74 µg/L for men 40-49 years to 1.82 µg/L for men 80 years and older. Free PSA GM increased from 0.22 µg/L for men 40-49 years to 0.51 µg/L for men 80 years and older, while complexed PSA increased from 0.40 µg/L for men 40-49 years to 0.99 µg/L for men 80 years and older. The adjusted mean for non-Hispanic white men had lower tPSA (1.03 µg/L) and cPSA (0.56 µg/L) than non-Hispanic black men (tPSA 1.25 µg/L and cPSA 0.72 µg/L). Hispanic men had higher cPSA (0.64 µg/L) than non-Hispanic white men. Obese men had lower age-adjusted mean total, free and complexed PSA (0.94, 0.27, and 0.51 µg/L, respectively) than men with normal BMI (tPSA 1.21, fPSA 0.32, and cPSA 0.68 µg/L).

Conclusion: The free and complexed PSA may provide additional information in conjunction with total PSA in screening for prostate cancer. Total, free and complexed PSA increased with age; total and complexed PSA were highest in non-Hispanic black men; and obese men had the lowest total, free, and complexed PSA.

A-052

Leptin and insulin hormones increase Sam68 expression and phosphorylation in human breast adenocarcinoma cells

F. Sánchez-Jiménez, A. Pérez-Pérez, C. González-Rodríguez, J. A. Virizuela, V. SANCHEZ-MARGALET. *VIRGEN MACARENA UNIVERSITY HOSPITAL, SEVILLE, Spain*

Background: Obesity and insulin resistance are well known risk factors for breast cancer development in postmenopausal women. High insulin levels, together with other hormones, such as leptin and cytokines, IGFs, estrogen and EGF, positively modulate the growth of these tumor cells. All these factors may act through signaling cascades that lead to the final effect of increasing growth and cell proliferation. Sam68 protein is a member of the signal transduction activator of RNA (STAR) family of RNA-binding proteins that can interact both with RNA and signaling proteins. According to this dual role, Sam68 has been involved in different carcinogenic mechanisms including alternative splicing or cell cycle regulation. Moreover, our group has previously described the role of Sam68 in the insulin and leptin signaling pathways as a receptor substrate, and it has been shown to participate in proliferation, cellular growth and antiapoptotic effects mediated by these hormones in different cellular types.

Objective: We aim to study the expression of Sam68 and its phosphorylation level upon insulin and leptin stimulation, seeking for a possible role of Sam68 in leptin and insulin receptor signaling in human breast adenocarcinoma cells.

Methods: We used the human breast adenocarcinoma cell line MCF7. We studied leptin-mediated and also insulin-mediated Sam68 phosphorylation by immunoprecipitation and immunoblot with anti-phosphotyrosine antibodies as well as polyU affinity precipitation. Quantitative RT-PCR and immunoblot were used to study the effect of leptin and insulin on Sam68 expression. siRNA was used to downregulate Sam68 expression and its effects on leptin and insulin activation of MAPK and PI3K pathways. Phosphorylation of some of the main proteins of these pathways (ERK1/2 and MEK as well as PKB and P70s6K respectively) was tested by using immunoblot with antibodies against phosphorylated proteins and anti-tubulin as loading control.

Results: Sam68 protein quantity and gene expression were found to be increased under leptin as well as insulin stimulation, by using 1 nM dose after a 24 hours stimulus. Moreover, both insulin and leptin stimulation promoted an increase in Sam68 tyrosine phosphorylation in MCF7 cells and negatively regulated RNA binding of Sam68, as previously observed in other systems. Sam68 downregulation resulted in lower activation of MAPK and PI3K pathways under both hormones stimulation. Sam68 was necessary for the complete leptin and insulin phosphorylation of the main proteins of these pathways.

Conclusion: These results suggest the participation of Sam68 in both leptin and insulin receptor signaling in human breast cancer cells, where Sam68 could mediate the trophic effects of these hormones in proliferation and cellular growth. Thus, Sam68 could be also considered as a future prognostic marker or therapeutic target in this kind of non genetic breast cancer.

A-053

MiR-28-5p, a potential biomarker for renal cell carcinoma, acts as a tumor suppressor in renal cell carcinoma for multiple antitumor effects by targeting RAP1B

C. Zhang[1], C. Wang[1], C. Wang[1], C. Wu[1], Q. Yang[1], C. Zhang[2]. *[1]Jinling Hispital, Nanjing, China, [2]Nanjing Univertisy, 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 investigate whether serum miR-28-5p could be a useful biomarker for the diagnosis of RCC and evaluate the functional significance of the miR-28-5p in RCC. **Methods:** This study included 33 RCC patients and 33 healthy controls. First, we analyzed tissue miR-28-5p levels in tumor tissues and matched normal tissues from the 33 RCC patients. Second, we investigated the serum miR-28-5p levels in the 33 RCC patients and the 33 normal controls. TaqMan probe based-RT-qPCR was used to measure serum miRNA levels. A combination of let-7d, let-7g and let-7i (let-7d/g/i) was used as endogenous control for normalizing the data of RT-qPCR. We also examined the expression level of miR-28-5p in some human RCC cell lines. The CCK8 proliferation, transwell and wound healing assays were used to explore the potential functions of miR-28-5p in RCC cells. Luciferase reporter assays were employed to validate regulation of a putative target of miR-28-5p. The effect of modulating miR-28-5p on endogenous levels of this target were subsequently confirmed via Western blotting. **Results:** MiR-28-5p expression was relatively decreased in RCC specimens compared with adjacent normal tissues ($P<0.01$). Consistent with the results from tissues, serum miR-28-5p levels were decreased in RCC patients compared to controls ($P<0.001$). ROC curve analysis showed an AUC of 0.90 (95% confidence interval, 0.85-0.95) and a sensitivity and specificity of 95 and 86%, respectively. MiR-28-5p was found to be also downregulated in human RCC cell lines A498 and Caki-2 as compared with normal cell line HK-2. Luciferase reporter assays showed that miR-28-5p directly regulated RAP1B. In RCC clinical specimens, the expression of RAP1B protein was significantly higher in cancer tissues than in non-cancerous tissues. Statistical analysis results indicated that the RAP1B protein level was negatively correlated to the miR-28-5p expression in RCC tissues ($P<0.05$). RAP1B protein was found to be upregulated in A498 and Caki-2 cells, and knockdown of RAP1B inhibited cell proliferation and migration, suggesting that RAP1B has oncogenic functions in RCC. Ectopic expression of miR-28-5p could result in increased RAP1B protein expressions, and inhibited proliferation and invasion of A498 and Caki-2 cells, while the downregulation of miR-28-5p with the inhibitor had the opposite effect. The miR-28-5p induced cell proliferation and migration could be rescued by RAP1B. **Conclusion:** MiR-28-5p may potentially serve as a novel biomarker for RCC and may act as a tumor suppressor in RCC progression by inhibiting the RCC cell proliferation and migration through targeting oncogeneRAP1B. Our findings indicate that targeting miR-28-5p by a genetic approach may provide a novel strategy for the treatment of RCC.

A-054

Serum thyroglobulin measurement in autoantibody positive samples by LC-MS/MS and immunoassay: is positivity rate different between the methods?

M. M. Kushnir[1], A. L. Rockwood[2], A. W. Meikle[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT, [3]Departments of Pathology and Medicine, University of Utah, Salt Lake City, UT*

Background: Measurement of thyroglobulin (Tg) in serum and plasma is used to monitor patients after treatment for differentiated thyroid carcinoma (DTC). A complicating factor in using Tg as biomarker of the recurrence of DTC is related to the presence of endogenous anti-Tg autoantibodies (Tg-AAb) in blood of many patients. Tg-AAb can interfere with immunoassay (IA) measurements and cause false-negative results. LC-MS/MS methods for Tg are expected to overcome Tg-AAb interference with measurement of Tg, but there were no studies supporting better utility of the LC-MS/MS methods for Tg in Tg-AAb positive samples.

Methods: Recently we developed LC-MS/MS method for measurement of Tg in serum samples (Clin Chem 2013;59:982-90). The lower limit of quantification is 0.5ng/mL; total imprecision is below 10%. We performed comparison of LC-MS/MS and Beckman IA using Tg-AAb negative and positive samples; and reviewed

historical data on analysis of Tg in Tg-AAb positive samples using Beckman IA (n=1367) and LC-MS/MS (n=6180) methods, by comparing Tg positivity rates for the assays. Tg calibrators were standardized between the methods. Measurement of Tg-AAb was performed by Beckman Antibody-II assay.

Results: In a set of Tg-AAb negative samples Tg concentrations determined with Beckman IA agreed well with LC-MS/MS (IA=1.00*LC-MS/MS-2.35, r=0.982, $S_{y.x}$=9.52); IA underestimated Tg concentrations in Tg-AAb positive samples. In a set of Tg-AAb positive samples tested negative for Tg using IA, concentrations determined by LC-MS/MS method were at or above 0.5ng/mL in 23% of samples. Positivity rate for different Tg cutoff concentrations and differences in the positivity rate between the methods are shown in Table.

Conclusions: Higher Tg positivity rate in Tg-AAb positive samples was observed by LC-MS/MS method as compared to the IA; the difference is likely caused by underestimation of Tg concentrations caused by interference of Tg-AAb with the IA.

Table. Tg positivity rate in Tg-AAb positive samples analyzed by LC-MS/MS and Beckman Coulter immunoassay.

	Percent of samples with concentration above the cutoff		
Tg cutoff concentration, ng/mL	LC-MS/MS, %	Beckman Access, %	Difference, %
>0.5	37.5	37.0	0.5
>0.6	37.1	34.8	2.3
>1	33.4	30.0	3.4
>2	26.4	23.9	2.5
>5	18.3	16.6	1.7
>7	15.1	13.8	1.3
>10	12.5	11.4	1.1
>15	10.1	9.2	0.9
>20	8.1	7.6	0.5

A-055

Value of a new biochemical parameter (serum Heavy chain/Light Chain pairs) in the follow-up of Multiple Myeloma after treatment

R. P. Garay, I. J. Ventura, E. A. Diez, E. Landeta, I. Amariko, A. G. Vicuña, A. S. Izarra. *Hospital Universitario Cruces, Bilbao, Spain*

Background:Multiple Myeloma (MM) has an incidence of 1-10% of all cancers. The laboratorial assays usually used for its diagnosis and follow-up are: detection of the monoclonal protein by Serum Protein Electrophoresis (SPE) and Immunofixation (IF) in serum and 24h urine, total immunoglobulins levels by nephelometry and serum Free Light Chains (sFLC). Determination of the bone marrow plasma cells, lytic lesions by MRI, complete blood count, creatinine and calcium levels are also used. Recently, a new technique that allows the analysis of immunoglobulin heavy chain/light chain pairs (Hevylite®) has been developed.Objectives:The sFLC determination has been introduced in 2006 in the Stringent Complete Response subcategory by the International Myeloma Working Group (IMWG), corresponding to a Complete Response plus normal FLC ratio and absence of clonal cells in bone marrow. The reasons for its inclusion are that it is a highly sensitive marker for sFLC and an excellent indicator of clonality (Durie et. al., Leukemia 2009). A recent study has further corroborated its correlation with a more stringent level of response with clinical prognostic impact (Kapoor et. al., JCO 2013). The aim of this study is to evaluate the utility of the Hevylite ratio (HLCr) together with sFLC in the follow-up of 3 MM IgG patients under treatment and follow-up.

Methods:sFLC and HLC were measured by turbidimetry (FreeliteTM and Hevylite®, on a SPAPLUS, Binding Site). The monoclonal protein was identified and quantified by electrophoresis (Capillarys Hydrasys Focusing, Sebia). Samples from 3 MM patients were analyzed:

-Patient 1: 58 years old woman with an IgG-L type MM stage II-A. 13 samples collected from June 2011 to July 2013. 1st line treatment (Velcade-Dexamethasone-Adriamycin) and TASPE (July 2012).

-Patient 2: 61 years old man with IgG-K MM stage II-A, ISS-2. 14 samples from May 2011 to November 2013. 1st line treatment (Velcade-Dexamethasone-Adriamycin) and TASPE (May 2012).

-Patient 3: 76 years old woman with IgG-K MM stage II, ISS-2. 14 samples from March 2013 until January 2014. 1st line treatment (Bortezomib-Melphalan-Prednisone) and 2nd line treatment (Cyclosphophamide-prednisone).

Results:-Patient 1: in sCR since December 2012. sFLC and HLC ratios normalized with absence of monoclonal protein by SPE and IF.

-Patient 2: achieved CR after TASPE, at this time in biochemical relapse. After treatment, the HLCr normalized in May 2012, the SPE became negative in August 2012 and the IF in November 2012, achieving a CR. The sFLC ratio is abnormal throughout follow-up, and currently there is a monoclonal protein detected by IF and SPE.

-Patient 3: Refractory MM with stable disease: abnormal sFLC and HLC ratios with 4.23 g/dL of monoclonal protein.

Conclusion:The inclusion of the Hevylite assay allows the quantitative follow-up of monoclonal immunoglobulins, particularly interesting when patients achieve a deepness of response where the standard SPE and IF become negative. Results obtained with Hevylite assay are in agreement with the other results. This preliminary data suggests that the new HLC assay may add specificity to the Stringent Complete Response. More studies are required to establish its prognostic value in the response evaluation.

A-056

Performance of the Roche Elecsys Thyroglobulin (Tg) II immunoassay

B. C. Netzel, M. A. Lasho, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Serum thyroglobulin (Tg) measurement is considered the gold standard in the follow-up of patients with differentiated thyroid cancer following total thyroidectomy and radioactive iodine ablation. In athyrotic patients, Tg is an excellent tumor marker because it is produced exclusively by the follicular cells of the thyroid. Performance of currently available Tg immunoassays varies due to different assay sensitivities, standardization against the certified reference material (CRM-457), and Tg autoantibody (TgAb) interference, among others. In this study we evaluated the analytical performance of the Roche Elecsys Tg II immunoassay.

Methods: The Roche Elecsys Tg II immunoassay (Roche Diagnostics, Indianapolis, IN) is a quantitative, two step, double antigen sandwich assay standardized against CRM-457 for the measurement of thyroglobulin in serum and plasma using the electrochemiluminescence immunoassay "ECLIA" technology. The assay uses 35 µl of sample and has a total assay time of 18 minutes. Testing was performed on the Roche Diagnostics cobas e411 analyzer. Accuracy was investigated by recovery studies using CRM-457. Imprecision studies were conducted using Liquichek™ Tumor Marker Control (Bio-Rad, Hercules, CA) and Roche PreciControl quality control (QC) materials. Analyte measurement range (AMR) studies were conducted by diluting a high concentration Tg serum sample with a negative Tg serum sample. LOQ studies were conducted using a low concentration Tg serum sample. Method comparison studies with the Beckman Access Tg assay (Beckman Coulter, Brea, CA) and an in-house developed LC-MS/MS Tg assay was performed using de-identified serum samples collected for routine Tg determination.

Results: Average recovery of CRM-457 was 106% (range 101-111%). Inter-assay imprecision studies produced coefficient of variation (CV) of <6% (range 1.4-5.5%) at concentrations of 6.4, 21.8, 85 and 188 ng/mL. The AMR of the assay was 0.1 to 500 ng/mL with Passing-Bablock regression fit of y = 0.96x -0.32 (r^2=0.999). Serial dilutions (x2 to x64) to expand the AMR produced an average recovery of 97% (range 91-104%). LOQ was determined to be 0.1 ng/mL (CV = 18%). The assay was compared to the Beckman Access Thyroglobulin assay (N=37, range 0.1-500 ng/mL). The Spearman correlation coefficient was 0.990 with a slope of 1.28 and intercept of -0.14 by Passing-Bablock regression fit. Comparison with the in-house Tg LC-MS/MS assay (N=129, range 0.5-500 ng/mL) produced a Spearman correlation coefficient of 0.943 with a slope of 1.58 and intercept of -0.69 by Passing-Bablock regression fit.

Conclusion: The Roche Elecsys Tg II shows good analytical performance and provides reliable Tg measurement for the management of thyroid cancer patients.

A-057

Single Cell Analysis of Heterogeneous Circulating Tumor Cell Populations

L. M. Millner[1], K. S. Goudy[2], M. W. Linder[3], R. Valdes Jr[3]. *[1]University of Louisville, Louisville, KY, [2]PGXL Technologies, Louisville, KY, [3]University of Louisville and PGXL Technologies, Louisville, KY*

Introduction: Enumeration of circulating tumor cells (CTCs) in blood is used in breast cancer patients as an independent predictor of outcome. Present methods do not distinguish subtypes and only detect epithelial-type CTCs. This is significant because CTCs experience epithelial to mesenchymal transition (EMT), a process that increases motility, disease progression, and decreases epithelial marker expression. This process may also change the CTC susceptibility to chemotherapeutics and eligibility for treatment. For example, patients with overexpression of HER2 are eligible for treatment with Herceptin. Examining CTCs as a bulk population may mask individual overexpression of markers used for targeting therapy. Here we describe a comprehensive method for characterizing the molecular heterogeneity of CTCs which could play an important role in directing personalized cancer therapeutics.

Objective: To develop a method for identifying single cell heterogeneity within a population of circulating tumor cells.

Methods: As a model, we used the breast cancer cell line MDA-MB-231 (MDA). This cell line was chosen because it has low expression of EpCAM and may represent cells that have experienced EMT. MDA cells are typically negative for EpCAM and HER2 protein expression but do express CD44. Cells were sorted into single cell populations using DEPArray Technology (Silicon Biosystems) and analyzed by single cell RT-PCR for 3 targets (EpCAM, ErbB2 (HER2), and CD44) and a housekeeping gene (ACTB). Using DEPArray technology the mean fluorescent intensity (MFI) of EPCAM, CD44 and HER2 protein expression was measured on 3,656 individual cells and MFI signals greater >1000 were considered positive.

Results: ACTB transcript expression as a positive control was confirmed in each of the eleven cells. EpCAM transcript expression was not detectable in any of the cells. CD44 transcript expression was observed in 10/11 (91%) and ErbB2 (HER2) expression was observed in 4/11 (36%) cells. We then measured the mean fluorescent intensities (MFI) for each of the cell surface target antigens on 3,656 individual cells. The MFI values [mean ± SD (range)] were: EpCAM, 525±91 (369-870); CD44, 3722±1598 (1223-13963), and HER2, 562±154 (389-6312).

Conclusion: The wide range of MFI signaling for HER2 demonstrates a discrete subpopulation of cells expressing high levels of HER2 within a population that on average expresses no or very low levels of HER2. This single cell analysis method may provide identification of a subpopulation of Herceptin-responsive cells within an apparently non-responsive group. Single cell subtyping has the potential to facilitate individually tailored therapies based on each patient's heterogeneous CTC profile.

A-058

Prognostic Biomarker Isocitrate Dehydrogenase-1 Mutations in Patients with Glioblastoma Multiforme

J. Polivka[1], J. Polivka[2], V. Rohan[2], M. Pesta[3], T. Repik[2], O. Topolcan[4]. *[1]Department of Histology and Embryology and Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [2]Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and Faculty Hospital Plzen, Plzen, Czech Republic, [3]Department of Biology, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [4]Central Imunoanalytical Laboratory, Faculty Hospital Plzen, Plzen, Czech Republic*

Background Glioblastoma multiforme (GBM) is the most malignant primary brain tumor in adults with high mortality. Standard therapy (surgery, radiotherapy and chemotherapy with temozolomide) has only limited effectiveness and the median survival of patient with GBM is 12.1 - 14.6 months. Recent GBM whole-genome studies revealed some novel prognostic and predictive biomarkers such as the recurrent mutations in metabolic enzyme IDH - Isocitrate dehydrogenase (isoforms IDH1 and IDH2). The distinctive mutation IDH1 R132H was uncovered to be a strong prognostic biomarker for glioma patients. Therefore we investigated the prognostic role of IDH1 R132 mutation in our GBM patient cohort.

Methods The IDH1 R132H mutation status was assessed in the Formalin-Fixed Paraffin-Embedded (FFPE) tumor samples from 44 GBM patients treated in the Faculty Hospital Plzen between 2008 and 2013. The real-time PCR with TaqMan® mutation detection assays and TaqMan® mutation detection IPC reagent kit was used. The IDH1 R132H mutation status was correlated with the progression free survival (PFS) and overall survival (OS) of patients using Kaplan-Meier survival analysis and Wilcoxon test.

Results The IDH1 R132H mutation was identified in 20 from 44 GBM tumor samples (45,4%). The majority of mutated tumors were secondary GBMs (16 in 18, 89.9%). Low frequency of IDH1 mutations was observed in primary GBMs (4 in 26, 15.3%). Patients with IDH1 R132H mutation had longer PFS - 136 vs. 51 days ($P<0.021$) as well as OS - 270 vs. 130 days ($P<0.024$).

Conclusion The prognostic value of IDH1 R132H mutation in GBM patients was observed in our study. Patients with this mutation had significantly longer PFS and OS than patients with wild-type IDH1 and suffered more likely from secondary GBMs. The IDH1 mutation status could be used as a strong prognostic factor for patients with GBM and should be further studied in larger patient cohort.

Supported by MH CZ - DRO (Faculty Hospital Plzen - FNPl, 00669806) and by the project ED2.1.00/03.0076 from the European Regional Development Fund.

A-059

New monoclonal antibodies detect all immunoglobulin free light chains in urine samples from over 13,000 patients

J. Campbell, J. Heaney, Y. Wang, M. Cobbold, M. Goodall, T. Plant, M. Drayson. *University of Birmingham, Birmingham, United Kingdom*

A decade or so ago, the first automated assay was launched for the quantitation of serum κ and λ immunoglobulin free light chains (FLC). FLC measurement is now a fundamental procedure in the diagnosis and monitoring of patients with plasma cell dyscrasias including multiple myeloma and related subtypes including light-chain-only, oligosecretory and non-secretory myeloma. Despite these advances, the assay, which uses sheep polyclonal anti-human FLC antibodies, has a number of well-observed limitations. It has been proposed that monoclonal antibodies (mAbs) may overcome these limitations. The development of FLC specific mAbs is difficult because the mAbs must demonstrate specificity for epitopes that are exposed on FLC but hidden on LC bound to whole immunoglobulin. This is complicated by the paucity of constant domain epitopes available; which can be further reduced by polymerisation of FLC, particularly FLC λ, thus reducing the number of potential binding sites. Production of mAbs specific for FLC has been described previously but other groups have either found that their mAbs did not detect FLC from all neoplastic plasma cell clones tested, or, have not tested sufficient clones to be confident that the mAbs would detect the FLC from 100% of neoplastic clones. Hence, the purpose of this study was to prospectively assess the clinical utility of new highly-specific mouse anti-human FLC mAbs on a large number of consecutive patient samples. Anti-κ and anti-λ FLC mAbs were covalently coupled to different polystyrene Xmap® beads and assayed, simultaneously, in a multi-plex format by Luminex® (mAb assay). The mAbs displayed no cross-reactivity to bound LC, the alternate LC type, or other human proteins and had improved sensitivity (<1mg/L) over the gold standard for identifying paraprotein, immunofixation electrophoresis (IFE; approximate sensitivity is 10mgL). The competitive inhibition format gave a broad calibration curve (up to 437.5 mg/L) and prevented anomalous results for samples in antigen excess (i.e. high FLC levels). The mAb assay had no false negatives and identified all monoclonal FLC in 13,090 urine samples tested (22.8% with monoclonal κ and 9.0% with monoclonal λ by IFE), and also detected all samples with polyclonal FLC. In a small cohort of Bence Jones positive samples (n=100), the mAb assay correlated excellently with densitometry, the gold standard for quantitating urine FLC. Importantly this shows that the mAbs are close to the ideal of detecting FLC from all patients and neoplastic plasma cell clones, and may be the first published mAbs with this clinical utility. Given the overall effectiveness of the anti-FLC mAbs, further clinical validation is now warranted on these mAbs in other assay platforms they are incorporated, including Seralite, a rapid (10-minute) and portable FLC test to be used at the point-of-care.

A-060

Development of RT-qPCR Assays for The Detection of Circulating Tumor Cells in Breast Cancer

S. Ahn[1], S. Park[2], H. Wang[3], S. Park[1], S. Kim[4], Y. Kim[1], H. Kim[1], G. Kim[1], J. Kim[1], H. Jin[5], S. Kim[6], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of, [3]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [4]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Surgery, College of Medicine, Yonsei University, Seoul, Korea, Republic of*

Background: Cancer cells which become detached from the primary tumors and enter into the systemic circulation are called circulating tumor cells (CTCs). The human epidermal growth factor receptor 2 (*HER2*, also known as *erbB2*) is crucial for treatment of breast cancer patients. HER2 is over-expressed in 20 to 30% in blood samples of breast cancer patients. Especially, HER2 gene's over-expression is associated with a poor clinical outcome. Therefore, the detection of HER2 expressing CTCs in the blood may have important prognostic and therapeutic treatment implications. In this study, apart from using HER2, EpCAM, CK-19, Ki-67, and hTERT were used for detection CTCs in peripheral blood of breast cancer patients. Furthermore, correlation between HER2 and CTC markers mRNA level in the blood were determined.

Methods: Human breast carcinoma cell line SK-BR-3, MCF-7 and MDA-MB-231 were used for the development of the assay and the confirmation of HER2, EpCAM,

CK-19, Ki-67, hTERT and GAPDH expression. A Total of 188 breast cancer patients who include 34 ductal carcinoma in situ (DCIS) patients, 93 stage I patients, 58 stage II patients and 3 stage III patients. A total of 50 healthy donors who did not have a breast cancer were also enrolled for this study. All blood samples were handled for extracting total RNA using TRIzol Reagent (Invitrogen, Carlsbad, California, USA) then the cDNA was synthesized. The mRNA expression levels of HER2, EpCAM, CK-19, Ki-67, and hTERT relative to GAPDH were measured by RT-qPCR TaqMan assay.

Results: Among a total of 188 patients, 39 patients (20.7%) displayed an over-expression of HER2 mRNA, while none of the healthy blood donors over-expressed HER2 mRNA. In 37 out of 39 HER2 positive patients, not only HER2 mRNA, but also at least one other type of marker was overexpressed at the same time. Among the 149 HER2 negative patients, 114 patients (76.5%) were positive at least one other type of marker. The HER2 mRNA levels in blood had a correlation with Ki-67 mRNA level (Pearson r=0.4358, R square=0.2360) and hTERT mRNA level (Pearson r=0.2988, R square=0.0893) in blood. As the breast cancer stage progress, patients who were over-expressed tumor association markers, such as hTERT, Ki-67, and HER2 were tend to increasing. On the other hand, expression of CTC epithelial markers, such as EpCAM and CK-19 not seem to have correlation with stage of cancer.

Conclusion: In conclusion, HER2 expression in the blood occurs concurrently with CTC markers. In this reason, CTC markers could be used for detection CTCs in blood of breast cancer patients. The results from this study seems to suggest that detection of CTCs using CTC markers and HER2 allow for more effective management of and better prognosis for breast cancer.

A-061

Analytical Evaluation of a Newly Developed ELISA for the Detection of Soluble Tumour Necrosis Factor Receptor 2 (sTNFRII) in Sera from Patients with Ovarian Cancer

A. Chacko, P. Ratcliffe, L. Stevenson, J. Lindsay, P. Lowry, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: There has been significant interest in discovery of biomarkers and biomarker panels for early detection of precancerous ovarian tumours. Various screening strategies combining serum-based markers with other clinical parameters are being tested in on-going trials. To date, no serum-based markers have been proven to improve diagnostic performance over the standard ovarian cancer marker CA-125, either alone or as part of a biomarker panel. Previous studies have suggested that sTNFRII levels may increase during tumour progression in different cancer types, including ovarian cancer. This study reports the analytical evaluation of a new developed enzyme-linked immunosorbent assay (ELISA) for the specific and sensitive detection of sTNFRII and its applicability to the study of patients with ovarian cancer. Methods: Sheep were immunized with the extracellular domain of the sTNFR II recombinant protein. Lymphocytes were collected and fused with heteromyeloma cells. Hybridomas were screened for immunoreactivity against native human sTNFRII. Hybridomas which showed strong reactivity to native antigen and <1% reactivity to cross reactants were selected for cloning to produce stable monoclonal hybridomas. An optimal antibody pair was identified for development of a sandwich ELISA platform for detection of sTNFRII in human serum. sTNFRII levels were determined in sixty one serum samples (34 from ovarian cancer patients and 27 healthy female controls). Statistical analysis was performed and box plots / Receiver Operating Characteristics (ROC) curves were constructed using GraphPad Prism. Results: The assay detected native sTNFRII across an assay range of 0-16ng/ml. The limit of detection,defined as the analyte concentration corresponding to an absorbance equal to blank mean plus 2xSD (n=20), was 0.324ng/ml. Intra-assay precision data (n=12) showed recovery at 103.6% ±10.4% (10.0% CV) for 2ng/ml, and 86.5% ±5.1% (5.9% CV) for 4ng/ml sTNFRII. Patient sera were run at a dilution of 1 in 5 to best match the expected clinical range for sTNFRII, giving an effective assay range of 1.6-80ng/ml. Median sTNFRII levels were significantly increased in ovarian cancer patients compared to healthy females (22.7ng/ml v 5.3ng/ml, Mann Whitney $p<0.0001$), which gave an area under the curve (AUC) of 0.907 for ovarian cancer versus healthy females. Conclusion: The results indicate that this newly developed ELISA is applicable to the specific detection of sTNFRII in human serum. The significant increase in median sTNFRII levels in serum from ovarian cancer patients, when compared to controls, indicates that this assay may be suitable for further studies into the use of sTNFRII as a marker for cancer diagnosis and monitoring.

A-062

Examination of Thyroglobulin and Thyroglobulin Antibody Testing Processes for an Urban Endocrine Center

S. E. Wheeler, L. Liu, H. Blair, O. Peck Palmer. *University of Pittsburgh, Pittsburgh, PA*

BACKGROUND: The American Society of Cancer estimates in 2014 ~62,980 individuals will be diagnosed with thyroid cancer. Patients and physicians require accurate and timely in-house thyroglobulin results. Specifically, in differentiated thyroid carcinoma (DTC), the most common thyroid cancer, thyroglobulin (Tg) is used to assess disease recurrence in patients who have undergone thyroidectomy. Commercially available Tg immunoassay methods are most common but are susceptible to Tg antibody (TgAb) interference. In most laboratories TgAb is quantified and manufacturer cutoffs are used to categorize a specimen as TgAb negative (Tg analyzed by immunoassay) or TgAb positive (Tg measurement is referred to an alternate methodology). Common methodologies include the Tg radioimmunoassay (RIA) method and the recently available LC/MS/MS methodology. Examination of alternative test options is critical as referring samples to outside laboratories increases result turnaround time and patient costs.

OBJECTIVE: To determine the optimal testing algorithm for a large endocrine center, we examined the correlation between the current in-house immunoassay methodology for Tg measurement with RIA and LC/MS/MS methodologies using patient specimens categorized with low or high TgAb concentrations.

MATERIALS AND METHODS: Excess samples (n=40; -80°C storage) were obtained from outpatient adults as well as 10 healthy volunteer specimens. Patient specimens were divided into 2 groups: 20 TgAb <20 U/L specimens; 20 TgAb >20 U/L specimens. Samples were analyzed for TgAb using a solid phase enzyme labeled chemiluminescent sequential immunometric assay (Siemens Immulite 2000 XPi, Erlangen Germany; manufacturer's cutoff of 20 U/L). Tg was analyzed using a simultaneous one-step immunoenzymatic assay (UniCel™ DxI 800 automated analyzer, Beckman Coulter, CA; all samples), a RIA method (USC Endocrine Laboratories, CA; used only for samples TgAb >20 U/L), and LC/MS/MS methodology (Quest Diagnostics, Nichols Institute, CA; all samples).

RESULTS: Overall high correlation was demonstrated between the DXI800 and LC/MS/MS methodologies (R^2=0.99; slope = 1.309). Specimens with low TgAb (<20 U/L) demonstrated good correlation between the DXI800 and the LC/MS/MS methodologies (R^2=0.99; slope = 1.312). We examined the effects of TgAb interference (TgAb >20 U/L) and found a good correlation between the DxI800 and the LC/MS/MS methodology (R^2 = 0.97; slope = 1.243). However,Tg measurement between the RIA and LC/MS/MS methods was lower (R^2 = 0.82) particularly for specimens with Tg concentrations >13ng/mL (>13 ng/mL; R^2 = 0.67). TgAb interference was reflected in the method comparison of the DxI 800 and RIA (R^2 = 0.76; slope = 1.216) methods. We observed high variability between TgAb methods similar to previous studies.

CONCLUSIONS: The high correlation between LC/MS/MS and DXI800 suggests that both methods may be appropriate for our patient population. However, before LC/MS/MS testing is placed into routine use our findings warrant validation in a larger TgAb defined population.

A-063

The upregulation of ICAM-1 mediated by leptin is Rho/ROCK-dependent and enhances gastric cancer cell migration

C. Wang, Z. Dong, Y. Yang, L. Du, G. Zheng, G. Zheng, W. Li, X. Zhang, Z. Li, L. Wang, J. Li, H. Liu. *Qilu Hospital, Shandong University, Jinan, China*

Background: Gastric cancer (GC) ranks as the second leading cause of cancer-related death in the world. Adipocytes provide fatty acids for rapid tumor growth, and the dysfunction of lipid metabolism can lead to the pathogenesis of human GC. Leptin is an adipokine of the obesity (ob) gene, and our previous study showed that leptin promote GC cell invasion by AKT/MT1-MMP pathway. However, the exact effect and the underlying mechanism of leptin in GC metastasis remain unclear. Intercellular adhesion molecule-1 (ICAM-1) is overexpressed and plays crucial roles in tumor metastasis. This study aimed to characterize the influence of leptin on ICAM-1 expression in GC and elucidate its underlying molecular mechanism.

Methods: Archived paraffin-embedded GC tissues and matched adjacent normal gastric tissues were collected from 84 patients who underwent surgery for primary gastric carcinoma. The expression of leptin and ICAM-1 were detected by immunohistochemistry, and the correlation of two proteins was further analyzed. The

effect of leptin on GC cell (AGS and MKN-45 cells) migration was measured by transwell. The level of ICAM-1 at both mRNA and protein were detected by RT-PCR and western blot after treatment with leptin. Moreover, the cell surface ICAM-1 and sICAM-1 were detected by flow cytometry and ELISA. ICAM-1-siRNA was designed and transiently transfected in GC cells. RhoA GTPase activity was detected using the G-LISA RhoA activation assay kit. Correlations of leptin and ICAM-1 expression with clinicopathologic factors were analyzed by Kruskal-Wallis test or Mann-Whitney U test, as appropriate. Chi-squared test was applied to analyze the correlation of leptin and ICAM-1 respectively. Other data from experiments were analyzed by paired Student's t-test or one-way ANOVA wherever appropriate. $P<0.05$ was statistical significance.

Results: Immunohistochemical analysis revealed that leptin (48/84, 57.1%) and ICAM-1 (54/84, 64.2%) were overexpressed in GC tissues, and they were positively correlated with each other ($P<0.001$), as well as with the clinical stage and lymphatic metastasis. In transwell assay, leptin promoted GC cell (AGS and MKN-45) migration in a time- and dose-dependent manner. Furthermore, leptin induced GC cell migration by upregulating ICAM-1 expression (mRNA: 4.06±0.54-fold for AGS, $P<0.001$; 2.56±0.33-fold for MKN-45, $P=0.005$. Protein: 3.07±0.25-fold for AGS, $P<0.001$; 2.9±0.26-fold for MKN-45, $P=0.003$), and knockdown of ICAM-1 by small interference RNA (siRNA) blocked this process (AGS: 53.9%±3.6%, $P=0.020$; MKN-45: 42.79%±3.78%, $P=0.005$). Notably, the surface expression of ICAM-1 (AGS, $P<0.001$; MKN-45, $P<0.01$), as well as the soluble ICAM-1 (sICAM-1) (AGS, $P<0.05$; MKN-45, $P<0.01$), was also enhanced by leptin. Moreover, leptin increased ICAM-1 expression through Rho/ROCK pathway, which was attenuated by pharmacological inhibition of Rho (C3 transferase) at 0.25 μg/mL (AGS, $P<0.01$; MKN-45, $P<0.01$) or inhibition of its downstream effector kinase Rho-associated protein kinase (ROCK) (Y-27632) at 3.3 μM (AGS, $P<0.01$; MKN-45, $P<0.01$), suggesting an essential role of Rho/ROCK pathway in this process.

Conclusions: Our findings indicate that leptin enhances GC cell migration by increasing ICAM-1 expression through Rho/ROCK pathway, which may provide preliminary experimental clues for the development of new therapies against the metastasis of GC.

A-064

hCG candidate epitopes for improving the measurement of hCG: results from the second ISOBM TD-7 workshop

P. M. Hemken[1], E. Paus[2], C. Sturgeon[3], W. Stewart[4], J. Skinner[1], L. Harwick[1], S. Saldana[1], C. Ramsay[1], K. Rupprecht[1], K. H. Olsen[2], J. M. Bidart[5], U. H. Stenman[6], P. Berger[7]. *[1]Abbott Laboratories, Abbott Park, IL, [2]Oslo University Hospital, Oslo, Norway, [3]Royal Infirmary, Edinburgh, United Kingdom, [4]Ninewells Hospital and Medical School, Dundee, United Kingdom, [5]Institut Gustave-Roussy, Villejuif, France, [6]Helsinki University Central Hospital, Helsinki, Finland, [7]University Innsbruck, Innsbruck, Austria*

The purpose of this collaboration was to determine specificity profiles and epitopes recognized by diagnostically relevant antibodies (Abs) directed against human chorionic gonadotropin (hCG). This provided the basis for improving the measurement of hCG by harmonization of epitopes of the Abs used and to build broad assay specificity consensus for use as a tumor marker, pregnancy and pregnancy related disorders.

Eight companies and research groups submitted 69 Abs directed to hCG and hCG-related variants. Each of these Abs were characterized in detail by the participants of the Second International Workshop (WS) on hCG of the International Society of Oncology and Biomarkers Tissue Differentiation 7 (ISOBM TD-7).

To determine the specificities of the Abs, the First WHO International Reference Reagents for six hCG variants, hCG, hCGn, hCGβ, hCGβn, hCGβcf, and hCGα were used. Seventeen reference monoclonal (m)Abs were used to assign molecular epitope localizations for the ISOBM-mAbs in the WS. This was performed by comparing ISOBM-Ab specificity, sandwich compatibility, mutual inhibition profiles and affinities, to mAbs of known epitope specificities.

The data shows that 48 mAbs recognized hCGβ, 8 hCGα-, and 13 αβ-heterodimer-specific epitopes. Twenty-seven mAbs were of pan hCG specificity. Two of these pan hCG mAbs had very low cross-reactivity with hLH (<0.1 %; epitope β_1), 12 with low hLH cross-reactivity (<1.0 %; epitopes $\beta_{2/4}$), and 13 with high hLH cross-reactivity (>>1 %; epitopes $\beta_{3/5}$). Four mAbs recognized epitopes on hCGβcf-only (e.g., epitopes β_{11} and β_{13}) and six mAbs epitopes on the remote hCGβ-carboxyl-terminal peptide (epitopes β_8 and β_9).

For routine diagnostic measurements, methods are used that either detect hCG-only, hCGβ-only, or hCG together with hCGβ or hCG together with hCGβ and hCGβcf. Sandwich assays that measure hCG plus hCGβ and eventually hCGβcf should recognize the protein backbone of the analytes preferably on an equimolar basis, should not cross-react with hLH and not be susceptible to blunting of signal by nonmeasured variants like hCGβcf. Such assays can be constructed using pairs of mAbs directed against the cystine knot-associated epitope β1 in combination with epitopes β2 or β4 on hCGβ peptide loops1+3 protruding from the central cysteine knot.

In summary, the results of this hCG ISOBM TD-7 WS[1] in combination with those of the First WS[2] enable recommendations to be made regarding epitope combinations to be used for the design of immunoassays for hCG and its variants.

References: 1. P. Berger, E. Paus, P. M. Hemken, C. Sturgeon, W. W. Stewart, J. P. Skinner, L. C. Harwick, S. C. Saldana, C. S. Ramsay, K. R. Rupprecht, K. H. Olsen, J.-M. Bidart, U.-H. Stenman. Candidate epitopes for measurement of hCG and related molecules: the second ISOBM TD-7 workshop. Tumor Biol. 2013; 34:4033-4057.

2. P. Berger, C. Sturgeon, J.-M. Bidart, E. Paus, R. Gerth, M. Niang, A. Bristow, S. Birken, U.-H. Stenman. The ISOBM TD-7 workshop on hCG and related molecules. Towards user-oriented standardization of pregnancy and tumor diagnosis: assignment of epitopes to the three-dimensional structure of diagnostically and commercially relevant monoclonal antibodies directed against human chorionic gonadotropin and derivatives. Tumor Biol. 2002; 23:1-38.

A-066

High sensitivity detection of residual disease in multiple myeloma using mass spectrometry

J. R. Mills, D. R. Barnidge, D. Murray, J. A. Katzmann, A. Dizpenzieri, D. L. Murray. *Mayo Clinic, Rochester, MN*

Background:Traditionally, detection of an M-protein (monoclonal immunoglobulin) has been used to diagnose and monitor multiple myeloma (MM). As therapies for MM have improved, more sensitive methods have been used to define response: immunofixation electrophoresis (IFX) of serum and urine, normalization of the serum immunoglobulin free light chain (FLC) ratio, and high sensitivity flow cytometry to detect clonal plasma bone marrow cells. It is hoped that these more sensitive approaches will differentiate those patients with minimal residual disease (MRD) versus no residual disease (NRD), the latter which could mean cure. Flow cytometry of plasma cells requires bone marrow aspiration, which is inconvenient and expensive and is potentially limited by sample bias. More sensitive methods to differentiate MRD from NRD using serum would be advantageous.

Objectives:To develop a high sensitivity method to detect residual M-protein secreted from malignant plasma cells as a means to monitor minimal residual disease (MRD) in serum.

Methods:We developed a microLC-ESI-Q-TOF mass spectrometry assay to detect the presence and accurate mass of malignant-specific monoclonal immunoglobulins, a method we termed monoclonal-immunoglobulin-Rapid-Accurate-Mass-Measurement (miRAMM). Briefly, serum immunoglobulins are enriched and then reduced with DTT, and samples are separated using an Eksigent-Ekspert LC system using a Poroshell C3 1x75mm column running at 25μL/min with gradient of aqueous (0.1 % formic acid) to organic (90:10 ACN/IPA) over 24 minutes. The accurate mass of monoclonal light chains is determined by deconvolution of the mass spectra of multiply charged ions across the retention times of immunoglobulin light chains. Serum samples from 14 patients (cohort #1) positive for M-proteins by serum protein electrophoresis (SPEP) and IFX were collected. Neat and diluted serum (diluted 1:1000 in pooled serum from healthy donors) was analyzed by IFX, SPEP, FLC and miRAMM. Cohort #2 included pre-and post-therapy serum from 21 MM patients who had been classified to have achieved stringent complete response (sCR) post; sCR is defined as an undetectable M-protein by SPEP, a negative FLC ratio, a negative IFX, and absence of clonal plasma cells by 4-color flow cytometry from bone marrow. The accurate mass of disease-associated light chains determined by miRAMM pretherapy was used to test for MRD post-therapy in the sCR patients.

Results:For patients in cohort #1 we were able to identify and determine the accurate molecular mass of the monoclonal light chains in all cases (14/14; 100%). To further test the relative sensitivities of each assay, these same patients' samples were subject to 1:1000 dilution into pooled human serum and retested by SPEP, IFX, FLC ratio, and miRAMM. A monoclonal was detected in 0/14, 2/14 (14%), 0/6, and 13/14 (93%), respectively. In addition, of 21 patients in cohort #2 who had been classified to have achieved sCR by conventional means, including 4-color flow cytometry of bone marrow, 67% had detectable residual monoclonal light chains by miRAMM.

Conclusion:This study demonstrates that miRAMM is a more sensitive approach to detect MRD compared with current methods. This method provides additional value in that the accurate mass measurement provides a unique molecular mass identifier of an individuals' malignancy.

A-067

Identification of four molecular subclasses of Luminal Breast Cancer with different likelihood of recurrence and response to Tamoxifen

R. A. Abbud-Antaki, A. Deluca. *Falcon Genomics, Inc., Allison Park, PA*

Background: Genomic studies have revealed four main molecular subtypes of breast cancer with significant heterogeneity within each class. Several genomic assays are now being used in the clinic for prediction of recurrence in breast cancer patients, and determination of which patients could be spared from Chemotherapy. The objective of this study is to develop a reliable clinical diagnostic test for breast cancer patient classification.

Results: We report on a gene expression-based test for classification of breast cancer tumors that identifies additional subsets of Luminal breast cancer with differences in prognosis and response to Tamoxifen therapy. For these studies, we employed Affymetrix HG-U219 GeneAtlas microarrays to examine gene expression profiles from breast cancer tissue (n=17) and normal adjacent tissue (n=6). When we performed Supervised hierarchical clustering using the previously published "intrinsic gene" signature, we could not obtain definite classification for each patient tumor. This has motivated us to come up with a quantitative approach to breast cancer patient classification.

We first examined expression of genes that are commonly used in the pathological evaluation of breast cancer tissue. These were: ESR1, PGR, ERBB2, AR, luminal keratins (KRT 8, 18, 19), basal keratins (KRT 5, 6B, 14), and Cadherins (E-CDH, OB-CDH, and P-CDH). We then classified our patient tissues according to luminal, basal KRTs, E-CDH, and OB-CDH. This gave us 8 different classes with 380 differentially expressed genes. Only 30 out of the 380 genes were found in the "intrinsic" gene list. The genes that were differentially expressed between these classes were not limited to those related to the luminal and basal phenotypes; other differentially expressed gene clusters included proliferation and stromal genes.

We then validated the ability of this signature to classify published breast cancer gene expression datasets (GSE2034, GSE5327, GSE1561, GSE26971, GSE9195, and GSE6532). Supervised Hierarchical Clustering and Gene Set Enrichment Analysis using our 380-gene signature revealed that Luminal cancer patients could be further divided into 4 groups (L1-L4). L1 samples, also known in the literature as Luminal-B, expressed high levels of proliferation genes and showed the highest likelihood of recurrence among untreated patients, but responded well to Tamoxifen treatment. However, the likelihood of recurrence after Tamoxifen for these patients only became similar to other untreated Luminal groups (L3-L4). However, the likelihood of recurrence for L2 patients was similar irrespective of Tamoxifen. L3 patients, on the other hand, showed statistically significant increase in survival after Tamoxifen, but it was not as good as L4 patients. Some patients had additional genetic abnormalities that made them susceptible to relapse. L4 patients had a better prognosis, with significantly improved survival after Tamoxifen.

Conclusions: Our 380-gene classifier is capable of providing comprehensive classification of breast cancer patients. This test could be used in the clinical setting for identification of the major molecular classes of breast cancer and further sub-classification of luminal patients into 4 classes with different rates of recurrence. This will allow for the early identification of patients that would not respond to Tamoxifen therapy.

Tuesday, July 29, 2014

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

Clinical Studies/Outcomes

A-068

Cystatin C as a marker of renal function in adult Nigerians with Hypertension and Diabetes

A. O. Tomi-Olugbodi[1], O. O. Oladipo[2], A. O. Olugbodi[3]. *[1]Department of Pathology, Drexel College Of Medicine, Hahnemann University Hospital,, Philadelphia,, PA, [2]Clinical Chemistry Department of Pathology/ Laboratory Medicine, Staten Island University, NY, [3]Department of Internal Medicine,Reading Hospital and Medical Center,, Reading, PA*

Background/Objective: Cystatin C (Cys C) has been suggested to be a better marker of the glomerular filtration rate (GFR) compared to the widely used serum creatinine. The aim of this study was to compare the accuracy of Cys C with that of serum creatinine in the assessment of GFR in patients with hypertension and diabetics.

Method: Twenty hypertensives and twenty diabetics were compared with forty age-matched healthy controls. Serum Cys C and serum creatinine were estimated in all study subjects and compared with the actual GFR as estimated by the Cockcroft and Gault's algorithm. The strength of significance of correlation was assessed using Pearson correlation (a p value of< 0.05 was accepted as significant).

Results: The serum Cys C correlated well with serum creatinine ($R = 0.757$, $p<0.01$) and with the estimated GFR ($R = -0.733$ and $- 0.710$ respectively; $p<0.01$). However, in the ROC analysis, the AUC of serum cystatin C (0.727) was found to be superior to that of plasma creatinine (0.539).

Conclusions: Serum Cys C and serum creatinine were well correlated in evaluating GFR in hypertensive and diabetic patients. However, serum Cys C was more closely correlated with the GFR and may therefore be a more accurate test of renal function in hypertensive and diabetic patients.

A-069

The best markers for systemic inflammatory response syndrome (SIRS) criteria

K. Saito[1], N. Nomura[1], K. Ishizuka[1], K. Yoshioka[1], S. Yuasa[1], T. Inaba[2], M. Nakanishi[3], N. Fujita[2]. *[1]HORIBA Ltd., Tokyo, Japan, [2]Department of Infection Control and Laboratory Medicine, Kyoto Prefectural University of Medicine, Tokyo, Japan, [3]Department of Medical Instrumental Research and Technology, Kyoto Prefectural University of Medicine, Tokyo, Japan*

Background: The present study was undertaken to find out and compare the usefulness of C-reactive protein (CRP) and hematology parameters in patients with SIRS. Patient presenting systemic inflammatory response syndrome (SIRS) exhibit two or more of the following criteria: Temperature greater than 38°C or less than 36°C, Heart Rate greater than 90 beats/minute, Respiratory rate greater than or equal to 20 breaths/minute or $PaCO_2$ less than or equal to 32 mmHg, WBC greater than or equal to 12,000/μL or less than or equal to 4,000/μL, and suspected or proven infection.

Some of these patients were also under treatment of antineoplastic drug. This type of treatment induces bone marrow suppression and therefore influences the immunity response to inflammation and infection. Therefore we examined WBC, LYM#, NEU#, ALY#, LIC#, PLT and CRP as biomarkers in patients with combination of SIRS and/ or antineoplastic drugs in order to evaluate if the bone marrow suppression could affect the predictive value of the different parameters and analyze which would be the most valuable

Methods: We examined 51 patients that we classified into distinct 4 groups depending on the combination of presence or not of SIRS and Antineoplastic drug medication:

5 patients with SIRS(+) and Antineoplastic drug medication (+);

14 patients with SIRS(+) and Antineoplastic drug medication (-).

13 patients with SIRS(-) and Antineoplastic drug medication (+).

18 patients with SIRS(-) and Antineoplastic drug medication (-).The area under the receiver operator characteristic curve (AUROC) was determined with 4 groups. Whole blood patient samples were tested for WBC, LYM#,NEU#,ALY#,LIC#,PLT and CRP using Pentra MS CRP(HORIBA Lid.)

Results: The AUROC value by 14 patients with SIRS(+) and Antineoplastic drug medication (-) and 18 patients with SIRS(-) and Antineoplastic drug medication (-) were 0.81 for CRP, 0.65 for PLT and 0.61 for WBC. And also the AUROC value by 5 patients with SIRS(+) and Antineoplastic drug medication (+) and 13 patients with SIRS(-) and Antineoplastic drug medication (+) were 0.82 for PLT, 0.8 for CRP and 0.77 for WBC. The AUROC of both PLT and CRP was 0.87. The AUROC of both WBC and CRP was 0.80.The ALY# and LIC# did provide useful information for evaluating that the patient had SIRS or not. The LYM# and NEU# had almost the same performance as WBC.

Conclusion: Some patients with SIRS(-) based on SIRS criteria also had inflammatory disease such as cholecystitis and otitis media. We evaluated patients with SIRS(-) by CRP. The results show, patients with SIRS(-) and CRP(>5mg/L) and including inflammatory disease such as cholecystitis and otitis media and patients with SIRS(-) and CRP(<5mg/L) without inflammatory disease. This shows that CRP is an excellent marker for SIRS criteria. Since WBC is affected by bone marrow suppression of Antineoplastic drug medication and CRP is not affected by it. The AUROC combination between CRP and PLT is better than the AUROC combination between CRP and WBC. Therefore, our conclusion is that CRP and PLT provide better results as supportive markers for SIRS criteria.

A-070

A case of Glutaric Aciduria type 1 in a Black South African girl

M. J. Turzyniecka. *IALCH-NHLS and UKZN, Durban, South Africa*

Background: Glutaric Aciduria type 1 is an autosomal recessive inborn error of metabolism caused by a deficiency of glutaryl-Co enzyme A dehydrogenase (GCDH). If unrecognised it results in a severe extrapyramidal disease and mental retardation. It has been well described in North American and European Caucasian populations but only recently identified as potentially one of the commonest inherited metabolic disorders in black South Africans. **Methods and Results:** We present a case of a 9 month old Black South African girl who was admitted for investigations of sudden hypotonia following a fall from her mother's back. There was no family medical history of note. She was delivered at term by normal vaginal delivery and had normal developmental milestones until the fall. She had no previous medical history. She had relative macrocephaly (head circumference 46cm - 97th centile) and was globally hypotonic with hyperreflexia. She had normal renal and liver function tests, lactate, ammonia, amino acid profile and negative reducing substances screen. She had high urine glutaric acid at 4061umol/mmol creatinine (reference: <30) and 3-OH glutaric acid at 49 umol/mmol creatinine (reference: < 2.5). MRI of brain showed T2 bilateral hyperintensity of the basal ganglia, cerebral peduncles and central tegmental tracts, symmetrical restricted diffusion of globus pallidus in keeping with acute on chronic changes of pre-existing Glutaric aciduria type 1. **Conclusion:** Macrocephaly is an important clinical sign of many neurometabolic disorders and one of the earliest signs of Glutaric Aciduria type 1 (GA type 1) thus warrants screening in all cases of macrocephaly of unknown origin regardless ethnicity. GA type 1 typically presents with acute cerebral injury precipitated by an intercurrent infectious illness. It is a preventable and treatable disorder requiring patients with GA type 1 to low protein (lysine and tryptophan) diet, riboflavin and L-carnitine supplementation. It has been linked to A293T mutation in the glutaryl-CoA dehydrogenase gene in black South Africans.

A-071

Assessment Of Serum And Urine Sialic Acid In Sickle Cell Anaemia Patients

s. E. idogun, E. Oriseseyigbemion-Sakpa. *university of Benin Teach Hospit, BENIN, Nigeria*

Aim and objectives: To assess serum and urine sialic acid and compare their values with other biochemical markers of nephropathy in patients with sickle cell anaemia.

Patients and methods: It was a cross sectional descriptive study that involved adult (18 – 60 years) sickle cell anaemia patients that were clinically stable without sickle cell anaemia crisis. The control participants were healthy volunteers that were aged-matched. Urine and blood specimens were obtained from the participants. The urine albumin analysis was by Lowry method, urine and plasma creatine was by Jaffe', Kinetic method, while the serum and urine sialic acid was by standard Ehrlich (P – dimethyl amino benzaldehyde) method. Data were analysed using SPSS version 16.

Results Sixty eight of the subjects were sickle cell anaemia patients while 30 respondents served as control. Table 1, shows the plasma and urine results of the studied analytes in the sickle cell anaemia patients and controls. There was a negative

correlation between serum sialic acid and urea (r = -0.15, P = 0.41), creatinine (r -0.17, P = 0.34) albumin creatinine ratio (r = -0.19, P =0.81), but these were not significant. However, there was a positive significant relationship between urine sialic acid and albumin creatinine ratio (r = 0.44, P = 0.01).

Conclusion: Serum sialic acid unlike urine sialic acid does not correlate well with other well established marker of nephropathy (creatinine and urea). Monitoring of urinary sialic acid in patients with sickle cell anaemia who are in steady state is therefore an important adjuvant test in detecting the early onset of sickle cell nephropathy.

Table 1: Plasma and urine results of the studied analytes in control (non SCA) and SCA patients

	Control (non-SCA anaemia) respondents (n = 30)	SCA respondents (n = 68)	p-value
Plasma urea (mmol/L)	3.33±0.16	6.43±0.54	<0.001
Plasma creatinine (μmol/L)	73.98±2.95	86.70±6.03	0.06
SSA (mmol/L	1.93±0.67	1.58±0.96	0.04
ACR (mg/mmol)	2.19±0.10	4.50±0.24	<0.001
USA (mmol/L)	0.78±0.04	1.13±0.05	<0.001
USCR (mmol/mol)	60.52±3.39	169.39±13.59	<0.001

ACR = Albumin creatinine ratio

USA = Urine sialic acid

USCR = Urine sialic and creatinine ratio

SCA = Sickle cell anaemia

A-072

Interleukin-18 a Potential Marker of Liver Cirrhosis in Chronic Hepatitis C Patients.

E. M. Abdalla, S. E. Younes, M. M. Abdo, S. A. M. Ahmed. *Suez Canal University, Ismailia, Egypt*

Background: WHO reported that Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma. In patients with liver cirrhosis, liver biopsy is the gold standard method to establish the diagnosis. But liver biopsy has many disadvantages, it is invasive, coasty and difficult to be standardized**.** That is why there has been increasing interest in noninvasive assessment of liver cirrhosis by the use of alternative serum markers. IL-18, a proinflammatory cytokine can be synthesized by injured hepatocytes and increases the susceptibility of liver endothelial cells to undergo apoptosis. An increased circulating level of IL-18 is likely to play a pathogenic role in patients with chronic liver disease.

Aim of the work: This study aimed to prove that Interleukin-18 can be used as a potential marker of liver cirrhosis in chronic hepatitis C patients. IL-18 levels were measured in 20 HCV infected patients with cirrhosis and compared to 20 non-cirrhotic HCV patients and 20 healthy controls.

Results: Serum IL-18 levels were significantly higher in cirrhotic (836 pg/ml) and non-cirrhotic CLD patients (751 pg/ml) than in healthy controls (278 pg/ml). IL-18 level is significantly increased with increase in histological stage of liver cirrhosis and its concentration may predict the degree of hepatocellular damage. A Positive correlation founded

between serum IL-18 levels and Child Pugh score suggest that IL-18 can be used as an additional non-invasive marker for monitoring the degree of liver cirrhosis in chronic hepatitis C patients and as a monitoring tool to assess response to therapy. Low cut off value of serum IL-18 at 495 pg/ml (which showed sensitivity of 100%) can be used as a screening value under which most of cases are probably negative cases for liver cirrhosis. While, a high cut off value of serum IL-18 at 875 pg/ml (which showed specificity of 95%) can be used as a diagnostic value above which 95% of cases are probably positive cases for liver cirrhosis.

Conclusions: IL-18 level as a non-invasive marker can be used for

follow up of chronic HCV patients and assessement of the severity of the disease and degree of liver cirrhosis instead of liver biopsy which has been proved to be invasive, coasty and difficult to standardize. Usage of low cut off value of serum IL-18 at 495 pg/ml as a screening value under which most of CLD cases are probably negative for liver cirrhosis and no need for liver biopsy. Usage of high cut off value of serum IL-18 at 875 pg/ml as a confirmatory test with liver biopsy above which 95% of cases are probably positive for liver cirrhosis. Usage of IL-18 level between 495 and 875 pg/ml as an indication for liver biopsy to diagnose cases with liver cirrhosis.

A-073

Risk map in three emergency laboratories

E. Gonzalez[1], Á. Salas García[2], E. Guillén Campuzano[1], M. Buxeda Figuerola[1], L. Juan Pereira[1], I. Caballé Martín[1]. *[1]Catlab, Terrassa, Spain, [2]Consorci Sanitari de Terrassa, Terrassa, Spain*

Background: Patient Safety is considered one of the key aspects of the quality policies of Health Systems The objective is to perform an analysis of potential errors in three emergency laboratories to estimate the impact on patient safety.

Methods: The Clinical Laboratory of Catlab consists of a central laboratory and three hospital emergency laboratories. It is calculated an estimation of the potential risks in each of the three emergency laboratories: Laboratory (1) 609794 clinical analysis/2013, Laboratory (2) 424882 clinical analysis/2013 and Laboratory (3) 256857 clinical analysis/2013.

The Failure Mode and Effect Analysis (FMEA) is the methodology that analyzes the quality, safety and reliability of the functioning of a system, identifying potential errors and their causes from their effects. By using the FMEA, it is calculated the risk priority number (NPR), which is the product of the estimation of the incidence, gravity and detectability (I*G*D). The NPR will allow us to prioritize the importance of the possible errors.

We analyze the possible potential errors that can arise in the different processes that are developed in the laboratories, according to the item that is affected, the causes and their effects.

Results:

Processes	Laboratory(1)	Laboratory(2)	Laboratory(3)
Strategics	27%	17%	31%
Support	10%	6%	10%
Pre-preanalytical	17%	23%	19%
Preanalytical	17%	24%	10%
Analytical	8%	14%	8%
Postanalytical	12%	9%	19%
Post-postanalytical	9%	7%	8%

Conclusion: There are differences between the pre-analytical errors in Laboratory (2) and the Laboratories (1) and (3). The Laboratory (3) makes a greater estimation of the error in the post-analytical processes. It is relevant the importance of the results of the strategic processes in the Laboratories, especially in the Laboratories 1 and 3.

The results obtained using the FMEA will allow us to implement a posteriori improvement actions.

A-077

Value of suspecting undiagnosed G6PD deficiency in very severe hyperbilirubinemia with hepatitis A.

A. Hazra, J. Chakraborty, L. Banerjee. *ESIC PGIMSR Manicktala Kolkata, Kolkata WB India, India*

Background: India has a high burden of both hepatitis A and G6PD deficiency (upto 15%) with no Government funded screening for G6PD. In adults only 2% of infectious hepatitis cases cross a bilirubin value of 30 mg/dL, and we present a case with total Bilirubin more than 60 mg/dL.

History: A 16 year old Indian boy who presented with 7 day history of fever, nausea, vomiting, malaise, anorexia, abdominal pain and yellow urine. On examination he showed deep icterus, and hepatomegaly. Total Bilirubin at presentation was 17.26 mg/dL of which about 60% was conjugated. AST was 7300 IU/L and ALT 6700 IU/L and ALP 640 IU/L. HEV and HAV IgM came back strongly reactive. His Hemoglobin was already 6.3 g/dL when initially checked after admission and two days after it dropped further to 5.3 g/dL and his Bilirubin shot up to 66.95 total of which about 70% was conjugated. next day the transaminases were coming down, with AST 745 mg/dL, ALT 1751 mg/dL, ALP was down to 350 mg/dL. LDH which was not checked initially turned out to be 1364 U/L this time, and Reticulocytes 19%. Putting together the dropping Hb, very severe Bilirubin, and high LDH and Reticulocytosis, an additional hemolytic etiology was suspected. DAT and a G6PD screen were added on in the pre-transfusion sample. DAT came out negative but the G6PD screen was strongly positive for deficiency and a quantitative spectrophotometric G6PD test fetched a level ~30% of lower reference rage even at this hemolytic phase. The patient was also screened for Wilson's disease by Serum Ceruloplasmin and 24 hr Urine copper which all came negative. The patient gradually recovered with transfusion and supportive therapy and his diagnosis of G6PD in addition to HepatitisA.

Conclusion: G6PD deficiency should be suspected in subjects with a history of hemolysis with Hepattis A, even when the majority of fraction of bilirubin is

conjugated . We also suggest to vaccinate all G6PD deficient patients for hepatitis A. Whether to screen blood bag for G6PD before transfusion to Hepatitis patients is of debate. Note Hepatocye damage would be aggraveted due to hepatocellular G6PD deficiency since the deficiencys is not isolated to only Erythroid tissue.

A-079

Neutrophil Gelatinase Associated Lipocalin and atherosclerosis: a study of its association with known cardiovascular risk factors and metabolic syndrome.

M. C. Freire[1], I. Bendet[1], M. L. Moreira[1], L. L. Leite[1], A. J. L. Jorge[2], M. G. Rosa[2], E. T. Mesquita[2]. *[1]DASA, RIO DE JANEIRO, Brazil, [2]Universidade Federal Fluminense, Niterói, Brazil*

Background: The Neutrophil Gelatinase Associated Lipocalin (NGAL) is a glycoprotein involved in the processes of innate immunity and inflammation. Initially described in neutrophils and epithelial cells, it is also produced by macrophages and smooth muscle cells in atherosclerotic lesions. It activates proteolytic processes linked to metalloproteinase 9 (MMP-9), considered to be responsible for vascular remodeling and rupture of atherosclerotic plaque, leading to clinical presentations of cardiovascular diseases (CVD).

Objectives: To evaluate the associations of NGAL with parameters known as cardiovascular risk factors and criteria for metabolic syndrome.

Methods: Observational, cross-sectional study of a sample (n = 219) of outpatiens assisted by the Family Medicine Program in the city of Niterói, Brazil, aged 45 years and older. All data and samples were collected between August 2011 and August 2012. NGAL serum levels were determined by sandwich ELISA (Bioporto Diagnostics), samples were frozen at -80°C until first thawed. We evaluated its associations with several risk factors.

Results: Of the parameters tested, we observed significant positive correlation between NGAL means and age, creatinine, leukocyte count, Framingham estimated risk, and a negative correlation with HDL cholesterol (Table 1).

Discussion: To stablish preventive strategies for CVD is one important goal of health systems, and it depends on accurate individual risk assessment. There are consistent data from studies in animals and humans that support the importance of NGAL in the patho-physiology of atherosclerosis and CVD. The biological effect of NGAL in atherosclerosis is suggested by the co-localization of NGAL and MMP-9 expression in human carotid atherosclerotic plaques with increased gelatinase activity in these tissues. The quest for understanding the role of this new biomarker in predicting cardiovascular events is of paramount importance, and at this point of the study we could show its association with HDL and to Framingham risk estimates.

Table 1: Mean differences of log NGAL values

category	log NGAL			
	N	mean	SD	*P*
Leukocyte count				
<11.000	200	4.98	0.55	0.001
>11.000	10	5.60	0.48	
Serum creatinine				
<1,2 mg/dL	199	4.97	0.54	0.002
>1,2 mg/dL	19	5.38	0.58	
Low HDL cholesterol (NCEP-ATPIII)				
no	156	4.94	0.55	0.003
yes	62	5.19	0.52	
10 years estimated risk of Myocardial infarction and death by Framingham score				
<10%	129	4.93	0.56	0.039
>10%	69	5.11	0.56	

A-080

Standardization of paroxysmal nocturnal hemoglobinuria assay

L. B. Sousa[1], M. A. Viana[1], R. L. Albuquerque[1], E. X. Souto[1], M. D. Freire[2], N. Gaburo[1]. *[1]DASA, Sao Paulo, Brazil, [2]DASA, Rio de Janeiro, Brazil*

Introduction: Paroxysmal Nocturnal Hemoglobinuria (PNH) is caused by clonal expansion of hematopoietic stem cells that present somatic mutations of the phosphatidylinositol-glycan-class A (PIG-A), resulting of biosynthesis deficiency of the molecule glycol-phosphatidyl-inositol (GPI) and partial or inability to express GPI-anchored proteins**.** Conventional assays to diagnose PNH present high specificity, but low sensibility. Flow Citometry has been used to evaluate the expression of GPI-anchored proteins in different blood lineages, because it is a highly specific and sensitive technique, and it is the gold standard for PNH management and diagnose.

Objective: standardize and validate the PNH immunophenotyping assay using the reagent FLAER (fluorescent aerolysin) for implementation in the routine of a diagnostic laboratory.

Methodology: A total of 80 peripheral blood samples within 24 hours collection from population with ages between 20 and 40 years old were used. Two protocols of surface markers were tested and all the monoclonal antibodies were titrated (Flaer, CD55, CD64, CD24, CD14, CD16, CD45). The acquisition was performed on BD FACSCanto II (BD, San Jose, CA) and the data was analyzed in the Infinicyt (Cytognos S.L., Salamanca -Spain) software.

Results: In the study population, were obtained seven PNH positive samples (9%) and 73 PNH negatives (91%). In all cases the FLAER reagent presented excellent performance in detection of neutrophils and monocytes with PNH phenotype, and there was no conflict between the FLAER staining and GPI-anchored proteins expression. The possibility to differentiate cells type I, II and III in erythrocyte population, when the MEM43 clone from the monoclonal antibody CD59-PE is used was also observed (FI = 2589. 62 negative samples and FI= 954,72 positive samples).

Conclusion: The evaluation of the FLAER reagent performance in detection of PNH clone through Flow Citometry, in this study, presented clear and precise distinction between normal cells and deficiency GPI cells, corroborating with the current literature.

A-081

Effects of Vitamin D Treatments on Arginine Derivatives in patients with Stage 3 and 4 Chronic Kidney Disease

C. Heideloff, J. M. El-Khoury, J. Hyland, J. Simon, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Vitamin D nutritional status has been linked to chronic kidney disease (CKD) and cardiovascular disease (CVD) in epidemiologic studies. Arginine derivatives, especially symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) have been associated with CKD and CVD. However, data available in literature regarding the relation between vitamin D blood levels and arginine derivatives is conflicting. **Objective:** In this prospective study, our objective was to examine if vitamin D supplementation and increase in 25-hydroxyvitamin D blood levels significantly change arginine, ADMA, SDMA, and the ratios of these biomarkers. **Methods:** This study was approved by our Institutional Review Board. A total of 16 CKD patients (stage 3 and 4) with vitamin D deficiency (<20 ng/mL) and insufficiency (<31 ng/mL) were enrolled in our study. Patients were instructed to take either vitamin D2 or vitamin D3 at 50,000 IU/month for 6 months if they were vitamin D insufficient or 50,000 IU/week for a month then 50,000 IU/month for 5 months if they were vitamin D deficient. 25-Hydroxyvitamin D2, 25-Hydroxyvitamin D3, arginine (ARG), ADMA, and SDMA were measured at baseline and after 24 weeks of supplementation. All analytes were determined by liquid chromatography-tandem mass spectrometry methods. **Results:** Vitamin D supplementation increased 25-hydroxyvitamin D levels from a mean of 24.8 ng/mL at baseline to 39.6 ng/mL after 24 weeks of treatment ($p<0.0001$). Arginine, SDMA, ADMA and their ratios were not significantly different before and after the treatments ($p>0.05$). **Conclusion:** Though vitamin D supplementation in stage 3 and 4 CKD patients significantly improved vitamin D status it did not significantly affect arginine, ADMA or SDMA blood levels.

A-084

A study of the parasitic causes of anaemia in children under five(5) years of age in the Bolgatanga municipality, Ghana

C. NKRUMAH[1], S. ALHASSAN ANEYIRE[2]. *[1]METHODIST HOSPITAL, WENCHI, Ghana, [2]COLLEGE OF HEALTH, KINTAMPO, Ghana*

Background: Anemia is a condition affecting children especially those less than 5 years, and pregnant women in the world (WHO, 2000). In tropical and developing countries, anemia is particularly prevalent with 50% or more of pre-school children and pregnant women being moderately or severely anemic (Cheesbrough, 2006). It is against this background that this research is being conducted.

Methods: This study represents the results of a descriptive cross-sectional study to establish the prevalence, magnitude and causes of anemia among children less than

5 years in the Bolgatanga municipality. Simple random sampling technique was used to obtain specimen from 100 participants following informed consent. Specimens analyzed included stool (wet preparation with physiological saline) for intestinal parasites and blood samples for hemoglobin measurement and detection of the presence of malaria parasites using Rapid Diagnostic Technique (RDT) kits. Actual measurement of their hemoglobin (Hb) was done using a Sysmex analyzer. Anemia was defined as haemoglobin concentration less than 11.0g/dl (Cheesbrough, 2006).

Results: The prevalence of anemia among the children was 62%. Of the anemic children, 35 (56%) showed presence of malaria parasites in their bloodstream whiles 18(29%) had intestinal parasites. 42(66%) of the anemic children had Hb between 10.0 and 11.0g/dl (mildly anemic), 13.0 (23%) had Hb between 7.0 and 9.9g/dl (moderately anemic), with 7 (11%) of them being severely anemic (Hb below 7g/dl). All the parasites detected in the stool samples were hookworm. The presence of these intestinal parasites and malaria parasites in the stool and blood respectively had a significant association with low Hb ($p<0.005$). **Conclusion**: Based on the results of the study, the prevalence of anemia was high in the study population. Malaria parasitaemia was a major cause of anemia in our environment followed by hookworm infestation. The study recommends among other things that the Ministry of Health should embark on intensive health education programs in the communities on early detection of malaria and hookworm infestation and enforce their prevention.

A-086

Fecal Biomarker Testing Identifies Exocrine Pancreatic Insufficiency in Patients with Possible Irritable Bowel Syndrome

J. G. Goepp, T. McBride, E. Fowler, A. Peace-Brewer, D. Landis. *Genova Diagnostics, Asheville, NC*

Objective: 1) To use fecal biomarker testing to identify a subset of patients with symptoms of irritable bowel syndrome (IBS) that might be explained by the presence of exocrine pancreatic insufficiency (EPI), and 2) to identify within that subgroup additional fecal biomarkers that might suggest a primary disease process capable of causing secondary EPI.

Relevance: IBS, long considered a "diagnosis of exclusion," is now recognized as an "umbrella diagnosis," identifying a heterogeneous group of patients who may in fact have one or more of several underlying, treatable diagnoses. EPI is one such diagnosis, but few studies have examined its prevalence or biological context in the setting of possible IBS. Because EPI may occur secondary to another gastrointestinal problem such as inflammation or parasitic infection, fecal biomarkers suggesting such processes may further clarify the diagnostic picture in patients presenting with symptoms consistent with IBS. A fecal biomarker panel with appropriate components may thus represent an important role for clinical laboratory medicine in diagnosis and management of conditions producing symptoms consistent with IBS.

Methods: One-year analysis of de-identified stool testing data for fecal pancreatic elastase-1 (FPE1) in all patients for whom stool specimens were submitted for testing bearing at least one of 13 ICD-9 codes indicating the potential for IBS. FPE1 testing was conducted using a standard dual monoclonal antibody ELISA technique with binding sites for two unique epitopes on the human PE-1 molecule. When biomarker data on other conditions were available on the same specimen, they were analyzed for significant associations with FPE1 status using Fisher's Exact Test, with significance set at $p < 0.05$.

Validation: A total of 9112 records were identified that included at least one of the 13 IBS-related ICD-9 codes and also a result of FPE1 testing. FPE1 in the range 200 mcg/g defined normal. Definitions of abnormal for other biomarkers were: calprotectin >120 mcg/g, eosinophil protein X (EPX) > 7 mcg/g, H. pylori, B. hominis, and other parasites = present.

Results: Objective 1: Some degree of EPI was suggested in 858 (9.4%) of all records, with FPE1 <100 mcg/g stool in 289 (3.2%) and FPE1 100 to 199 mcg/g in 569 (6.2%). Objective 2: FPE1 of <100 was significantly associated with abnormal results for fecal calprotectin and EPX, as well as with evidence of infection with Blastocystis hominis, non-Blastocystis parasites, H. pylori, and entamoeba. FPE1 of 100-199 was significantly associated with parasitic infection as well.

Conclusions: Among patients in whom IBS was a consideration, fecal biomarker testing revealed that 9.4% had indications of some degree of exocrine pancreatic insufficiency by FPE1. Fecal biomarker testing was also more likely to suggest presence of an inflammatory or parasitic gastrointestinal condition in patients with abnormal FPE1, suggesting that in some cases EPI itself might be secondary to such underlying processes, and indicating potential lines for further evaluation of possibly treatable diagnoses. Further prospective studies with definitive patient follow-up are recommended.

A-087

Association between the Delta Estimated Glomerular Filtration Rate and the Prevalence of Monoclonal Gammopathy of Undetermined Significance in Korean Males

T. Jeong, W. Lee, S. Chun, W. Min. *University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Republic of*

Background: The prevalence of monoclonal gammopathy of undetermined significance (MGUS) varies with age, gender, and ethnicity. We investigated the association between the reduction in the estimated glomerular filtration rate (eGFR) and the prevalence of MGUS in healthy Korean males.

Methods: We enrolled 723 healthy Korean males who visited the hospital for regular health checkups. Serum creatinine concentration, serum electrophoresis, serum immunofixation, and the serum free light chain assay were performed. Data, including age, date of health checkup, and previous serum creatinine concentrations were obtained from electronic medical records. We calculated delta eGFR per year and the prevalence of MGUS was compared based on the delta eGFR per year and age group.

Results: Thirteen (1.8%) of seven hundred and twenty-three participants exhibited the monoclonal band on serum immunofixation. Prevalence of MGUS by age group was 0.00% (0/172 for 40s), 1.63% (6/367 for 60s), and 3.80% (7/184 for > 60-years). The median decrease in delta eGFR per year was 5.3%. The prevalence of MGUS in participants in their 50s with > 5.3% decline in delta eGFR per year was significantly higher than those with < 5.3% decrease in delta eGFR per year (3.16% vs. 0.00%; $p = 0.049$). The prevalence of MGUS in participants in their 50s with > 5.3% decrease in delta eGFR per year was similar to that of healthy males in their 60s.

Conclusion: Using the rate of reduction in delta eGFR per year in healthy Korean males who had their serum creatinine level checked regularly may increase the MGUS detection rate in clinical practice.

A-090

An Update on a Candidate BK Virus DNA Standard Reference Material

J. Harenza[1], L. Cook[2], P. M. Vallone[1]. *[1]NIST, Gaithersburg, MD, [2]University of Washington, Seattle, WA*

Background: The polyomavirus, BKV, is widespread in the population due to primary infection during childhood and largely remains latent in the majority of individuals. However, illnesses as a result of BKV occur in immunocompromised patients, organ transplant, HIV/AIDS- infected, or diabetic patients. Two common illnesses resulting from BKV, hemorrhagic cystitis and nephropathy, occur in transplant patients, as prescribed anti-rejection medications can weaken the immune system. This often leads to renal allograft failure. To treat BKV, accurate quantitation of viral load is necessary for proper adjustment of anti-rejection medications in an effort to improve an individual's immune function. However, current BKV standards are only available for the Ia genotype of the virus, and it has been well-documented that using assays specific to Ia can lead to underestimation of viral load of other genotypes, due to primer and/or probe binding site polymorphisms. Therefore, we propose a candidate reference material panel of BKV genotypes to aid in precise measurements of viral load.

Methods: Viral DNA was extracted from six available clinical isolates and distinct genotypes (Ia, Ic, III, IV, V, VI) of the BK virus. Single site restriction digestion was performed to linearize each viral genome and long-range PCR was performed to amplify entire genomes. Each amplicon was ligated into a plasmid backbone, transformed into, and subsequently propagated in, XL-10 Gold E. coli cells. DNA was extracted, purified, and re-linearized to enable sequencing and accurate copy number measurement. Using the Illumina MiSeq platform, whole genome sequencing was performed for each genotype. New primer and probe sets, specific to each genotype, were designed using this sequence information and optimized for quantitative PCR (qPCR) and digital PCR (dPCR).

Results and Future Goals: We have amplified and sequenced six full-length BKV genomes. Next-generation sequencing information allowed us to design and optimize efficient (between 90-110%) primer and probe sets for both qPCR and dPCR platforms. Homogeneity and stability studies will be performed for each DNA construct using the newly developed assays, and the materials will be certified for genome copy number using dPCR.

Conclusion: The availability of a panel of BKV DNA genotypes as potential reference materials will enable traceability for manufacturers of calibrant materials. This will ultimately improve upon the consistency and accuracy of viral load quantitation measurements across laboratories, leading to improved dosing regimens in infected patients.

A-092

Can Procalcitonin or Lactate help with Sepsis Evaluation in Cancer Patients?

D. R. Mendu, M. Fleisher, S. I. McCash, Q. Xiang, L. Miller, M. S. Pessin, L. V. Ramanathan. *Memorial Sloa-Kettering Cancer Center, New York, NY*

Objective: Severity of illness due to sepsis in patients with cancer is a major clinical complication and contributes to high mortality in this patient population. We evaluated two biomarkers used to help discriminate between bacterial infection and noninfectious acute-phase reactions: lactate (LA) and procalcitonin (PCT).

Methods: A cohort of 40 random patients with malignant disease admitted to Memorial Sloan Kettering Cancer Center was studied. Out of these 40 patients, 10 were considered as "controls" using the sepsis diagnostic criteria of lactate < 2.0 mmol/l, and negative bacterial cultures: and PCT< 0.5 ng/mL per manufacturer's recommendations. Six out of the forty patients had viral infections. LA and PCT were measured at the time of admission followed by an additional sample collection for each patient within 24 hours. Heparinized blood samples were obtained and the plasma used for LA analysis on the Nova pHOX and for PCT determined by an Enzyme Linked Fluorescent Antibody analysis on the mini Vidas (BioMérieuxS, France). Concordance studies between LA and PCT were done.

Results: Our study demonstrated that elevated LA and PCT levels have only a 50% concordance. PCT was elevated in 50% of the patients and demonstrated a lack of concordance with plasma Lactate. Additionally, we observed that the PCT was elevated in patients who were negative for bacterial infection, based on microbiological cultures, and did not correlate well with LA concentrations >4 mmol/L. The six patients with viral infection had significantly elevated PCT levels and the LA levels were < 4 mmoL/L. In order to examine the possible effect of pre-analytical factors that may explain the LA/PCT discordance, we analyzed LA stability in five healthy volunteers at different time points at room temperature. Within 15 minutes, the LA in heparinized blood increased steadily, and by 30 minutes, LA concentrations were almost 40 % higher than the initial LA measurement. Conclusions: Our preliminary findings suggest that PCT levels were consistently high in cancer patients without sepsis. Our data also indicates a poor correlation between LA and PCT in cancer patients evaluated for suspected bacterial infection and neutropenia. Increased LA concentrations could be due to pre-analytical variation, oxygen deficit due to mitochondrial oxidative phosphorylation, inhibition by interleukins (ILs), tumor necrosis factor (TNF) or a combination of the latter. These data support the already known poor specificity of LA as an accurate indicator of sepsis. Further clinical investigation is also needed to explain elevations in PCT in the absence of bacterial infection in patients with cancer.

Based on these data, the significance of PCT in cancer patients without infection is unclear and requires further investigation. Our data also suggests a need to investigate the relationship between plasma LA and PCT in the clinical evaluation of sepsis in cancer patients.

A-095

Urinalysis/Microscopy Reflex to Urine Culture on i R I C E L L® Complete Urinalysis™ Workcells: Are We There Yet?

R. Khoury, A. Gandhi, B. P. Salmon, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

Background: Urinary Tract Infection (UTI) is serious health problem, it is very common among the geriatric population, it is the second most common type of infection (second only to respiratory tract infection),and can cause serious complications and it is a significant cause of morbidity and death, with the expected death rate as high as 3% in those who develop pyelonephritis. The high mortality rate is largely due to delayed presentation and the development of bacteremia/sepsis.

Urinalysis (UA) is one of the most frequently requested tests; it is estimated that between 200 and 300 million UA are performed yearly in the United States. It is an invaluable tool in the diagnosis of UTI, malignancy, calculi, and detecting systemic diseases affecting the kidneys.

Methodology: 1,634 specimens were collected for urinalysis and culture from residents in Long-Term Care Facilities over a period of 2 weeks. Urinalysis and microscopy was done using iRICELL™ automated system. All the specimens were cultured; the culture was done using MicroScan Walkaway96 conventional panels. We used urine culture as the reference standard, no growth or <10,000 colony-forming unit/mL were considered negative, cultures with > 50,000 colonies colony-forming unit/mL were considered positive. The urinalysis instrument generates a "urine culture candidate report" when at least one of the five bacteriuria parameter (Nitrite, Leucocyte Estrase, and microscopy values: white blood cells, bacteria and all small particles); the report was used to assess its effectiveness for urine culture screening.

Results: 801 (49.0%) had positive culture. 1344 (82.3%) specimens were identified as urine culture candidates. Of the 1,344 that were identified as a candidates, 772 (57.4%) had positive culture, 547 (40.7%) were negative, 25 (1.9%) were contaminated. 290 (17.7%) specimens were not selected as culture candidates because of the negative urinalysis; of these specimens 29 (10%) had positive culture and 259 (89.3%) were negative.

Conclusion: using the candidate for urine culture would have avoided urine culture on about 15% of the patients but would have missed the diagnosis for 10% of the patients that were considered as having normal specimens. Urinalysis and microscopy still lack the sensitivity and specificity to be used alone to diagnose urinary tract infection, more work needed to establish better parameters and algorithm to be used when screening patients for UTI using urinalysis.

A-096

Interleukin-6 (IL-6) Combined with Clinical and Demographic Parameters Predicts Death in Critically Ill Patients with Systemic Inflammatory Response Syndrome

J. M. Colón-Franco[1], S. Litt[2], D. A. Anderson[2], D. Plummer[2], W. D. Dupont[2], T. W. Rice[2], A. P. Wheeler[2], A. Woodworth[2]. *[1]Medical College of Wisconsin, Milwaukee, WI, [2]Vanderbilt University Medical Center, Nashville, TN*

Background: Increasing sepsis severity is associated with significant mortality. Currently, there are few prognostic biomarkers for septic patients. Objective: To determine the prognostic utility of 5 inflammatory biomarkers, clinical and demographic data to predict mortality among Medical ICU (MICU) patients with systemic inflammatory response syndrome (SIRS). Methods: This retrospective cohort study enrolled 201 MICU patients with SIRS. Among them, 91 were septic and 45 died during their admission. TNFα, IL-6, IL-10, CRP and LBP were measured on the Immulite 1000 (Siemens Healthcare Diagnostics) in residual plasma collected on the day of SIRS and 1 or 2 days prior to meeting SIRS criteria (days -1 and -2). Procalcitonin (PCT) was measured on the Vidas B•R•A•H•M•S® Assay (Biomeriéux). The prognostic strength of individual biomarkers and logistic regression models that combined baseline co-variables (SIRS criteria, age, and sex; Model A) and biomarker concentrations (Model B) were evaluated. The change in serial biomarker concentrations (days -2, -1 and 0) and the difference in median concentrations for each day were determined for survivors and non-survivors by two way ANOVA and Mann-Whitney tests respectively. Results: Table 1 lists the areas under the receiver operating characteristic curves (AUCs) for individual biomarkers and models. The evolution of IL-6 over time was significantly different in survivors and non-survivors (time-survival interaction p=0.02). Median IL-6 concentrations were significantly different in both groups during all study days (p<0.01, <0.001, and <0.0001 on days -2, -1, and 0 respectively). Conclusions: IL-6 was the best single predictor of mortality. PCT showed limited prognostic ability. When combined with baseline demographics and clinical data, IL-6 showed improved ability to predict death. Additional biomarkers (model AB) did not improve the model's prognostic strength. Serial measurement of IL-6 combined with other clinical parameters accurately predicts mortality in patients with systemic inflammatory response syndrome.

A-098

Fatigue in rheumatoid arthritis and its relation to interleukin-6 serum level

d. I. hashad, A. Helal, E. Shahine, M. Hassan, R. Abdel Moneim. *FACULTY OF MEDICINE, alexandria, Egypt*

Objectives: This work aimed to investigate the occurrence and level of fatigue and its relation to IL-6 serum level in rheumatoid arthritis (RA) patients. **Patients and methods:** The study included 60 patients with RA diagnosed according to the 2010 ACR-EULAR classification criteria for RA and 30 healthy controls. Patients were included if they were above 18 years and fulfilled a score P6 over 10 of the 2010 ACR-EULAR classification criteria for RA. Disease activity was assessed using 28 joint disease activity score (DAS28), erythrocytes sedimentation rate (ESR) and C-reactive protein (CRP). Fatigue was assessed using the Bristol Rheumatoid Arthritis Fatigue Multidimensional Questionnaire (BRAF-MDQ) and serum IL-6 level was measured in patients and controls using enzyme-linked immunosorbent assay (ELISA) technique. **Results:** The BRAF-MDQ was significantly higher among patients (mean =50.6± 15.2) than controls (mean= 7.8± 3.7) (p< 0.001). Patients' mean IL-6 serum level was 35.05 ±21.23 pg/ml and 4.72 ±3.09 pg/ml among control subjects (p< 0.001). DAS 28 ranged between 4.33 and 7.67. Mean 1st hour ESR was

43.57 mm and CRP was positive in 76.7% of patients. Significant correlations were found between BRAF-MDQ score and serum IL-6 level (r = 0.947, p <0.001), ESR (r =0.509, p< 0.001) as well as CRP positivity (r =0.411, p=0.005) in RA patients. Serum IL-6 level correlated with ESR (r = 0.463, p< 0.001) and CRP (r =0.376, p= 0.01) among patients. **Conclusion**: Fatigue is a common symptom and scores higher among RA patients than healthy controls and should be measured in all RA patients with simple fatigue questionnaires matching with different cultures. Fatigue becomes more prominent as serum IL-6 level increases independently of the disease duration and activity.

A-099

Ferritin in sera and CSF: Its importance as both predictive and etio-diagnostic biomarker in ischemic stroke, single center prospective study

H. M. Demerdash[1], O. Mansour[2], M. Megahed[3], T. Zytoun[3]. *[1]pharos University, Alexanddria ,Egypt, Alexandria, Egypt, [2]Faculty of medicine , Alexanddria ,Egypt, Alexandria, Egypt, [3]Faculty of medicine , Alexandria ,Egypt, Alexandria, Egypt*

Background:Iron and ferritin are known to have an important role in stroke as well as in other disorders. This prospective study was designed to determine whether determination of CSF ferritin levels might help to estimate the severity and prognosis of stroke.Methods:Thirty-two patients with a diagnosis of acute stroke due intrinsic atherosclerotic vessel pathology were included in the study within 24 h from onset of symptoms. Plasma and CSF ferritin were assayed; and correlated them with the known marker Aβ1-42 at admission. Clinical status was determined by the Canadian Stroke Scale at admission and on day 21.

Results:Serum ferritin level was found to be higher in patients with large lesion size (P < 0.01), deteriorated neurologic status during clinical follow-up (P = 0.03) and ICAD stroke patients (P < 0.01). CSF and Serum ferritin level were correlated with neurologic deficit (r = 0.50, P < 0.001). No correlation was found between Aβ1-42 and ferritin levels (r = 0.07, P = 0.7). Serum ferritin level (P = 0.007; OR = 1.02; 95% CI, 1.01-1.03) and large size of lesion (P = 0.021, OR = 11.92; 95% CI; 1.46-197.12) were independently associated with stroke due to ICAD pathology, Increased serum ferritin levels correlate to severity of stroke and the size of the lesion.

Conclusion:our results supported that a raised level of CSF and serum ferritin may imply a poor prognosis in terms of neurologic deterioration or ICAD induced stroke patients.

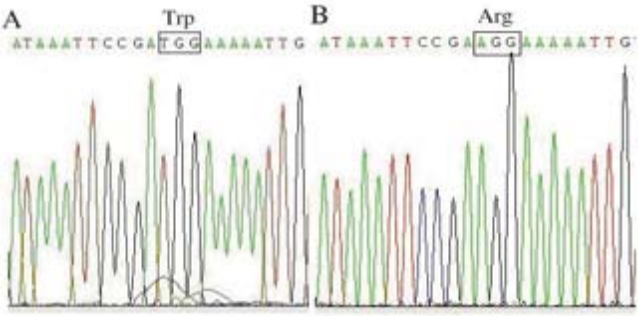
A-100

A Novel Missense Mutation in the Androgen Receptor Gene Causes the Complete Androgen Insensitivity Syndrome

X. Liu[1], Z. Cai[2], L. Xie[3], L. Ji[1]. *[1]Peking Unversity Shenzhen Hospital, Shenzhen, China, [2]Hengyang Blood Center, Hengyang, China, [3]Shenzhen Research Institute of Population and Family Planning, Shenzhen, China*

A 22-year-old, phenotypically female individual was presented at hospital because of primary amenorrhea. The physical examination revealed normal female external genitalia, sparse pubic hair and developed breasts with juvenile nipples and pale areolas. A mass was found in the left inguinal canal. The gynecological examination revealed a short, blind-ending vagina 7.0 cm in length. Ultrasonography of the pelvis showed no uterus, fallopian tube, or ovaries. The hormonal profiles were as follows: follicle-stimulating hormone (FSH) 23.4 IU/L, luteinizing hormone (LH) 39.6 IU/L, testosterone (T) 23.0 nmol/L, estradiol (E_2) 0.177 nmol/L. FSH and LH were above the upper normal limit. T was elevated compared with the normal female level and was in the normal range for males. E_2 was in the normal range for females. Cytogenetic analysis from the peripheral blood lymphocytes revealed a 46,XY karyotype in the patient.

DNA (genomic DNA was extracted from peripheral blood of the patient) sequencing of polymerase chain reaction (PCR) products revealed a single-nucleotide substitution (A→T) at position 2184 in exon 3, resulting in the conversion of arginine (AGG) to tryptophan (TGG) at amino acid position 608 in the DNA-binding domain of the AR (Figure 1). The novel missense mutation of exon 3 in the AR gene, resulting in the nonfunctional protein, is responsible for the clinical symptoms of CAIS.



The study extends the spectrum of exon 3 mutations in the AR gene.

Figure 1. DNA sequence of exon 3 of the androgen receptor(AR) of the patient(A) and the normal sequence (B). The mutant sequence shows conversion of arginine (AGG) to tryptophan (TGG).

A-101

Association Between Serum Uric Acid Levels and Non-Alcoholic Fatty Liver Disease

E. Sertoğlu[1], C. N. Ercin[2], G. Celebi[2], H. Gurel[2], H. Kayadibi[3], H. Genc[4], M. Kara[5], T. Dogru[2]. *[1]Ankara Mevki Military Hospital, Anittepe Dispensary, Biochemistry Laboratory, Ankara, Turkey, [2]Gulhane School of Medicine, Department of Gastroenterology, Ankara, Turkey, [3]Adana Military Hospital, Department of Medical Biochemistry, Adana, Turkey, [4]Izmir Military Hospital, Department of Gastroenterology, Izmir, Turkey, [5]Etimesgut Military Hospital, Department of Gastroenterology, Ankara, Turkey*

Background: Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of conditions ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) and cirrhosis. It has become one of the most common forms of chronic liver disease affecting 20-30% of the Western population. Elevated serum uric acid (SUA) levels have been suggested to be an independent risk factor for the development of NAFLD in healthy adults. In this study, we aimed to investigate the relationship between SUA and liver histology in non-diabetic and normotensive patients with NAFLD.

Materials and Methods: A total of 242 male patients, 102 with non-alcoholic steatohepatitis (NASH) and 140 with non-NASH were included. The histopathological examination of the cases were carried out according to Kleiner NAFLD activity score (NAS) combining steatosis, lobular inflammation and ballooning hepatocyte degeneration. Hyperuricemia was diagnosed as SUA more than 7 mg/dL.

Results: The prevalance of hyperuricemia was 33.4%. SUA levels in patients with NASH were significantly higher than those of non-NASH (p= 0.035). There was also no difference between the NASH and non-NASH groups in terms of hyperuricemia prevalence (p= 0.107). Univariate and multivariate analyses both demonstrated that hyperuricemia had a significant association with younger age (OR, 0.930; 95% CI, 0.884-0.979) (p= 0.005), higher body mass index (OR, 1.173; 95% CI, 1.059-1.301) (p= 0.002) and hepatocellular ballooning (OR, 1.678; 95% CI, 1.041-2.702) (p= 0.033). Area under the curve for hepatocellular ballooning was OR, 0.626; 95% CI, 0.544-0.708) (p= 0.005) and NAS was OR, 0.579; 95% CI: 0.507-0.652) (p= 0.035). Hyperuricemia was insignificant in predicting other hepatic necro-inflammatory changes.

Conclusions: SUA level seems to be a useful metabolic parameter in the differentiation of NASH and non-NASH. In addition, hepatocellular ballooning, which is considered to be an earlier predictor of hepatocyte injury, was the unique histological parameter associated with hyperuricemia. It is also thought to be a biochemical evidence in the pathogenesis of liver damage. Further studies on the involvement of SUA in NAFLD will not only expand our understanding of the mechanism of NAFLD, but will also assist in the eventual development of new prevention and treatment strategies for NAFLD by modulating the SUA levels.

A-102

The Elecsys® Periostin assay as a companion diagnostic for the novel asthma drug lebrikizumab

J. Sherman[1], C. Holweg[2], H. Kincaid[3], S. Sidobre[3], L. Ma[3], R. Maciuca[2], A. Geistanger[4], J. Matthews[2], S. Palme[4], K. Yen[2]. *[1]Roche Professional*

Diagnostics, Indianapolis, IN, [2]Genentech, South San Francisco, CA, [3]Covance Central Laboratory Services, Inc, Indianapolis, IN, [4]Roche Professional Diagnostics, Penzberg, Germany

Objective: The Elecsys® Periostin assay is being developed as a companion diagnostic for the anti-IL13 drug lebrikizumab in asthma. The current clinical trial assay was used to evaluate the precision performance according to CLSI EP 5-A2 under field conditions. The assay was used in the Phase IIb lebrikizumab trials (LUTE and VERSE) to stratify asthma patients by baseline serum periostin levels.

Methods: The Periostin assay is an automated electrochemiluminescence immunoassay. The assay requires a total of 18 minutes to perform. It employs two monoclonal antibodies targeted to Periostin via the sandwich principle. The assay is used for the in vitro, quantitative determination of periostin in human serum.

Imprecision testing was conducted using a total of eight human serum pools covering the entire measuring range (10-160 ng/mL). This experiment was carried out at three external routine laboratories, on three **cobas e** 601 analyzers. The experimental setting included two replicates in two runs per day for twenty-one days (EP-5), using two reagent lots. Using this design, repeatability, intermediate precision and reproducibility were estimated based on a variance-components model.

It has been shown that high serum Periostin levels (≥ 50ng/ml) are associated with benefit from lebrikizumab in asthma (Phase II lebrikizumab trial MILLY, Corren et.al. NEJM, 2011). LUTE and VERSE were randomized, multicenter, double-blind, placebo-controlled, parallelgroup clinical trials. Patients were aged 18 years and older whose asthma remained uncontrolled despite daily treatment with inhaled corticosteroids (ICS) therapy and a second controller medication. Patients were randomized in a 1:1:1:1 ratio to receive subcutaneous lebrikizumab at 250 mg, 125 mg or 37.5 mg dose or placebo, administered every 4 weeks. The primary endpoint was reduction in exacerbation rate and the main secondary endpoint was percent change in forced expiratory volume (FEV_1). Efficacy analysis for each endpoint was assessed in subgroups of periostin high (≥50 ng/ml) and low (<50 ng/ml) patients.

Results: Repeatability for the Periostin assay varied from 1.24% to 1.64% CV, intermediate precision varied from 2.30% to 2.56% CV and overall reproducibility varied from 3.10% to 3.95% CV. The precision obtained fulfilled the assay specification and met the requisite target to segment subjects properly to the correct periostin stratum.

A total of 463 patients were enrolled in both studies, of which 42% of patients were classified as periostin-high. Compared with placebo, the exacerbation rate was reduced by 60% (95% CI: 18, 80) in periostin-high patients (combined dose levels) and by 5% (95% CI: -81, 47) in periostin-low patients. FEV_1 at 12 weeks increased in the periostin-high group by 9.1% (95% CI: 2.2, 15.9) over placebo, compared with a 2.6% (95% CI: -2.7, 7.9) increase over placebo in the periostin-low group.

Conclusion: The Periostin assay demonstrated good precision and was used for clinical sample testing to stratify patients in the lebrikizumab Phase IIb trials. Lebrikizumab treatment reduced the exacerbation rate and increased FEV1 in patients with uncontrolled asthma on ICS and a second controller, particularly in those who were periostin-high, confirming the findings from the Phase II trial MILLY.

A-103

Equivalence between high sensitivity CRP and low sensitivity CRP tests in infants

S. N. Narla, P. Nelson, D. Brass, B. Horne, Y. Zhu. *Medical University of South Carolina, Charleston, SC*

Background: C-reactive protein (CRP) is an acute-phase protein that rises in response to inflammatory processes. Most clinical laboratories perform two different assays for measuring CRP, low sensitivity CRP (ls-CRP) and high sensitivity CRP (hs-CRP). The hs-CRP is usually used in combination with other biomarkers to assess risk of developing myocardial infarction in patients presenting with acute coronary syndromes and risk of cardiovascular disease in adults who do not manifest disease at present. Along with the monitoring of inflammatory process, ls-CRP is also used in the diagnostic evaluation of sepsis in neonates and infants. CRP values in healthy infants and neonates vary from a range of 0.2 mg/dL to 1.0 mg/dL. Therefore, for the diagnosis of sepsis, a concentration >1.0 mg/dL is considered abnormal and serial testing is frequently performed. The objective of this study is to verify whether the hs-CRP method is equivalent to ls-CRP in monitoring inflammatory processes in infants.

Methods: For this study, 33 serum samples with ls-CRP higher than 0.7 mg/dL from a patient population of <1 year old were collected. These serum samples were analyzed by both ls-CRP and hs-CRP assays on the UniCel DxC800 Systems by turbidimetric immunoassays. In both methods, CRP reacts with a specific antibody to form insoluble antigen-antibody complexes. The turbidity was measured at 340 nm for ls-CRP with an analytical measurement range of 0.7mg/dL to 20 mg/dL and at 940 nm for hs-CRP with the range of 0.02 mg/dL to 6 mg/dL. Equivalence of these two methods for the chosen population was evaluated with an allowable total error of 0.6 mg/dL or 25 %, whichever is higher. Also, 18 samples with ls-CRP values <0.7 md/dL were analyzed by both methods.

Results: 33 specimens were compared over a range of 0.22 to 23.35 mg/dL by ls-CRP. The difference between the two methods was within the allowable error for 31 of 33 (94 %) specimens, with a linear regression equation y=0.886x+0.3713 and the correlation coefficient (R) of 0.9908.The error index was determined by the ratio of the difference between two methods (hs-CRP – ls-CRP) to the allowable total error and the average error index was -0.13 with a range of -1.03 to 1.25. For 12 samples with concentrations < 2.4mg/dL by ls-CRP, the mean bias was -0.16 mg/dL with a standard deviation of 0.39 mg/dL, out of which 2 samples had error >0.6 mg/dL. For 21 samples with concentrations > 2.4mg/dL by ls-CRP the mean bias was -1% with a standard deviation of 12%. Also, all the 18 samples with CRP concentrations <0.7mg/dL by ls-CRP presented values <0.7 mg/dL by hs-CRP method.

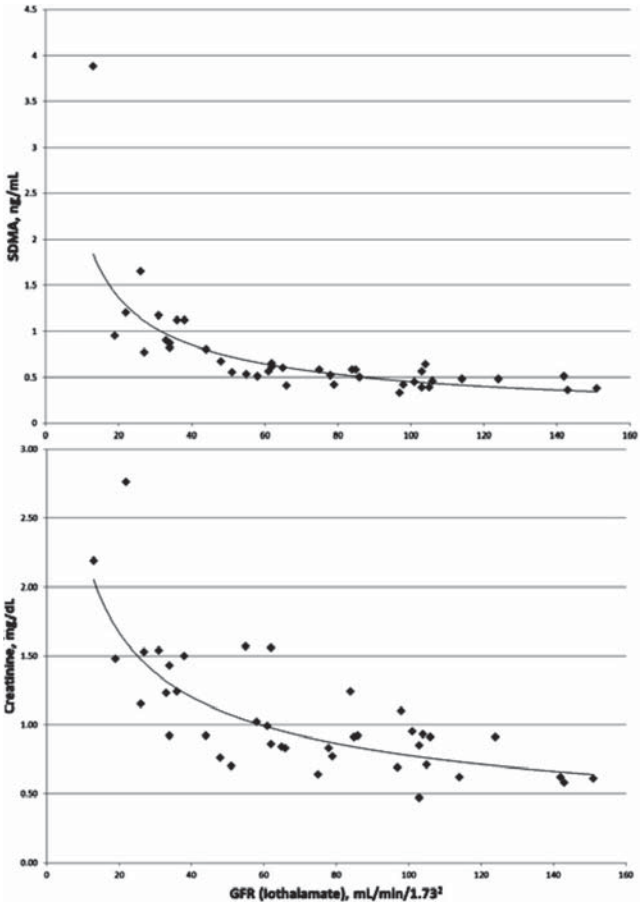
Conclusions: CRP concentrations in the patient population of <1 year old infants can be analyzed by both Beckman Coulter UniCel DxC ls-CRP and hs-CRP methods to monitor inflammatory processes. Overall, the hs-CRP assay is equivalent to the ls-CRP method for the majority of patients (94%). However, in about 17% patients with CRP < 2.4 mg/dL, the bias may be greater than the allowable total error.

A-105

SDMA Outperforms Serum Creatinine-Based Equations in Estimating Kidney Function Compared with Measured GFR

D. A. Payto, J. M. El-Khoury, D. R. Bunch, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Symmetric dimethylarginine (SDMA), a catabolic product of post-translational modified arginine containing proteins, is an emerging biomarker for renal function. Recent studies have shown that there is a correlation between SDMA and renal function in chronic kidney disease (CKD) patients. However, there are no published reports that have investigated the performance of SDMA versus creatinine in estimating glomerular filtration rate (GFR) in both CKD and non CKD patients. The objective of this study was to determine the correlations of measured GFR with SDMA levels, creatinine levels, eGFR (MDRD), and eGFR (CKD-EPI). **Method:** EDTA plasma samples were obtained from 21 male and 19 female adult subjects who were 21-76 years old. These subjects had GFR measured by radioactive iothalamate renal clearance, ranging from 13-151 mL/min/1.73^2. Arginine, asymmetric dimethylarginine (ADMA), and SDMA in EDTA plasma were assayed using a previously validated LC-MS/MS method. **Results:** A power regression analysis showed R^2=0.7544 and R^2=0.5827 the correlation of measured GFR with SDMA and creatinine, respectively. A linear regression analysis was used for the correlation of measured GFR with eGFR by MDRD and eGFR by CKD-EPI with R^2=0.6534 and R^2=0.7183, respectively. **Conclusion:** Correlation of measured GFR with SDMA was higher than those with creatinine, eGFR (MDRD), and eGFR (EPI) in a population of CKD and non CKD patients. In Conclusion: SDMA outperforms creatinine and creatinine-based equations in estimating kidney function compared with measured GFR.



A-106

Prevalence of Metabolic Syndrome and its Components according to Different Definitions among Nepalese Type 2 Diabetic Patients

D. R. Pokharel[1], D. Khadka[2], M. Sigdel[1], N. K. Yadav[1], L. B. Sapkota[1], P. Rayamajhi[1], S. K. Jha[1], A. Yadav[1], P. S. Shukla[1]. *[1]Manipal College of Medical Sciences and Teaching Hospital, Pokhara, Nepal, [2]Gandaki Medical College and Teaching Hospital, Pokhara, Nepal*

Objective: The objective of this study is to determine the prevalence of the metabolic syndrome (MetS) and its components according to WHO (1998), NCEP ATP III (2005), IDF (2005) and newly introduced Harmonized (2009) criteria and determine their agreement and disparity in diagnosing MetS among Nepalese patients with type 2 diabetes mellitus (DM).

Methods: Patients with type 2 DM without any other acute or chronic illness were recruited for the study from the Manipal Teaching Hospital (MTH), Pokhara, Nepal. Clinical data were obtained by interviewing the patients with structured questionnaire, anthropometric and blood pressure measurements and biochemical analyses of the blood samples. The analyzed biochemical parameters included fasting serum glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides and high density lipoprotein cholesterol. Statistical analysis included usage of Student's t and Chi square tests, kappa statistics and 95% confidence intervals.

Results: 1061 (male: 589, female: 472) type 2 diabetic patients aged between 30-89 years were included in the study. The total age adjusted prevalence was 69.9 %, 73.9%, 66.8% and 80.3% according to WHO, NCEP ATP III, IDF and Harmonized definitions, respectively. The Harmonized definition outperformed other definitions in diagnosing MetS. Prevalence generally increased with the age and remained highest in the age range of 50-69 years in both the sexes. It was significantly higher in females (p=0.000) according to WHO, NCEP ATPIII and Harmonized definitions. Patients of Dalit community had the highest prevalence according to NCEP ATP III (p=0.033) and Harmonized (p=0.037) definitions whereas patients of Mongol and Newar communities outnumbered other communities according to WHO (p=0.001) and IDF (p=0.037) definitions, respectively. The overall agreement was substantial between Harmonized and NCEP ATP III (κ=0.62), moderate between Harmonized & IDF (κ=0.54) and NCEP ATP III & WHO (κ=0.51), fair between NCEP ATP III & IDF (κ=0.33) and Harmonized & WHO (κ=0.37) and slight for WHO & IDF definitions (κ=0.26). The most frequent component of MetS was central obesity according to the WHO (98.8%), IDF (99.9%) and Harmonized (85.6%) definitions respectively. However, decreased HDL was the most frequent component (93.5%) according to NCEP ATPIII definition. On the other hand, hypertension was the least frequent component according to all four definitions (WHO: 66.2%, NCEP ATP III: 72.1%, IDF: 65% & Harmonized: 67%). The overall diagnostic potential of the NCEP ATP III definition was the best among others [sensitivity: 90.8%, specificity: 98.9%, positive predictive value: 99.9% and negative predictive value: 49.9%] while comparing with Harmonized definition as the gold standard.

Conclusion: The prevalence of MetS in type 2 DM is very high in both genders irrespective of the definitions used and increases with age thus posing a potential threat of increased cardiovascular diseases in this group of patients. The patient education and control of the modifiable risk factors for MetS should be given due priority by the clinicians in the management of subjects with type 2 DM.

A-107

Serum 25-Hydroxy Vitamin D3 Levels in Patients with Psoriasis

S. Abusoglu[1], A. Unlu[1], F. Akyurek[1], F. T. Akyurek[1], G. S. Kurtoglu[2]. *[1]Selcuk University Faculty of Medicine, Konya, Turkey, [2]Meram Training and Education Hospital, Konya, Turkey*

Background: Vitamin D has classically been associated with phosphorus-calcium metabolism and bone physiology. However, the finding of vitamin D receptors at different sites suggests that vitamin D also has important extraskeletal functions. Vitamin D plays a pivotal role in modulating dendritic cell function and regulating keratinocytes and T-cell proliferation. Psoriasis is considered a prototypic T helper (Th) 17-mediated disease with a putative role played by vitamin D deficiency in its pathogenesis. The most efficient laboratory approach consists of a well-defined evaluation of immune response in order to help diagnosis, to monitor evolution, and to evaluate the effects of individualized therapeutic treatments. Vitamin D has been found to regulate immune function in a number of inflammatory and autoimmune disorders. The objective of this study was to find out the serum vitamin D levels in patients with psoriasis.

Methods: Blood samples were collected and serum was seperated with centrifugation from 51 healty and 93 patients with psoriasis. Patients with chronic disease and calcium metabolism disorders were excluded. This study was approved by local ethic committee. 100 µL internal Standard (d6-25-hydroxyvitamin D3) and 1000 µL acetonitrile were added to 250 µL of serum, calibrator, control for protein precipitation, vortexed for a minute and centrifuged at 13.000 rpm for 10 minutes. 40 µL of supernatant was injected into HPLC analytical column for chromatography. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode. This method's coefficient of variation and % bias values were 9.35,1.29; 3.81,3.21 and 2.29,2.60 for 10, 32 and 150 µg/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum 25-hydroxy vitamin D3 levels were significantly lower in patient group (10.3 ±5.6) compared to control group (13.7 ±7.8) (p=0.004) according to Mann-Whitney U test. Also, there was no statistically significant correlation between Psoriasis Area Severity Index (PASI) and serum vitamin D levels (p=0.99) according to Spearman correlation analysis.

Conclusion: Vitamin D may play an important role of psoriasis pathogenesis. Vitamin D has been used to treat psoriasis in the topical form with great success. Low levels of vitamin D may also have important implications in the pathogenesis of psoriasis. Vitamin D3 acts mainly on the vitamin D receptor to regulate keratinocyte growth and differentiation, but also

has an influence on immune functions of dendritic cells and T lymphocyte. Vitamin D deficiency may be common in patients with psoriasis. Screening for vitamin D deficiency may be useful for comprehensive management.

A-108

CEDIA® Cyclosporine Applications for the Beckman Coulter AU480, AU680 and AU5800 Analyzers

D. L. Cheng, T. Miwa, C. Wong. *Thermo Fisher Scientific, Fremont, CA*

Background: Cyclosporine is an immunosuppressant used in the treatment of autoimmune diseases and the reduction of tissue rejection following organ transplantations.

Although its mechanism of action is still under investigation, cyclosporine appears

to affect the metabolism of T-helper lymphocytes and T-suppressor lymphocytes, resulting in the inhibition of the immune system. Although cyclosporine is safely used over an established narrow range of concentrations, inadequate dosing may lead to organ rejection, while overuse may lead to a number of adverse effects including nephrotoxicity, and hepatotoxicity. The probability of adverse effects on users' health increases with drug concentration. Therefore, it is crucial to monitor cyclosporine levels to achieve optimal immunosuppressive effects in patients.

Methods: The CEDIA Cyclosporine PLUS Assay is based on the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. Cyclosporine in the human whole blood sample competes with cyclosporine conjugated to one inactive fragment for antibody binding site. Once cyclosporine in the sample binds to antibody, inactive enzyme fragments reassociate to form active enzymes. The amount of active enzyme results in an absorbance change that is directly proportional to the amount of cyclosporine in the sample, and is measured spectrophotometrically. The Beckman Coulter AU480/AU680/AU5800 analyzers are new applications for the CEDIA Cyclosporine PLUS Assay. Performance measures of the CEDIA Cyclosporine PLUS Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers were conducted in low-range (25-450 ng/mL) and high-range (450-2000 ng/mL) assay studies. Analyzer performance was determined for precision, linearity, limit of detection, and accuracy. Correlation studies using the AU480/AU680/AU5800 analyzers were conducted against the reference analyzer, Hitachi 911.

Results: All studies were evaluated using CLSI guidelines. Five levels of cyclosporine controls were used in the studies. The precision ranged from 7.1 to 3.1%CV for within-run and 12.7 to 8.6%CV for total run. Low range linearity was measured and confirmed over a range of 9.08-427.85 ng/mL on the AU480/AU680/AU5800. High range linearity was measured and confirmed over a range of 590.1-2027.6 ng/mL on the AU480/AU680/AU5800. The limit of detection on the AU480/AU680/AU5800 yielded 8.3 ng/mL. Accuracy was measured using patient correlation against the reference analyzer Hitachi 911, which yielded a Deming's Regression for each analyzer. (Low Range Cyclosporine): AU480 = 1.03*(Hitachi 911) - 0.40 (N = 115, r = 1.00), AU680 = 0.97*(Hitachi 911) + 13.00 (N = 100, r = 1.00), AU5800 = 1.00*(Hitachi 911) - 0.50 (N = 116, r = 0.99). High Range Cyclosporine: AU480=1.03*(Hitachi 911) + 90.96 (N = 126, r = 0.97), AU680=1.07*(Hitachi 911) + 30.23 (N = 126, r = 0.97), AU5800=1.05*(Hitachi 911) + 9.00 (N = 126, r = 0.97).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the CEDIA Cyclosporine PLUS Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers, and will provide an effective monitoring system for patients receiving cyclosporine therapy.

A-109

Chemical composition of urinary tract calculi assessed in a Caribbean teaching hospital

L. L. Dilworth, D. McGrowder, M. Tapper, R. Thompson, D. Stennett. *The University of the West Indies Mona, Kingston, Jamaica*

Background: Urolithiasis is a heterogeneous and fairly common urological disorder with an estimated lifetime prevalence of between 5 to 10%, with the risk being higher in men. Environmental and genetic factors contribute to calculi formation. Research regarding kidney stones has gained increased attention in light of its complex molecular genetic basis as well as recent associations between urolithiasis and cardiovascular diseases. Importantly, the proper management of patients with renal stone disease involves the analysis of urinary calculi by varied methods. The main objective of this study was to assess the chemical constituents particularly inorganic minerals of urinary tract stones observed at the University Hospital of the West Indies over a 4-year period. The study is validated by the observation that there is a paucity of data on the types and composition of calculi recovered from patients within this region.

Methods: The study was conducted on 288 urinary tract stones sent to the chemistry laboratory for analysis from both male and female patients. Samples were washed with deionized water, air-dried and powder obtained via pulverization in an agate mortar. Qualitative chemical analysis of the stones for calcium, magnesium, phosphate, oxalate, uric acid, cystine and bicarbonate was done based on standard methods. Carbonate content was determined by the effervescence test by exposing the stone powder to concentrated hydrochloric acid. After the mixture was boiled, the filtrate was used for detection of calcium with ammonium oxalate, magnesium with potassium phosphate and ammonia, phosphate with ammonium molybdate and oxalate with calcium chloride. The powder was also boiled with N-potassium hydroxide and Folin's uric acid reagent with sodium cyanide added to the filtrate to detect uric acid. Cystine was detected with sodium nitroprusside and sodium cyanide.

Results: The incidence of stones from males and females was in the ratio of 1.4:1. Calcium, the main constituent was present in 96.2% of the stones followed by phosphate 67.7%, oxalate 56.3%, magnesium 28.2% and uric acid 17.3%. Mixed calcium phosphate accounted for 42.4% of the stones while there were 25.4% pure calcium phosphate stones. Mixed calcium oxalate accounted for 43.4% of the stones while there were 12.9% pure calcium oxalate stones. Mixed uric acid was present in 16.3% and urinary stones containing bicarbonate accounted for 11.8%. One cystine stone was recorded.

Conclusion: The study revealed that a relatively high proportion of the urinary tract stones in the sample population consisted of both pure and mixed calcium phosphate, followed by calcium oxalate and uric acid. The main contributory factor to the frequency of these stones seems to be hypercalciuria resulting from hypercalcaemia. Results show that males are more likely to present with urolithiasis. Ongoing studies are geared at garnering more detailed information on both the composition and structure of the urinary tract stones using solid state nuclear magnetic resonance spectroscopy and X-ray diffraction crystallography.

A-110

Screening for Hemoglobin Variants [HVs] While Measuring Hemoglobin A1c (HA1c).

N. Yadak[1], C. Hoang[2], D. Moore[2], E. S. Pearlman[2]. *[1]University of Tennessee Health Sciences Center, Memphis, TN, [2]Veterans Affairs Medical Center, Memphis, TN*

Context: As part of an effort to develop an in-lab protocol for the reporting of HA1c results in the presence of HVs, we sought to assess the frequency and identities of HVs in the VAMC population.

Methods: Over seven weeks, 5188 patients were assayed for HA1c using HPLC [Tosoh; South San Francisco, CA] according to manufacturer's directions. Specimens with an HV flag were referred (Lab Corp; Raleigh, NC) for hemoglobin ID [H-ID]. Estimates of the frequency of AS and AC (8 and 2.8% respectively) among African-American [A-A] and the population prevalence of DM (9.5%) are literature derived. A total of 40,648 HA1c assays were done in calendar year 2012.

Results: A total of 208 (4%) HV flags were generated. The median age was 61 YO [27, 90], 188 (90.4%) were male and 192 (92.3%) A-A while 6 were of unknown race (UR). Of these, 181 [87%] had sufficient specimen for H-ID and results included AS (118/113 A-A, 4 UR), AC (42/40 A-A, 1 UR) while the remaining 21 comprised HPHF (9), D-LA (3), SC (2), G-Norfolk (1), Kokomo (1), A2' (1) and no HV identified [NVI] (4). Of a total of 57,680 veterans registered at the VAMC, 35.7% are self-identified as A-A. Diabetic patients (5480) were assumed to be tested 3 times (on average) in any 12 month period and the remaining 24,208 assays were attributed to once-tested patients. Thus in any given 12 month period 29,668 patients out of the total of 57,680 (51.47%) patients would be tested. Requests were assumed to be uniformly distributed over 52 weeks. Making all these assumptions the expected number of AS/A-A patients was [29,668]•[0.357]•[0.08]•[7/52] = 114 [actual=113]. A similar calculation yields an expected 40 instances of AC trait [actual=40]. HPHF and Kokomo specimens generated an HV flag but not a numeric result for HA1c.

Conclusion: In our population that was 35.7% AA, 4% of patients assayed for A1c generated an HV flag. More than 90% occurred in AAs and 85% were S and C trait. Four (2.2%) specimens were NVI. The observed and (crudely) estimated number of AA patients with S and C trait were in close agreement [chi-sq. = 0.084; p>.9].

A-111

Tubular Damage Is Present In Patients with MGUS and Asymptomatic Multiple Myeloma Even in the Absence of Impaired Estimated Glomerular Filtration Rate; Alterations of Neutrophil Gelatinase-Associated Lipocalin and Cystatin-C in Myeloma Patients Post IMiD- and Bortezomib-Based Regimens

I. Papassotiriou[1], D. Christoulas[2], E. Kastritis[2], G. P. Papassotiriou[2], A. Margeli[1], N. Kanellias[2], E. Eleutherakis-Papaiakovou[2], M. A. Dimopoulos[2], E. Terpos[2]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Department of Clinical Therapeutics, University of Athens, Medical School, Athens, Greece*

Background: We have recently shown that urinary and serum Neutrophil gelatinase-associated lipocalin NGAL were elevated in the vast majority (90% and 70%, respectively) of newly diagnosed patients with multiple myeloma (MM), while serum cystatin-C (CysC), an accurate marker of GFR, was elevated in 70% of them. However, there is no information for the value of these markers in patients with MGUS, asymptomatic MM (AMM), as well as in symptomatic MM post treatment.

Patients and Methods: Thus, we measured urinary and serum NGAL and serum CysC in 40 patients with MGUS; 36 with AMM and 120 healthy controls. Furthermore, we measured serum NGAL and CysC in 39 newly diagnosed symptomatic MM patients before and after frontline therapy with novel agents. Serum and urinary NGAL was measured using an immunoenzymatic technique, while CysC was by means of immunonephelometry. The estimated GFR (eGFR) was calculated using the CKD-EPI equation. Patients were divided into the 5 CKD stages of the KDIGO classification, according to eGFR.

Results: Urinary NGAL was elevated in patients with MGUS (median: 14ng/ml, range 0.5-31ng/ml) and AMM (22.3ng/ml, 0.9-78ng/ml) compared to controls (5. ng/ml, 0.7-9.8ng/ml, $p<0.001$ for both comparisons). Similarly, serum NGAL was elevated in patients with MGUS (106ng/ml, 74.9-205.5ng/ml) and AMM (94.2ng/ml, 29.5-306.4ng/ml) compared to controls (63ng/ml, 37-106ng/ml, $p<0.01$). There was no difference between MGUS and controls or MGUS and AMM regarding CysC serum values, indicating that traditional indices of renal function could not detect early renal damage. However, 22 (55%) patients with MGUS and 24 (66%) with AMM had higher urinary NGAL values than the higher value of the controls. Similarly, 9 (22.5%) MGUS and 11 (30%) AMM patients had higher levels of serum NGAL than the higher value in the control group. As expected, patients with symptomatic MM had elevated serum NGAL and CysC ($p<0.001$). NGAL strongly correlated with CysC ($r=0.675$, $p<0.001$) and CKD stage (mean±SD values for stages 1/2, stage 3 and stages 4/5 were: 97±57ng/ml, 144±79ng/ml and 205±124ng/ml, respectively, $p=0.014$). CysC also correlated with CKD stage (0.96±0.29mg/l, 1.54±0.32mg/l and 2.51±1.00mg/l respectively, ANOVA $p<0.001$). Among patients with eGFR <50ml/min at baseline (n=22), 4/4 who received bortezomib-based regimens and 5/18 who received IMiD-based regimens achieved at least minor renal response. After 4 cycles of therapy, serum NGAL increased in patients who received IMiD-based therapy compared to baseline (255±264ng/ml vs. 147±104ng/ml, $p=0.021$), but not in patients who received bortezomib (119±68ng/ml vs. 159±111ng/ml $p=0.520$), regardless of myeloma response to treatment.

Conclusions: We conclude that the high levels of urinary and serum NGAL in MGUS and AMM indicate the presence of subclinical renal damage in these patients early in the course of their disease, when other markers of renal function, such as sCr or even the more sensitive CysC indicate that renal function is preserved. Thus, NGAL may be useful as an early marker that predicts the development of renal damage and the progression of the disease in these patients. NGAL seems also to increase in patients with renal impairment who receive IMiD-based regimens.

A-112

Association of Interleukin (IL-18) -607A/C and -137C/G Polymorphisms With Early Graft Function In Renal Transplant Recipients

H. Akbas, F. Davran, A. Dinckan, H. Kocak, D. Ozel, M. Gultekin, G. Suleymanlar. *Akdeniz University, Faculty of Medicine, Antalya, Turkey*

Background: Recent studies have suggested that increased levels of IL-18 in serum and renal parenchyma may predict acute rejection in patients with renal transplantation. IL-18 mediates a wide range of inflammatory and oxidative responses including renal injury, fibrosis and graft rejection. It has been reported that the promoter -607 and -137 polymorphisms of IL-18 influence the level of cytokine IL-18 expression. This prospective observational study aimed to assess the relevance of serial postoperative serum/urine creatinine, cystatin C and IL-18 measurements for monitoring early graft function in renal transplant recipients and to evaluate the pro-inflammatory property of IL-18 by measurement of serum IFN-γ and CRP. We also determined the effect of IL-18 -607 A/C and IL-18-137 C/G polymorphisms on graft function.

Methods: This study included 75 renal transplant recipients (28 female, 47 male; mean age: 38.28 ± 13.03) from living related donors. Blood and urine samples were collected immediately before and after transplantation at day 7 and month 1. Serum IFN-γ, IL-18, creatinine, cystatin C, CRP and urinary IL-18, cystatin C and creatinine levels were measured. Polymorphisms of the promoter region of the IL-18 gene, IL18-607A/C and -137C/G were determined by analysis of "real-time PCR/Melting curve". GFR values were estimated by Modified Diet in Renal Disease (MDRD), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and some cystatin-C-based formulas (Larsson, Rule, Hoek). SPSS 20.0 software was used for statistical analysis.

Results: Serum creatinine, cystatin C, CRP, IFN- γ, IL-18, urine cystatin C levels and urinary cystatin C/creatinine, urinary IL-18/creatinine ratios were significantly decreased after transplantation ($p<0.005$). Serum cystatin C and IL-18 levels were significantly higher in patients with IL-18-137 GG genotype before transplantation. While pretransplant levels of serum creatinine and IL-18 were found significantly higher in patients with IL-18-607 CC genotype, we also observed significantly higher serum IFN-γ levels and estimated GFR (MDRD and CKD-EPI) values in CA genotype ($p=0.012$). Receiver operating characteristic (ROC) analysis was performed to quantitate the accuracy of the different markers to detect changes in GFR. Posttransplant serum creatinine and cystatin C demonstrated a significantly greater AUC (area under the curve), sensitivity and specificity values than IL-18 and IFN- γ.

Conclusion: In this study, although we observed significantly differences in serum IL-18 levels and some GFR markers according to genotypes, the influence of polymorphisms on early graft function has not been clearly shown. Future larger studies are needed to confirm the association of cytokine gene polymorphisms with graft function. Prior to transplantation, screening of genetic predisposition which may have deleterious effect on graft function could lead to the development of new treatments for better graft survey and ultimately improve the outcome of renal transplantation.

A-113

May routine urine analysis reduce the number of unnecessary culture requests

A. Ozturk, Z. Ginis, T. Hanci, Z. Yildiz, M. Yavuz Taslıpinar, F. Ucar, G. Ozturk, A. Yalcindag, E. Alay, G. Erden. *Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey*

Background: Urinary Tract Infection (UTI) is the most common bacterial infection in the society. Bacterial infections can lead to the leukocytosis. Urine analysis is one of the most common tests for assessing urinary-tract infections. Urine culture is still the 'gold standard' for the detection of urinary tract infection, however, it is time- and labor-intensive and has a high number of unnecessary cultures. The aim of this study was to evaluate diagnostic performance of infection-related (associated) parameters of urine preliminary analysis (leukocyte esterase, nitrite, bacteria and leukocyte) in comparison to urine culture as the reference method and to investigate whether the presence of UTI cause leukocytosis.

Methods: The electronic database of our laboratory was searched between July 2013 and December 2013. Our hospital is a tertiary central hospital with 671 beds. Approximately 239.029 urinalyses were requested while the majority of these requests were from outpatients and emergency patients. A total of 3427 patients (1980 women, 1447 men, mean age 49.23±18.42) which were request urinanalysis, CBC and urine culture on the same day were enrolled in the study. All results were retrospectively reviewed. Leukocyte esterase and nitrite in chemical analysis were measured with fully automatic urine analyzer (IRIS iQ200 Diagnostics, USA). Pyuria (WBC) and bacteriuria in microscopy, leukocyte of CBC (Beckman Coulter LH780, USA) parameters were compared with urine culture. Diagnostic performance of parameters for detection of UTI were evaluated.

Results: Urine cultures were positive in 413 patients (%11.9). E. coli was the most frequently isolated bacteria (70.4%). Ratios of positive dipstick results for leukocyte esterase and nitrite in culture positive patients were 85% (n=352) and 40 % (n=166) respectively. The positive microscopy results for bacteria and leukocyte were 31 % (n=127) and 75 % (n=310), respectively. Positive predictive values of leukocyte esterase, nitrite, pyuria and bacteriuria tests were 69 %, 97 %, 74 %, and 91 % while negative predictive values were 80 %, 62 %, 75 %, 58 % respectively. The highest specifity rate was nitrite (99 %). Leukocytosis rate in patients with a positive urine culture were 23 % (n=96). A relatively high correlation was found between LE and microscopic WBC count ($r = 0.827$; $P < 0.001$).

Conclusions: Routine urine analysis is thought to be reliable in preliminary diagnosis of UTI and start empirical treatment without waiting for culture results. Also urine

analysis is easy to apply and quick test that could reduce unnecessary requests with predicting culture results. Dipstick urine analysis and urine microscopy can rule out UTI in considering most samples have no or unsignificant growth. With a systematic algorithm, laboratory workload, cost and unnecessary antibiotic prescriptions could be reduced.

A-115

Enhanced Liver Fibrosis (ELF) Score indicates progressive fibrosis in pre-clinical stages of Primary Biliary Cirrhosis

D. C. Baldo[1], A. Dellavance[1], F. Latini[2], J. A. Barreto[2], N. R. França[3], L. E. C. Andrade[3]. *[1]Fleury Medicine and Health, São Paulo, Brazil, [2]Beneficent Association of Blood Collection from Sao Paulo, São Paulo, Brazil, [3]Universidade Federal de Sao Paulo, São Paulo, Brazil*

Background: Liver fibrosis is a common consequence of most chronic liver diseases. The assessment of liver fibrosis is usually made by histologic analysis of liver biopsy samples. Percutaneous liver biopsy has inherent risks to the patient. Primary biliary cirrhosis (PBC) is a slowly progressive cholestatic disease associated with the development of cirrhosis and liver failure that may justify liver transplantation. PBC diagnosis is based on completion of at least two of the following criteria: characteristic histological findings; elevated alkaline phosphatase serum levels for over six months; and circulating anti-mitochondria autoantibodies (AMA). AMA is detected in roughly 95% of PBC patients, with a specificity of at least 98%. Occasionally AMA-positive (AMA⊕) subjects with normal liver enzymes are identified by the characteristic cytoplasmic pattern in the regular antinuclear antibody indirect immunofluorescence assay on HEp-2 cells. Biochemically normal (BN) AMA-positive individuals may represent earlier stages of CBP and may require confirmation by liver histology analysis. The Enhanced Liver Fibrosis (ELF) score is an alternative approach that combines three serum markers in an algorithm able to generate a score correlated with liver fibrosis state (mild, moderate and severe). **Aim:** To investigate liver fibrosis status, as assessed by ELF score, in BN AMA-positive individuals along a 4.5-year follow-up period.

Methods: ELF score was determined for 37 PBC patients, 68 BN/AMA-positive individuals, and 172 age- and gender-matched blood donors. BN/AMA-positive individuals and PBC patients had two samples obtained 4.57 (1.37 - 16.04) and 4.75 (1.91 - 8.37) years apart, respectively. ELF score was calculated according to a pre-established algorithm based on the serum levels of hyaluronic acid, procollagen III and inhibitor of metalloproteinase determined by chemiluminescence (Siemens Healthcare Diagnostics).

Results: ELF score was higher in baseline PBC samples (mean 9.63; 95%-CI: 9.09-10.17) than in baseline BN/AMA-positive samples (9.14; 95%-CI: 8.92-9.37) ($p=0.038$) and blood donor samples (8.91; 95%-CI: 8.81-9.02) ($p<0.001$), with no significant difference between BN and blood donors ($p=0.284$). There was a significant increase in ELF score in the follow-up of PBC patients ($p=0.010$) and BN/AMA-positive individuals ($p<0.001$).

Conclusion: PBC patients presented evidence of more severe liver fibrosis than BN/AMA-positive individuals did. The progression in liver fibrosis status in BN/AMA-positive individuals along the years indicates a subclinical inflammatory process in these individuals. ELF score appears to be a sensitive parameter for evaluation of the progression of liver involvement in BN/AMA-positive individuals.

A-116

Cardiac troponin I and B-type natriuretic peptide predict clinical outcomes in stable renal transplant recipients

P. Jarolim[1], B. L. Claggett[1], M. A. Pfeffer[1], A. Ivanova[2], M. A. Carpenter[2], A. G. Bostom[3], J. W. Kusek[4], L. G. Hunsicker[5], P. F. Jacques[6], L. Gravens-Mueller[2], P. Finn[1], M. J. Conrad[1], S. Solomon[1], A. S. Levey[7]. *[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [2]University of North Carolina, Chapel Hill, NC, [3]Rhode Island Hospital, Providence, RI, [4]National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD, [5]University of Iowa, Iowa City, IA, [6]US Department of Agriculture, Jean Mayer Human Nutrition Research Center on Aging, Boston, MA, [7]Tufts Medical Center, Boston, MA*

Background: Cardiac troponins and natriuretic peptides are becoming established as predictors of clinical outcomes (CO) in patients with cardiovascular (CV) disease; however their value in renal transplant recipients has not been established. Methods: Using a case-cohort design, we tested baseline specimens from 1114 stable renal transplant recipients enrolled in the FAVORIT study (The Folic Acid for Vascular Outcome Reduction In Transplantation) using the B-type natriuretic peptide (BNP) and high sensitivity troponin I (hsTnI) assays (both Abbott). CO included all-cause death, dialysis-dependent kidney failure (DDKF) and CV outcomes. The relationship between BNP and hsTnI values and CO were assessed via Cox regression models. Quartiles (Q) of the two biomarkers were included in models adjusted for age, gender, race, treatment, history of smoking, coronary heart disease, diabetes mellitus, A/C ratio, eGFR, BMI, blood pressure, lipid levels, graft vintage and donor type. Combinations of Q4 of BNP and hsTnI (BNP high, hsTnI high) and Q1-3 of BNP and TnI (BNP low, hsTnI low) were also evaluated. Results: Median concentrations and interquartile ranges for TnI and BNP were 5.6 (3.3, 10.5) ng/L and 56 (22, 129) pg/L. Both BNP and TnI levels were associated with age, donor type, history of CHD and diabetes mellitus, systolic BP, eGFR and urine ACR. Hazard ratios (HRs) for each fatal and non-fatal endpoint increased significantly with increasing quartiles of BNP after adjustment and remained significant after adding TnI to the model. HRs for the BNP/TnI combinations were strongly associated with all CO studied (Table). Conclusion: BNP is predictive for death, CV as well as renal outcomes in stable renal transplant recipients. Simultaneous elevation of BNP and TnI is relatively common and strongly predictive of CO.

Adjusted hazard ratios for combinations of low and high BNP and hsTnI results

Clinical outcome	BNP low/ hsTnI low (63%)	BNP low/hsTnI high (13%)	BNP high/hsTnI low (13%)	BNP high/hsTnI high (12%)
All-cause mortality	Ref	1.68 (1.03-2.72)	1.97 (1.19-3.26)	3.26 (1.92-5.54)
Mortality and DDKF	Ref	1.21 (0.77-1.91)	1.73 (1.09-2.76)	3.34 (2.04-5.48)
DDKF	Ref	0.75 (0.39-1.44)	1.32 (0.70-2.49)	2.64 (1.31-5.29)
CV outcomes	Ref	1.14 (0.67-1.92)	2.17 (1.27-3.69)	2.46 (1.38-4.36)
Mortality and CV outcomes	Ref	1.41 (0.90-2.20)	2.12 (1.33-3.36)	2.67 (1.63-4.38)

A-117

Results of Sample Testing for the Determination of Reference Intervals in Apparently Healthy Pediatric Subjects for ADVIA Centaur® Systems, Dimension Vista® and Dimension EXL® Systems Thyroid Assays

R. H. Christenson[1], D. Counts[1], B. Plouffe[2], S. Gafary[2], J. L. Burcham[2], R. Levine[2], T. Sullivan[3]. *[1]The University of Maryland, Baltimore, MD, [2]Siemens Healthcare Diagnostics Inc., Tarrytown, NY, [3]Norwich Pediatric Group, Norwich, CT*

Background: Age-specific reference intervals are necessary for appropriate interpretation of thyroid hormone measurements in the pediatric population and may vary due to methodological differences. A challenge for establishing pediatric reference intervals has been the availability of well-characterized samples from healthy pediatric subjects. Of the few studies that provide this information, most are based on specimens from patients who were hospitalized or required medical care. This study used methodology consistent with CLSI guidelines to pedigree and collect samples from apparently healthy pediatric subjects presenting for regular well child care.

Objective: To test well-characterized specimens from healthy pediatric subjects to establish pediatric reference intervals for various assays and instruments.

Methods: Eight US sites prospectively collected samples from apparently healthy pediatric subjects, under institutionally approved consent/assent procedures. Subjects were normal according to CDC weight- and height-based growth charts, were free of chronic and acute diseases, were not on medication, had no family history of thyroid dysfunction, no visible or palpable goiters, and were negative for anti-thyroglobuin and anti-thyroid peroxidase antibodies. Three age subgroups were analyzed with approximately equal numbers of males and females. Samples were shipped to a central laboratory and tested in singleton using multiple Siemens immunoassay systems. The lower and upper reference limits were defined as the 2.5^{th} and the 97.5^{th} percentiles of the distribution of test results for each of the two older subgroups. For the infant subgroup, a robust method (Horn and Pesce) was used to calculate the reference intervals.

Results:

System	Assay[a]	Infant	n	Children	n	Adolescent	n
System	**Assay[a]**	**≥1mo to <24mo**	**n**	**≥2yr to <13yr**	**n**	**≥13yr to <21yr**	**n**
ADVIA Centaur	FT3	3.28-5.19	72	3.34-4.80	190	3.04-4.65	129
ADVIA Centaur Dimension VISTA	FT4	0.94-1.44	72	0.86-1.40	190	0.83-1.43	129
	T3	1.17-2.39		1.05-2.07		0.86-1.92	
	T4	6.03-13.18	82	5.50-12.10	191	5.50-11.10	148
	TSH	0.741-5.24		0.628-3.90		0.438-3.98	
Dimension VISTA Dimension EXL	FT3	3.34-5.24	82	3.31-4.88	191	2.91-4.53	148
	FT4	0.88-1.48		0.81-1.35	190	0.78-1.33	147
	T4	7.4-14.3	75	6.8-12.5		6.0-11.6	
	TSH	0.781-5.72		0.704-4.01	185	0.516-4.13	147
Dimension EXL	FT3	3.47-5.29	75	3.35-4.82	185	2.91-4.70	147
	FT4	0.93-1.45	77	0.82-1.40	187	0.78-1.34	
	T4	6.6-13.4	75	5.8-11.8	186	5.4-10.6	

[a]Assay units: FT3, pg/mL; FT4, ng/dL; T3, ng/dl; T4, µg/dL; TSH, µIU/mL

Conclusion: Pediatric reference intervals were established for ADVIA Centaur, Dimension Vista and Dimension EXL thyroid assays using rigorously pedigreed samples. These data will assist with the appropriate interpretation of thyroid measurements in infants, children and adolescents.

A-118

Evaluation of six different formulas and equations for estimate low density lipoprotein cholesterol (LDLc) concentration.

O. M. Arroyo Huanaco, S. R. Alcantara Tito, B. J. Sánchez Jacinto, S. M. Flores Toledo, J. C. Jara Aguirre. *Universidad Peruana Cayetano Heredia - Facultad de Medicina Alberto Hurtado - Escuela de Tecnologia Medica, Lima, Peru*

Background: The National Cholesterol Education Program Adult Treatment Panel (ATP III) identified the serum LDLc concentrations as the primary criterion of diagnosis and treatment of patients with hyperlipidemia and also as one of the most important parameter for evaluation of coronary heart disease and cardiovascular risk assessment. Most of Peruvian clinical laboratory used the Friedewald formula and some LDLc precipate method for estimate serum LDLc concentration, but the Friedwald formula used has some limitations especially with extreme low and high triglycerides values (> 400 mg/dL). The aim of the study was compare six different proposal formulas and equations: Friedewald, Anandaraja, Chen, Puavilai, Vujovic and Cordova for estimate value of serum LDLc compared to LDLc precipitate method. **Methods:** A descriptive cross-sectional study was conducted and 220 serum samples from adolescent students of medical technology at the Peruvian University Cayetano Heredia were collected during the annual clinical evaluation performed on November 2012. Serum samples on fast conditions for lipid profile were obtained, the measurement of LDLc cholesterol and HDLc cholesterol were performed by precipitation methods (Wiener Lab, Argentina), Total Cholesterol (TC) and Triglycerides (TG) were measurement by enzymatic colorimetric endpoint method (Wiener Lab, Argentina), using a BT 3000 Plus automatic photometer analyzer at the Clinical Laboratory of Cayetano Heredia Clinic - Universidad Peruana Cayetano Heredia. Linear regression analysis and Blant Altman plots were performed to evaluate six different formulas and equations for LDLc estimation and compared to LDLc precipate method, using MS Excel and MedCalc Statistical Software. TG and LDLc obtained values for the statistical analysis and clinical classification were divided in levels according to ATP III. **Results:** Linear regression and Blant Altman plots showed a significant correlations and agreements, respectively. The correlation coefficient for serum sample group of TG ≤ 149 mg/dL were to Friedewald, Chen, Puavilai, Vujovic (R^2>0.95), Cordova (R^2=0.94), Anandaraja (R^2=0.81), respectively. For serum sample group of TG between 150-199 mg/dL were Friedewald, Puavilai (R^2=0.88), Chen, Vujovic (R^2=0.89) and Cordova (R^2=0.91), whereas TG (200-400 mg/dL) Friedewald, Chen, Anandaraja, Puavilai , Vujovic (R^2=0.87) and Cordova (R^2=0.86). Using Friedewald formula n=18 (8.18%), Anandaraja n=30 (13.63%), Chen n=44 (20%), Puavilai n=14 (6.36%), Vujovic n=24 (10.91%) and Cordova n=106 (48.18%) patients show a probably misclassification for hyperlipidemia. **Conclusion:** Six differents formulas showed significant correlations and agreements according Lineal regression analysis and Blant Altman plot. The Puavilai equation/formula showed good performance to estimate LDLc in all triglycerides levels and shows less misclassification of patients for hyperlipidemia compared to LDL precipitate method used in this study.

A-119

A Hope for Healing Using Amniotic Membrane and Stem Cells

A. O. B. S. Osman[1], M. M. S. El Ansary[2], H. G. Metwally[3], A. A. Gad[2], H. G. Al-Inany[2], A. H. B. Elbadawy[4]. *[1]Misr University for Science and Technology, Faculty of Medicine, Cairo, Egypt, [2]Cairo University, Faculty of Medicine, Cairo, Egypt, [3]Cairo University,Faculty of Medicine, Cairo, Egypt, [4]Assiut University, Faculty of Medicine, Assiut, Egypt*

Objectives: Testing a new technique for treatment of chronic non-healing ulcers using amniotic membrane (AM) alone or in combination with autologous mesenchymal stem cells (MSCs) as an application of clinical laboratory medicine in regenerative medicine.**Methodology:** After Institutional Ethical Committee approval, each patient signed informed written consent. The study was conducted on **37**chronic leg ulcers. Patients were randomly divided into **4**groups; **Group I**: (control group **11**ulcers), ulcers were treated with conventional wound dressings that were changed day by day for **8**weeks. **Group II**: (**14**ulcers) AM was placed in contact with ulcer and held in place with 2ry dressing; which was changed day by day. **Group III**: (**6**ulcers) autologous BM derived MSCs were injected into ulcer bed and ulcer edges. **Group IV**: (**6**ulcers) Autologous MSCs were injected into ulcer bed and ulcer edges, and freshly prepared AM was placed in contact with ulcer and held in place with 2ry dressing.**Patients were subjected to:*I. Assessment of ulcer healing and wound measurement:*-1. Percentage of the healed wound area and healing rate:Healed wound area%= (**Original wound size-final wound size) /Original wound size×100 **Healing rate in cm^2/day**= (Original wound size-final wound size) /Time consumed to reach final wound size**-2. Wound size:** Greatest length and greatest width were measured, and surface area was calculated in cm^2.**-3. Wound grading**: Based on Pressure Ulcer Scale for Healing (PUSH Tool) Ulcers classified as **Mild**, **Moderate, and Severe**. ***II- Pain Assessment:***On a scale from 1 to10, pain level is classified as (**No pain, Mild, Moderate, & Severe**).***III- Follow up:***-Healing rate and ulcer size-Pain Assessment -Taken of AM graft (Day2).-Ulcer images (Day0, and weekly till end of the study)**Statistical Analysis** It was performed with SPSS software version15.0 for Windows (SPSS Inc., CR). **Results:**Significant improvement in patients of groups **II**,**III**, and **IV** regarding pain &ulcer size .There was significant difference in healing rate &reduction in ulcer size between **group I** and **(group II &IV)** with (**P<0.001**) where wounds showed an overall decrease in wound size and improvement in wound bed with healthy granulations**. Group II** results showed complete healing of **14**ulcers in**14-60**days with mean of **33.3±14.7**, healing rate was **0.064 –2.22**and mean of **0.896 ±0.646**cm^2/day with **100%** reduction in ulcer size. In **group III**; **4**ulcers (**66.7%)** showed complete healing in **45-60**days with mean of **50.5±6.7**, while **2**ulcers **(33.3%)** showed partial healing with healthy granulations. Ulcers' healing rate was **0.084 – 0.787**cm^2/day, with mean of **0.303 ±0.254**. Reduction in ulcer size ranged from **50.4%** to**100%**, with mean of

83.9% ±24.9%. In **group IV**- in the combined treatment with AM and autologous MSCs- **3**leg ulcers **(50%)** showed complete healing in **14- 25**days with mean of **17.7±6.35** and **3**ulcers **(50%)** showed partial healing with healthy granulations. Range of Ulcers' healing rate was **0.359–1.23**cm^2/day with mean of **0.759 ±0.361**. Range of reduction in ulcer size in group **IV** was **46.5% – 100%**, and mean of **78.95%±24.2%**. **Conclusion:**Using AM alone or in combination with autologous MSCs represents promising simple, safe, effective, and novel therapeutic approach for closing and healing of persistent non-healing chronic leg ulcer.

A-120

Inflammatory Markers in Polycystic Ovary Syndrome

G. Ozturk[1], S. Ozdemir[1], O. Ozdemir[2], D. Kalkan[2], G. Erden[1], S. Sezer[3], C. R. Atalay[2]. *[1]Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey, [2]Ankara Numune Research and Training Hospital, Department of Obstetrics and Gynecology Ankara, Turkey, [3]Ankara Numune Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey*

Background: Previous studies have demonstrated polycystic ovary syndrome (PCOS) associated with a proinflammatory state. The clinical spectrum of PCOS includes components of the metabolic syndrome, such as central obesity, insulin resistance, dyslipidemia and hypertension. All these disorders are epidemiologically related to cardiovascular disease, most probably through low-grade intravascular chronic inflammation. We aimed to investigate whether inflammatory markers, including C-reactive protein (CRP), procalcitonin and ischemia modified albumin (IMA) are related and altered in polycystic ovary syndrome.

Methods: A case-control study including fifty women diagnosed with PCOS,

according to Rotterdam criteria, and thirty-three controls, matched for body mass index (BMI) and age. Serum samples for CRP, procalcitonin and IMA were collected from these women under spontaneous menstrual cycles between the third and seventh days and between 08:00 and 10:00 after an overnight fast, but at random times if they suffered severe oligo- or amenorrhoea. CRP levels were measured by immunoturbidimetric method (ADVIA® 2400 Chemistry System, Siemens Healthcare Diagnostics Inc.Tarrytown USA). Procalcitonin levels were measured by sandwich immunoassay method (ADVIA® Centaur CP System, Siemens Healthcare Diagnostics Inc. Tarrytown USA). MA levels were measured by spectrophotometric method. The $p<0.05$ was considered as statistically significant.

Results: PCOS patients had increased levels of testosterone and luteinizing hormone (LH) compared to healthy BMI matched controls. Mean CRP, procalcitonin and IMA levels were higher in patients with PCOS than controls, but these differences were not statistically significant.

Conclusion: In PCOS women, plasma levels of CRP, procalcitonin and IMA were not significantly increased when compared with age- and BMI-matched controls. Several previous studies suggest that chronic low-grade inflammation in PCOS is an important risk factor for long-term situation including cardiovascular complications, metabolic disorders and ovarian dysfunction. The precise mechanisms underlying these associations require additional studies to clarify the state of the cardiovascular system in women with PCOS compared with controls in large numbers of patients to determine the relative contribution of different factors including insulin resistance, androgen status and BMI.

A-121

Recommendations of U.S. Preventive Services Task Force for Health Screenings that Include Use of Laboratory Tests

S. Shahangian[1], V. A. Moyer[2]. *[1]CDC, Atlanta, GA, [2]U.S. Preventive Services Task Force, Chapel Hill, NC*

Background: Health screening tests greatly impact the public's health because they involve testing of asymptomatic populations for diseases or conditions where interventions may impact health outcomes. U.S. Preventive Services Task Force (USPSTF) is the leading independent panel of experts that conducts rigorous and impartial assessments of scientific evidence for effectiveness of clinical preventive services.**Methods:** All screening recommendations are rated by USPSTF into 1 of 5 grades: 'A' for strong recommendation with high certainty that net benefit is substantial, 'B' for recommendation with high certainty that net benefit is moderate or moderate certainty that net benefit is moderate to substantial, 'C' for individualized decision making with at least moderate evidence that net benefit is small, 'D' against screening with moderate to high certainty that net benefit is zero or harms outweigh benefits, and 'I' is a statement that no recommendation can be made due to insufficient, poor quality, or conflicting evidence, with the balance of benefits and harms of screening undetermined.**Results:** Laboratory screening tests for 35 diseases/conditions have been included in USPSTF's evaluations for different target populations, resulting in a total of 60 currently active recommendations for laboratory testing. Of these, there were 20 'A' and 'B' recommendations including: cervical cancer in women aged 30-65 years; chlamydial infection in sexually active women aged ≤24 years and older pregnant women at risk; colorectal cancer in adults aged 50-75 years; diabetes mellitus in adults with sustained hypertension, gonorrhea infection in sexually active women at increased risk, hepatitis B virus (HBV) infection in pregnant women; hepatitis C virus infection of persons at high risk and 1-time screening of those born in 1945-1965; HIV screening of pregnant women, 1-time HIV screening of adolescents and adults aged 15-65 years; risk of coronary heart disease in men aged ≥35 years, as well as both men aged 20-34 years and women aged ≥45 years who are at increased risk; and syphilis infection in pregnant women. The most common recommendation grades were D and I (17 each). D recommendations included: cervical cancer in women aged <21 years, those aged >65 years not at high risk and with adequate prior screening, those with hysterectomy leading to removal of cervix and no history of high-grade or cancerous lesion, and human papilloma virus screening of women aged <30 years; colorectal cancer in adults aged >85 years; genital herpes simplex virus infection in adults, pregnant women, and adolescents; gonorrhea infection in men and nonpregnant women at low risk for infection; HBV infection in men and nonpregnant women; hereditary hemochromatosis in adults; lead poisoning in children aged 1-5 years at average risk and pregnant women; ovarian cancer in women excluding those with known genetic mutations that show increased risk; pancreatic cancer; prostate cancer; and syphilis infection in nonpregnant persons not at increased risk.**Conclusion:** While common perception is that screening is recommended for major and prevalent diseases, the UPSTF either recommended against (28%) or insufficiently supported (28%) use of more than half of the evaluated laboratory markers.

A-122

Performance of the Elecsys® Vitamin D Assay in a Multicenter Evaluation

A. Algeciras-Schimnich[1], A. A. Valcour[2], P. Jarolim[3], M. Lessig[4], J. Kappes[5]. *[1]Mayo Clinic, Rochester, MN, [2]LabCorp, Burlington, NC, [3]Brigham and Women's Hospital, Boston, MA, [4]NLS Lab Director Nationwide Labs, Fort Lauderdale, FL, [5]Roche Diagnostics Operations, Indianapolis, IN*

Background: Increased awareness and investigation into the importance of vitamin D for many physiological processes has resulted in dramatically increased demand for vitamin D testing. New automated methods to measure 25-hydroxyvitamin D (25-OHD) levels have been developed to help laboratories accommodate this surge. The fully automated Roche Elecsys® Vitamin D assay, which allows for the quantitative determination of total 25-OHD in human serum and plasma, was recently introduced to the US market. The Elecsys Vitamin D assay is standardized against a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using National Institute of Standards and Technology (NIST) reference materials for calibration. The current study evaluated the performance of the Elecsys Vitamin D assay across several laboratories in the United States to determine its suitability for use in clinical practice. Functional sensitivity, imprecision, and clinical concordance with a well-established NIST standardized LC-MS/MS method were assessed. **Methods:** Functional sensitivity was assessed using 8 pooled samples covering a range of 0-16 ng/mL, which were tested once a day for 10 consecutive days. Precision was assessed, following CLSI EP5-A2/EP15-A2, from 5 pooled samples in the 10- to 50-ng/mL range. Deming regression analysis was used to compare the results of the Elecsys Vitamin D assay obtained at 2 sites on the cobas e411 analyzer with LC-MS/MS performed at Mayo Clinic. Four sample cohorts were used: subjects referred for routine vitamin D testing were included in cohort I, while predialysis patients (cohort II), pregnant women (cohort III), and patients in the intensive care unit (cohort IV) represented unique subpopulations. The clinical performance of the 2 methods was evaluated using 20 ng/mL and 30 ng/mL as cutoffs for vitamin D sufficiency. **Results:** The functional sensitivity of Elecsys Vitamin D, assessed at 2 sites, was 3.52 ng/mL and 3.37 ng/mL with coefficient of variation (CV%) of 15% and 16%, respectively. Elecsys Vitamin D demonstrated consistent reproducibility across lots (N=2), across the 2 sites tested, with CV% of 5.66% to 7.12% (SD for concentrations below 15 ng/mL; 1.40 ng/mL). Consistent repeatability (CV% of 1.30% to 5.48% [0.40 to 0.72 ng/mL]), within site/lot precision (CV% of 1.94% to 7.47% [0.49 to 1.26 ng/mL]), and within site/across 2 lots precision (CV% of 4.06% to 7.78% [1.00 and 1.10 ng/mL]) was demonstrated. Overall, acceptable consistency between Elecsys Vitamin D assay at both sites, and LC-MS/MS was observed for regression parameters in both the routine and specialty cohorts with a slope range of 1.03 to 1.22 and Pearson's r range of 0.857 to 0.932. The LC-MS/MS sufficient/insufficient classification based on the 20-ng/mL cutoff over cohort I (routine adults) was 67.3% sufficient/32.7% insufficient compared with 68.8%/31.2% observed with the Elecsys method at 1 site. Using the 30-ng/mL cutoff over cohort I, the LC-MS/MS classified 36.7%/63.3% as sufficient/insufficient, respectively, and the Elecsys showed agreeable numbers at 38.3%/61.7%. **Conclusion:**

The Elecsys Vitamin D assay showed robust technical performance, with good functional sensitivity and low imprecision. Analysis of multiple cohorts showed high clinical concordance with NIST standardized LC-MS/MS, supporting the suitability of the assay for use in clinical practice.

A-123

Homogeneous immunoassay for estradiol based on blue-emitting upconverting nanophophors and luminescence resonance energy transfer

V. Kale, L. Mattsson, R. Arppe, T. Soukka. *University of Turku, Turku, Finland*

Objective: The purpose of this study was to demonstrate a competitive homogeneous assay for 17β-estradiol (E2) based on photon upconversion luminescence. $NaYF_4$: Yb,Tm upconverting nanophosphor (UCNP) with strong emission at 475 nm under 980 nm excitation was used as a donor in upconversion luminescence resonance energy transfer (UC-LRET) with small molecular conventional fluorescent dyes energy acceptors.

Methods: Upconverting nanophosphors (UCNPs; $NaYF_4$: 20% Yb, 0.5% Tm) of 25-30 nm in size were synthesised using oleic acid as chelating agent and shape modifier.[1] The nanophosphors functionalized silica shell comprising carboxylic acid groups, which were covalently conjugated to anti-E2 Fab antibody fragments (Fab S16) using carbodiimide chemistry. E2 conjugated Alexa Fluor 488 (E2-AF488) and fluorescein isothiocyanate (E2-FITC) dyes were used as acceptor conjugates. The luminescence

spectrum of the donor (emission peak around 475 nm) overlapped with the excitation spectrum of the acceptors. In the homogeneous E2 assay, E2 dilutions in buffer were first incubated together with the Fab S16 coated upconverting donor, then E2-AF 488 or E2-FITC were added to the reaction and the sensitized acceptor emission was measured at 565 nm with anti-stokes luminescence plate reader (Hidex Oy, Turku, Finland) under 980 nm infrared excitation.

Results: By using 0.006 mg/ml UCNP donor with 16 nM E2-AF488 acceptor conjugate or 0.01 mg/ml UCNP donor with 4 nM E2-FITC acceptor conjugate in the assay reaction, standard curves with IC_{50} values of 2 nM and 1.7 nM were obtained, respectively. The working range of the assay was from 0.80 nM to 3.10 nM E2 concentrations with both the acceptors. Signal levels for the homogeneous assay using the E2-FITC acceptor conjugate were two times higher than by using the E2-AF488 acceptor conjugate. However, the ratio between maximum and minimum signals, or the signal-to-background ratio, was 12 for E2-AF488 and 8 for E2-FITC acceptor.

Conclusions: In this study, we introduced a blue emitting upconverting nanophosphor as an efficient donor in UC-LRET and demonstrated a competitive homogeneous upconversion based immunoassay for E2. Photon upconversion and luminescence resonance energy transfer enable sensitive homogeneous immunoassays, which eliminate the autofluorescence background with simple instrumentation and render UCPs an attractive reporter technology for clinical diagnostics.

References:1. Kale, V; Soukka, T; Hölsä, J, Lastusaari, M. J. Nanopart. Res.2013, 15, 1850.

A-124

Enhanced Liver Fibrosis (ELF) Score for the Evaluation of Liver Fibrosis in Autoimmune Hepatitis

E. Oliveira[1], R. M. Perez[2], P. M. Oliveira[1], A. Dellavance[3], L. E. C. Andrade[1], M. L. Ferraz[1]. *[1]Federal University of Sao Paulo, Sao Paulo, Brazil, [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3]Fleury Medicine and Health, Sao Paulo, Brazil*

Background: Evaluation of fibrosis is very important in chronic liver diseases, in order to define prognosis and therapeutic options and to establish strategies of follow-up. Liver biopsy has been considered the gold-standard for the evaluation of fibrosis. However, is an invasive method and prone to sampling error. These problems have motivated the search of non-invasive and more reliable methods for detecting and staging liver fibrosis. The ELF (Enhanced Liver Fibrosis) score is based on an algorithm of three markers: (hyaluronic acid, pro-collagen III e inhibitor of metalloproteinase 1) and has been evaluated in different etiologies of liver diseases. However, ELF score has not yet been evaluated in patients with autoimmune hepatitis (HAI). **Aim**: to evaluate the performance of ELF score in patients with AIH, using the liver biopsy as a gold-standard.

Methods: Patients with HAI and a liver biopsy performed in less than 12 months were included and had a serum sample collected for the ELF score (Siemens Healthcare Diagnostics). Patients with hepatitis B or C were excluded, as well as patients with alcohol consumption > 20g/day.

Results: 109 patients were evaluated and 89 were included (90% female, mean age 34y). According to the ELF cut-offs proposed by the manufacturer, 8% of patients had absent/mild fibrosis, 42% moderate fibrosis and 50% advanced fibrosis. ELF score overestimated fibrosis in 28% of cases and underestimated in 33%. The accuracy of ELF (AUROC) for significant fibrosis ($F \geq 2$) was 0,64 (CI 95%: 0,52-0,77; p= 0,05), for advanced fibrosis ($F \geq 3$) was 0,59 (CI 95%: 0,47-0,71; p= 0,17) and for cirrhosis was 0,66 (CI 95%: 0,54-0,77; p= 0,009). Coeficient of correlation for ELF and fibrosis was 0,24 (p=0,22), for periportal necroinflammatory activity was 0,28 (p= 0,009) and for lobular necroinflamatory activity was 0,43 (p<0,001).

Conclusion: ELF score had not a good performance as a marker of liver fibrosis in patients with AIH. Using proposed cut-offs, there was overestimation as well as underestimation of liver fibrosis in many cases when comparing to liver biopsy. It is possible that the biomarkers included in the ELF score could have been influenced by the intense necroinflammatory process observed among AIH patients. In fact, ELF score was more intensely associated to lobular activity (p< 0,001) then to liver fibrosis (p= 0,22). It is interesting to note that 4 patients with acute hepatitis and no fibrosis had a high ELF score, confirming that intense inflamation is a relevant confounder of indirect liver fibrosis assessment.

A-125

Organisms cultured from the synovial fluid of infected prosthetic joints.

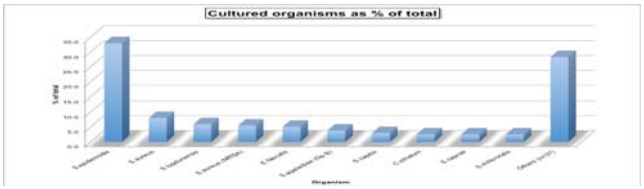
P. Kilmartin[1], S. Gulati[2], P. Citrano[2], K. Kardos[1], C. Deirmengian[1]. *[1]CD Diagnostics, Inc, Wynnewood, PA, [2]Citrano Medical Laboratories, Towson, MD*

Objective:Prosthetic joint infection (PJI) occurs after primary joint replacements at a rate of 1.0-3.0% and after revision joint replacements at a rate of 2.0-20.0%. Synovasure™ is a laboratory developed test (LDT) for the detection of alpha-defensins in the synovial fluid (joint fluid), which have been shown to be elevated in infected samples. There are two main objectives of this study; first to describe the variety and distribution of organisms infecting joint replacements nationally, and second to evaluate for organism-specific differences in the synovial fluid alpha-defensin level.

Methods:1698 synovial fluid specimens from 40 states were sent to our laboratory for the SynovasureTM alpha-defensin assay and were also cultured using the BacT/ALERT® FAN FA/FN culture bottles for recovery of aerobic and anaerobic organisms (Biomerieux). The organisms were identified and evaluated for susceptibilities using the VITEK® 2 ID/AST system (Biomerieux), a fully automated system that provides rapid microbial identification and susceptibility testing.

Results:47 different organisms from 237 culture-positive samples were identified (distribution summarized in graph). The data indicate that Staphylococcus epidermidis and Staphylococcus aureus account for 46% of the organisms cultured. Of S. aureus isolates, 41% were methicillin-resistant. In addition, there were no statistically significant organism-specific differences in the synovial fluid alpha-defensin levels in the culture-positive samples.

Conclusion:In a large national survey, we have identified Staphylococcus epidermidis (33%), Staphylococcus aureus (13%) and Staphylococcus lugdunensis (6%) as the most common organisms isolated from synovial fluid. The 40% rate of methicillin-resistance among S. aureus isolates is quite concerning given the poor outcomes associated with the treatment of these prosthetic infections. The elevation of alpha-defensin in the synovial fluid of infected joint arthroplasties does not appear to be influenced by the organism type.



A-126

Predictive roles of N-terminal proBNP and high sensitive Troponin T levels in determining mortality in chronic renal failure patients

S. Ozdem[1], V. T. Yilmaz[2], G. Uzun[1], L. Donmez[3], S. S. Ozdem[4], G. Suleymanlar[2], R. Cetinkaya[2], F. Ersoy[2]. *[1]Akdeniz University Medical School, Department of Biochemistry, Antalya, Turkey, [2]Akdeniz University Medical School, Department of Internal Medicine, Division of Nephrology, Antalya, Turkey, [3]Akdeniz University Medical School, Department of Public Health, Antalya, Turkey, [4]Akdeniz University Medical School, Department of Pharmacology, Antalya, Turkey*

Background: The mortality in chronic renal failure (CRF) patients is higher than that in normal population. Cardiovascular events are the major contributors in increased mortality rate in CRF patients since more than 50% of deaths are due to cardiovascular events. Cardiac troponins are highly specific and sensitive markers of myocardial damage. Increments in troponin levels in CRF are related to adverse clinical outcomes. B-type natriuretic peptide (BNP) is a cardiac neurohormone predominantly released from the ventricles in response to left ventricular volume expansion and pressure overload. It is released as a preproBNP and is cleaved into proBNP and a signal peptide. ProBNP is subsequently cleaved into BNP and the inactive N-terminal proBNP (NT-proBNP) peptide. Levels of BNP or NT-proBNP are known to be elevated in patients with left ventricular dysfunction. BNP may serve as an important plasma biomarker for cardiac stress and ventricular hypertrophy in patients with CRF. In fact, elevated levels of BNP indicate an increased risk of cardiac events and mortality in patients undergoing peritoneal dialysis. Markers with capacity to identify CRF patients with high mortality risk early in the course of disease may help in clinical management of these patients improving outcomes. In the present study we investigated the predictive values of NT-proBNP and high sensitive Troponin T (hsTNT) levels separately and in combination in determining all-cause mortality in CRF patients on peritoneal dialysis.

Methods: A total of 51 CRF patients (21 female, 30 male, mean age: 50.9 ± 16.5 years) and 41 healthy control subjects (22 female, 19 male, mean age: 45.7 ± 11.6 years) were included in the study. Serum levels of NT-ProBNP and hsTnT (ECLIA method) were measured in all study subjects. Optimal prognostic cut-off points for NT-ProBNP and hsTnT were determined. Overall survival rates were determined by using the Kaplan-Meier method with log-rank test for comparison among groups with different cut-off points of NT-ProBNP and hsTnT. **Results:** CRF patients had significantly higher levels of NT-ProBNP (5382.00 ± 1115.00 vs 77.00±9.99 pg/mL, p<0.00001) and hsTnT (0.070 ± 0.010 vs 0.004 ± 0.001 ng/mL, p<0.00001) than control subjects. Of the 51 patients enrolled, 24 died and 27 survived during a median follow-up period of 56.4 months (P25-P75: 32.3-57.3). Optimal prognostic cut-off points for NT-ProBNP and hsTnT were ≥ 1950 pg/mL and ≥ 0.050 ng/mL, respectively. Patients with elevated hsTnT levels had higher risk than patients with elevated NT-proBNP levels when compared with the group which had low levels of both markers (HR 4.00 [95% CI, 1.57−10.20], *P* =0.004 and HR 3.31 [95% CI, 1.40−7.82], *P* = 0.006], respectively). Patients with elevated levels of both hsTnT and NT-proBNP had a markedly increased all-cause mortality risk (HR 10.38 [95% CI, 2.82-38.27], *P* <0.0001). **Conclusion:** Our results have demonstrated for the first time that determination of both hsTnT and NT-proBNP levels is better at predicting CRF patients with high mortality risk than determination of either biomarker alone.

A-127

Enhanced Liver Fibrosis (ELF) Score for the Evaluation of Liver Fibrosis in Shistossomiasis Mansoni

T. B. Medeiros[1], A. L. C. Domingues[1], E. P. Lopes[1], A. Dellavance[2], L. E. C. Andrade[3], M. L. Ferraz[3]. *[1]Federal University of Pernambuco, Recife, Brazil, [2]Fleury Medicine and Health, São Paulo, Brazil, [3]Federal University of São Paulo, São Paulo, Brazil*

Background: Schistosomiasis mansoni has a worldwide distribution and affects over 70 million people, constituting a major cause of fibrogenic liver disease. The assessment of periportal fibrosis by ultrasound is the gold-standard for staging fibrosis in patients with schistosomiasis. However, although simple and non-invasive, ultrasound examination is not always available in endemic areas. **Aim**: to correlate the ELF score with patterns of periportal fibrosis obtained by ultrasonography in patients with Schistosomiasis mansoni.

Methods: Patients of both genders, aged> 14 years, coming from an endemic area for schistosomiasis with a history of exposure to contaminated water were included. An ultrasonography was performed by a single examiner, with unit Siemens Acuson X 150 with 3.5 MHz convex transducer. Periportal fibrosis was classified according to the six patterns of Niamey (graded from A to F, with A = no liver fibrosis and F = very advanced fibrosis). Exclusion criteria were the presence of serum markers of viral hepatitis, ethanol consumption > 210 g/week and steatosis. All patients had serum samples collected for determination of ELF score on the same day of ultrasonography. The evaluation of ELF markers (hyaluronic acid, procollagen III and inhibitor of metalloproteinase 1) was performed by chemiluminescence (Siemens Healthcare Diagnostics).

Results: 78 patients were evaluated; 50 (64%) were female, mean age 50 ± 14 years. According to Niamey classification, patients were divided into three groups: 7 patients (9%) presented pattern A/B of fibrosis, 33 (42%) pattern C/D and 38 (49%) pattern E/F. Groups showed the following ELF scores, according to the pattern of fibrosis: A/B = 8.55 ± 0.40, C/D = 9.31 ± 1.23, E/F = 9.52 ± 0.93. Significant difference was observed between groups A/B and E/F (p = 0.01).

Conclusion: In patients with Schistosomiasis mansoni the ELF score was able to discriminate patients with patterns A/B (absent or mild fibrosis) of those with E/F (advanced fibrosis).The test can be an useful tool for the diagnosis and for monitoring fibrosis progression in patients with Schistosomiasis mansoni in situations where ultrasound is not available.

A-128

Circulating blood platelet function in acute liver allograft rejection

Y. Wang. *Tianjin First Central Hospital, Key Laboratory for Critical Care Medicine of the Ministry of Health, Tianjin, China*

Background: Platelet surface receptor expression is increased in acute vascular events in various clinical settings. Endothelial injury is associated with increased adhesion and aggregation of platelets. In the present study, we investigated platelet aggregation, and markers of platelet activation in liver allograft recipients with acute rejection.

Methods: The whole blood samples from 20 recipients with biopsy-confirmed acute rejection (rejection group), 20 recipients with stable graft function (stable group) after liver transplantation, and 20 healthy volunteers (control group) were collected. To examine platelet function we measured platelet aggregation tests by using platelet aggregometer, platelet expression of glycoprotein (Gp)Ib, GpIIIa, GpIIb/IIIa, and P-selectin (granule membrane protein 140 [GMP140]) under unstimulated and activated conditions by quantitative flow cytometry, and plasma soluble P-selectin (sP-selectin) by enzyme-linked immunoadsorbent assay.

Results: When the platelet membrane GpIb, GpIIIa, and GpIIb/IIIa expression of rejection group, stable group, and control group were compared in a resting state and in a agonist [thrombin receptor-activating peptide (TRAP)]-activated state, no significant differences were observed among three groups ($P>0.05$). Platelet expression of P-selectin and sP-selectin levels were significantly increased in rejection group and stable group compared with control group ($P<0.05$). A significantly increased sP-selectin level was found in rejection group compared with stable group ($P<0.01$), and platelet P-selectin expression in a TRAP activated state ($P<0.05$). Platelet maximum aggregation values induced by all agonists (adenosine diphosphate, arachidonic acid, collagen, epinephrine) were higher in rejection group than stable group, but the difference did not reach statistical significance ($P>0.05$).

Conclusion: Acute rejection induces platelet activation without inducing circulation platelet aggregation, which reveals the state of ongoing pathogenesis of immune and inflammatory processes.

A-129

Interferences by hemolysis, lipemia and bilirubin on routine parameters on chemistry analyzers in a pediatrics hospital

S. Agarwal, G. Vargas, G. Buffone, S. Devaraj. *Texas Children's Hospital and Baylor College of Medicine, Houston, TX*

Background: Clinical laboratory assays can be affected by different interferences, most commonly, hemoglobin, lipids, bilirubin, auto-antibodies, and heterophile antibodies. Our goal was to study the effect of interference by hemolysis, lipemia and bilirubin on routinely performed parameters on various chemistry analyzers including VITROS 5600, BN Prospec, Dimension Xpand, Architect i1000SR, and Advia Centaur using pediatric samples in the Texas Children's Hospital laboratory. Further we wanted to establish cut-off indices above which these interferences confound analysis of pediatric samples. **Methods:** We tested the effect of hemolysis on K+, AST, LDH, TBIL, ALT, CK, Mg++, ALB/TP, ALKP, Fe, Lipase,NH3+ and phosphorus on VITROS 5600. Similarly for lipemia interference we analyzed HIV-1/2,Testosterone, Progesterone, Ceruloplasmin,Haptoglobin,C3 / C4, IgG / IgM / IgA, Vitamin D,AFP,aHBC,HCG,CK-MB,TSH, Albumin,Ferritin and Glucose. Lastly, for icteric interference we performed analyses for Estradiol, Folate, ALB/TP, ALT, GGT, Glucose, Na+, Cortisol, C4, Haptoglobin and HbA1c on the respective analyzers. Experiments were carried out with test samples from 3 different serum pools spiked with increasing concentrations of hemolysate (0.75g/l, 1.5g/l, 3.0g/l, 6.0g/l,), 20% Intralipid (400mg/dl, 1000mg/dl, 2000mg/dl) and commercially available Bilirubin (100uM, 250uM and 500uM). These were then analyzed on the various instruments in laboratory: VITROS 5600, Architect i1000SR, Prospec, Xpand, and Centaur. The Hemolysis-(H), Lipemia-(T) and Icterus- (I) indices were measured and documented on VITROS 5600. Lastly, for lipemic interference (>2000mg/dl) we further treated the samples with Lipoclear(1:5 ratio) and then re-analyzed the samples.

Results: We categorized hemolysis by H-index of 101-250 as mild, 251-500 as moderate, 501-999 as significant and H-index of >1000 as grossly hemolysed. Similarly, for Lipemia T-index of < 100 was considered mild,101-500 as moderate and T- index of >501 as severely lipemic. For Icteric evaluation I-index of 0-5 was characterized as normal, 5.1-9.9 as mild, 10.0 -19.9 as moderate and ≥ 20 as grossly icteric. For significant hemolysis, the main parameters affected and which shouldn't be reported included AST, LDH, TBIL, and NH3+. All the measured analytes were compromised by gross hemolysis and these samples should be rejected. None of the analyzed parameters were significantly affected by the level of Icterus. With regards to lipemia, mild lipemia (<400mg/dl) did not affect the assays while Ceruloplasmin, Haptoglobin, C3, C4, and Immunoglobins (IgG, IgA, IgM) were significantly affected by moderate (400-1000 mg/dl) and severe lipemia (>2000mg/dl), and hence should not be reported. Vitamin D also showed a significant decrease in moderately lipemic samples (decrease of 10-15%). However, since most samples are drawn in a postprandial state, we tested the effect of Lipoclear. Addition of Lipoclear to moderately lipemic samples significantly attenuated the effect of lipemia interference on the above mentioned analytes.

Conclusion: Accurate reporting of pediatric samples for the analytes affected by common interferences will lead to better clinical interpretation. Our results can be applied to other laboratories for the analysis and reporting of these parameters.

A-130

Serum Neopterin Levels in Patients with Pulmonary Embolism

S. Abusoglu[1], F. Akyurek[1], A. Unlu[1], Z. Buyukterzi[2], E. Kurtipek[2]. *[1]Selcuk University Faculty of Medicine, Konya, Turkey, [2]Meram Training and Education Hospital, Konya, Turkey*

Background: Neopterin (D-erythro-1-2-3-trihydroxypropylpterin) is produced from guanosine triphosphate by activated human monocytes, monocyte derived dendritic cells, and macrophages. Release and production of neopterin is stimulated mainly by interferon-c (IFN-c) released by activated Th1- lymphocytes during the cellular immune response. Pulmonary thromboembolism (PTE), is an extremely common medical problem. Yet despite its frequency, much remains to be learned regarding the pathogenic mechanisms that initiate pulmonary thromboembolism. Marked activation of endothelium, platelets, and leukocytes are key events in thrombogenesis in pulmonary thromboembolism. The role of clinical laboratories is to give accurate, fast and reliable results to clinicians. Also, early detection of diseases as pulmonary thromboembolism is important to take care before the serious complications. The aim of this study was to determine serum neopterin concentrations as an early biomarker on patients with pulmonary thromboembolism.

Methods: Blood samples were collected from 41 healthy control and 38 patients with pulmonary embolism. Patients with chronic disease and inflammatory disorders were excluded. This study was approved by local ethic committee. Serum neopterin levels were analyzed with flourometric detection by high perfomance liquid chromatography. Briefly, 100 µL trichloroacetic acid was added to 500 µL serum sample, vortexed and centrifuged at 10000 g for 10 minutes. Supernatant was injected to C18 chromatographic column on Agilent HPLC system. Chromatographic detection was perfomed at 353 and 438 nm excitation and emission wavelength. This method's coefficient of variation and % bias values were 4.21,3.50; 6.89,5.62 and 8.14,6.64 for 0.5, 5 and 10 µmol/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum neopterin levels were significantly lower in control group (5.72 ±2.29 µmol/L) compared to patient group (7.83 ±4.45 µmol/L) (p=0.049) according to Mann-Whitney U test.

Conclusions: Activated T cells and macrophages synthesise and release a number of cytokines whose main function is immunoregulation. As a marker of cellular immune response activation depending on IFN-γ release, neopterin may better reflect the disease. Our findings suggest that neopterin levels may be used as an immunological marker in follow-up the disease.

A-131

How non-standardized serum creatinine-based definitions of acute kidney injury contribute to disparate reported incidence rates of contrast-induced nephropathy

J. S. McDonald, R. J. McDonald, E. E. Williamson, D. F. Kallmes. *Mayo Clinic, Rochester, MN*

Background- Prior controlled studies of intravenous contrast-induced nephropathy (CIN), acute kidney injury (AKI) resulting from iodinated contrast material administration, have reported incidence rates ranging from 2% to 40%. This discrepancy may be partially explained by the lack of a standardized serum creatinine (SCr)-based definition of AKI. To date, there is little evidence regarding how different definitions of SCr-defined AKI and baseline renal function affect the incidence of AKI, and which, if any, of these definitions provide a reliable means to distinguish between CIN and contrast-independent AKI. In the current study, we examined how using different SCr definitions of AKI and baseline renal function affect AKI incidence rates in a propensity score-matched cohort of contrast-enhanced and unenhanced CT scan recipients.

Methods- All contrast-enhanced and unenhanced abdominal, pelvic, and thoracic CT scans performed at our institution from 2000-2010 and accompanying pre- and post-scan SCr results were identified. Contrast and noncontrast scan recipients of similar clinical and demographic characteristics were compared following propensity score-based 1:1 matching. AKI was defined using absolute (SCr ≥ 0.5mg/dl and ≥ 0.3mg/dl over baseline), relative (SCr ≥ 25% and ≥ 50% over baseline), and hybrid definitions (SCr ≥ 0.5mg/dl or ≥ 25%, and ≥ 0.3mg/dl or ≥ 50% over baseline). Baseline renal function was defined by using either the mean SCr result 24 hours or 7 days prior to scan. The incidence of AKI was compared between groups using Fisher's exact test.

Results- Following 1:1 matching, 21,372 patients were identified who underwent a contrast enhanced (N=10,636) or unenhanced CT scan (N=10,636). Using the six definitions of AKI, the incidence of contrast-dependent AKI ranged from 2% to 15% while the incidence of contrast-independent AKI ranged from 1% to 14%. Regardless of AKI definition, the rates of contrast-independent AKI were statistically similar to the rates of contrast-dependent AKI (ORs ranged from 0.91 (95% CI 0.66-1.24), p=.58 to 1.84 (0.91-3.75), p=.08). Higher incidences of AKI were observed if baseline renal function was defined using the mean 7-day pre-scan SCr result compared to using the mean 24-hour pre-scan SCr result.

Conclusion- Differing definitions of AKI and baseline renal function contribute to disparities in the reported incidence of AKI. However, no definition could extricate CIN from contrast-independent AKI causes in our cohort.

A-133

Intravenous Contrast Material Exposure is not an Independent Risk Factor for Dialysis or Mortality

J. S. McDonald, R. J. McDonald, E. E. Williamson, D. F. Kallmes. *Mayo Clinic, Rochester, MN*

Background – Contrast-induced nephropathy (CIN), defined as acute kidney injury (AKI) occurring immediately after exposure to iodinated contrast material, is generally reported to be a self-limited phenomenon. However, concern remains that CIN can cause irreversible nephrotoxicity resulting in dialysis and death, particularly in patients with specific pre-existing comorbidities including renal failure, diabetes mellitus, and congestive heart failure. In the current study, we sought to determine the true incidence of short-term dialysis and mortality following intravenous contrast administration among individuals with closely matched demographic and clinical characteristics using propensity score analysis.

Methods - All contrast-enhanced and unenhanced abdominal, pelvic, and thoracic CT scans performed at our institution from 2000-2010 and related pre- and post-scan serum creatinine (SCr) results were identified. Contrast and noncontrast scan recipients were compared following propensity score-based 1:1 matching to reduce intergroup selection bias. Patients with pre-existing diabetes, congestive heart failure, or chronic or acute renal failure were identified as high-risk patient subgroups for nephrotoxicity. The effects of contrast exposure on the incidence of AKI (SCr ≥ 0.5mg/dl above baseline within 24-72 hours of exposure), and dialysis or death within 30 days of exposure were determined using odds ratios and covariate-adjusted Cox-proportional hazard models.

Results - 1:1 propensity score matching yielded a cohort of 21,346 patients (10,673 contrast-enhanced exams/10,673 noncontrast exams). Within this cohort, the risk of AKI (O.R.=0.94 (95% CI: 0.83-1.07), p=.38), emergent dialysis (O.R.=0.93 (0.52-1.65), p=.89), and 30-day mortality (H.R.=0.97 (0.87-1.06), p=.45) was not significantly different between contrast and noncontrast groups. Although patients who developed AKI had overall higher rates of dialysis and mortality, contrast exposure was not an independent risk factor for either outcome (dialysis: O.R.=0.89 (0.40-2.01), p=.78; mortality: H.R.=1.03 (0.82-1.32), p=.63). Similar findings were observed among the subgroups of patients with renal failure, diabetes, and congestive heart failure.

Conclusion - Intravenous contrast material administration was not associated with excess risk of AKI, dialysis, or death, even among patients with comorbidities reported to predispose them to nephrotoxicity.

A-134

Neutrophil gelatinase-associated lipocalin (NGAL) and Pentraxin -3 (PTX-3) Levels in Chronic Renal Failure and their relationship with Inflammation

O. Sarı[1], N. -. Eren[1], S. Cigerli[1], B. Aslan[2], T. Basturk[1], M. Sevinc[1], A. Unsal[1]. *[1]şişli etfal trainig and research hospital, İstanbul, Turkey, [2]Quality Management Program-Laboratory Services, Toronto, ON, Canada*

Background: Early diagnosis of Chronic Renal Failure (CRF) provides to initiate the effective treatments that can decelerate the progress of the disease and improve prognosis. However, lack of early predictive biomarkers presents difficulties in early diagnosis. Therefore, finding biomarkers that considerably increases in the early stages of CRF and demonstrates correlation with the disease progress is necessary.

NGAL is a known biomarker of acute kidney injury that is secreted in response to renal tubular epithelial cells damage. Increased NGAL levels are determined in the blood and urine following acute renal injury. Pentraxin-3 (PTX-3) is an inflammatory mediator. In contrast to other members of the pentraxin family such as CRP and

Serum Amiloid Protein, PTX-3 is produced in the inflammation site and shows close correlation with the level of tissue damage. Inflammation plays an important role in the progression of the CRF and contributes to increased mortality and morbidity observed in CRF.

In this study, we investigated serum NGAL and PTX-3 levels and their relationship with the severity of renal damage in patients with early stage CRF.

Method: 20 (2 male and 18 female) non diabetic Stage 1 and 34 (17 male and 17 female) Stage 2 CRF patients were included in this study. Average age in the Stage 1 CRF group was 42.55±7.00, and in the Stage 2 CRF group 47.44±7.47. In both groups, serum NGAL and PTX-3 were measured by using the ELISA method from Bioscience Human and Aviscera Bioscience, respectively.

Results: NGAL levels in patients with Stage 2 CRF were higher than that of the patients with Stage 1 CRF ($p<0.01$). ROC analysis and diagnostic tests used for the calculation of a diagnostic cut off value for Stage 2 CRF. For the diagnostic cut - off value of 1037 pg/ml, sensitivity was 73.55%, specificity 70.00%, positive predictive value 80.65%, and negative predictive value 60.87%. Area under the curve was estimated as 73.3% and its standard erreor was 6.9%.In the patients with NGAL levels higher than 1037pg/ml, the probability of having Stage 2 CRF was found to be 6481 times higher than that of the patients with NGAL levels less than 1037 pg/ml.In Stage 1 and 2 CRF groups , 19.4% and 80.6% of the patients had NGAL levels higher than 1037, respectively. In Stage 2 cases, there was a positive correlation between serum creatinin and NGAL concentrations (r=0,362; p0.05). In Stage 1 and 2 CRF patient groups, CRP and PTX-3 concentrations did not show correlation with eGFR values. There was no statistically significant correlation between NGAL and PTX-3

Conclusion: The serum NGAL concentrations were found to be higher in Stage 2 CRF than Stage1 CRF group. Our results indicate that NGAL could be used as a strong and independent diagnostic biomarker for the early stage CRF and a risk for disease progression. We did not observe statistically significant correlation between serum NGAL and PTX-3 levels.

A-135

Serum Lipoprotein-Associated Phospholipae A2 and Methylarginine Levels in Patients with Pulmonary Embolism

A. Unlu[1], F. Akyurek[1], S. Abusoglu[1], Z. Buyukterzi[2], E. Kurtipek[2]. *[1]Selcuk University, Konya, Turkey, [2]Meram Training and Education Hospital, Konya, Turkey*

Background: Pulmonary thromboembolism (PTE) is a preventable disease with higher mortality and morbidity characterized by diagnostic difficulties and recurrence risk. Obstruction of pulmonary arteries is developed from the detached fragments of thrombus in deep veins of the lower extremities. Plasma lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet activating factor acetylhydrolase, is produced by inflammatory cells, co-travels with low-density lipoprotein (LDL), and hydrolyzes oxidized phospholipids, thereby propagating inflammation and potentially thrombosis. ADMA is derived from the catabolism of proteins containing methylated arginine residues. Higher ADMA concentrations have been measured in many cardiovascular and metabolic diseases. There has been limited markers for laboratory evaluation of pulmonary thromboembolism. The aim of this study was to find out the serum methylated arginine and lipoprotein-associated phospholipase A2 levels in patients with pulmonary embolism.

Methods: Blood samples were collected from 41 healthy control and 45 patients with pulmonary embolism. Patients with chronic diseases were excluded. This study was approved by local ethic committee. Serum lipoprotein-associated phospholipase A2 test was analyzed with a colorimetric kit on automated system (Abbott C16000). Reported intra- and inter-assay CV values for 171 and 456 ng/mL were 1.3;0.6 and 3.6;4.9, respectively. Method was linear up to 486 ng/mL. Serum methylated arginine levels were analyzed with liquid chromatography tandem mass spectrometry on ABSCIEX API 3200 system. Briefly, 1 mL deuterated d7-ADMA containing methanol was added to 200 µL serum sample for protein precipitation, vortexed and centrifuged on 13000 g for 10 minutes. 40 µL supernatant was injected to Shimadzu LC-20AD HPLC system. Chromatographic seperation was performed on C18 column for 5 minutes. This method's coefficient of variation and % bias values were 15.6,10.2; 9.72,7.88 and 6.45,6.02 for 0.4, 0.8 and 1.6 µmol/L, respectively. Statistical analysis was performed with SPSS v16.

Results: Serum phospholipase A2 levels were significantly lower in patient group (158.3 ±49.8 ng/mL) compared to control group (206.5 ±38.4 ng/mL) ($p<0.001$) according to independent sample t test. Serum asyymetricdimethylarginine levels were significantly lower in patient group (0.44 ±0.17 µmol/L) compared to control group (0.60 ±0.18 µmol/L) ($p<0.001$). Serum syymetricdimethylarginine levels were significantly lower in patient group (0.54 ±0.20 µmol/L) compared to control group (0.70 ±0.17 µmol/L) ($p<0.001$). Serum arginine levels were significantly lower in patient group (163 ±81 µmol/L) compared to control group (285 ±127 µmol/L) ($p<0.001$). Serum citrulline levels were significantly lower in patient group (9,14 ±6,20 µmol/L) compared to control group (21,81 ±7,26 µmol/L) ($p<0.001$). Serum arginine/ADMA ratio was significantly lower in patient group (379 ±145 µmol/L) compared to control group (477 ±205 µmol/L) ($p=0.013$).

Conclusion: ADMA has been evaluated in several different classes of pulmonary hypertension. In previous studies, no significant difference was reported between pulmonary embolism and healthy controls. Also, in this study serum methylated arginines and phospolipase A2 levels were found to be statsitically lower compared to control group. This might be due to venous characteristic of this disease. Serum asymmetric dimethylarginine levels may be affected by several factors. Arginine/ADMA levels may provide accurate data and be a reliable marker compared to single ADMA measurements.

A-136

Prevalence of metabolic syndrome among hypertensives in Ghana

C. NKRUMAH[1], W. OWIREDU[2]. *[1]METHODIST HOSPITAL, WENCHI, Ghana, [2]KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, Ghana*

Background: Metabolic syndrome can be found in approximately one-third of patients who do not have diabetes but have hypertension. There are numerous correlations between the metabolic syndrome and hypertension, although this is not always the case. As metabolic syndrome and hypertension are independent risk factors for the same disease process, namely cardiovascular disease, it is possible that patients suffering from both these disease entities may have a compounded risk. Our study will therefore attempt to determine the prevalence of metabolic syndrome and investigate the proposed association between these two disease entities.

Methods: This cross-sectional study was conducted at the Hypertension Clinic of the Department of Medicine, Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana between April 2009 and November 2010. Informed consent was obtained from 300 participants consisting of 200 hypertensives (diagnosed by a Consultant Physician based on WHO – International Society of Hypertension Guideline of blood pressure ≥140/90 mmHg or use of antihypertensive) and 100 apparently healthy normotensives served as control. Blood samples were collected in the morning after an overnight fast of at least 12 hours. Serum and plasma were stored at -80ºC after centrifugation at 2000g for 5 minutes until assayed. Fasting blood glucose (FBG), Apolipoprotein A-1 (Apo A-1), Apolipoprotein B (Apo B), Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) were measured on an Auto-Analyzer (Flexor junior, Vital Scientific N.V., The Netherland).

Results: The prevalence of MetS among the hypertensive patients were significantly higher than the normotensive control (56.5% vrs 9.0%, 54.5% vrs 5.0% and 65.5%vrs15.0%, $p<0.001$) using NCEP ATP III, WHO and IDF criteria respectively. Irrespective of the criteria applied, all the components of MetS were significantly higher among the hypertensive patients as compared to the normotensive control. Among the hypertensive patients, the highest prevalence of cardiovascular risk factor was abdominal obesity as measured by WHR (77.0%), followed by reduced HDL-cholesterol (74.0%). From the univariate analysis, females were at about 3 times at risk of developing hypertension as compared to the male counterpart (OR = 2.7; 95% CI = 1.6-4.4; p = 0.0000). Reduced apolipoprotein A1 served as a risk factor (aOR = 13.4; 95% CI = 1.5-121.4; p = 0.0210) whilst high apolipoprotein A1 protects the individual from developing hypertension (aOR = 0.1; 95% CI = 0.0-0.2; p = 0.0000). High apolipoprotein B poses about 9 times risk of developing hypertension as compared to the normal level (aOR = 9.3; 95% CI = 4.2-20.9; p = 0.0000). Both Impaired fasting glucose and diabetes each pose more than 10 times risk of developing hypertension as compared to normoglycaemia.

Conclusion: The study demonstrated that, hypertension is more than just elevated blood pressure in our setting; it is intimately associated with the metabolic syndrome. There is therefore the need for metabolic screening of all hypertensives and increase awareness on the critical importance of public health strategies aimed at reducing risk factors in the entire population. Early detection and treatment of the global risk profile should thus become a priority.

A-137

CEDIA® Mycophenolic Acid Applications on the Beckman Coulter AU480, AU680 and AU5800 Analyzers

D. L. Cheng, C. Wong. *Thermo Fisher Scientific, Fremont, CA*

Background: Mycophenolic Acid (MPA) is metabolized from Mycophenolate Mofetil (MMF) or Mycophenolate Sodium. It is an immunosuppressant used in the prevention of tissue rejection for patients who have undergone renal and heart transplants. MPA is a specific inhibitor of inosine-monophosphate dehydrogenase (IMPDH) - an enzyme used by B and T lymphocytes for de novo purine synthesis. This repression of B and T cell proliferation results in the desired immunosuppressive effects.

Methods: The CEDIA MPA Assay is based on the enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. MPA in the human plasma patient sample competes with MPA conjugated to one inactive fragment for antibody binding site. Once MPA in the sample binds to antibody, inactive enzyme fragments reassociate to form active enzymes. The amount of active enzymes results in an absorbance change that is directly proportional to the amount of MPA in the patient sample. This change is measured spectrophotometrically for a quantitative value of concentration. The Beckman Coulter AU480/AU680/AU5800 analyzers are new applications for the CEDIA MPA Assay. Analyzer performance was determined for precision, linearity, limit of detection, and accuracy on the Beckman Coulter AU480/AU680/AU5800 clinical chemistry analyzers, over the range of the assay (0.3-10 µg/mL). Results were measured against the reference analyzer Hitachi 917.

Results: All studies were evaluated using CLSI guidelines. Three levels of MPA controls were used in the studies. The precision ranged from 6.1-2.2%CV for within-run and 7.7-2.5%CV for total run. Linearity was measured and confirmed over a range of 1.8-11.9 µg/mL. The limit of detection on the AU480/AU680/AU5800 yielded 0.07 µg/mL. Accuracy was measured using patient correlation against the reference analyzer Hitachi 917, which yielded a Deming's Regression for each analyzer: AU480 = 0.992*(Hitachi 917) - 0.096 (N = 107, r = 0.998), AU680 = 0.995*(Hitachi 917) - 0.043 (N = 107, r = 0.998), AU5800 = 0.993*(Hitachi 917) - 0.089 (N = 107, r = 0.998).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the CEDIA MPA Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers, and will provide an effective monitoring system for patients receiving MMF or Mycophenolate Sodium therapy.

A-138

Fructosamine and Glycated albumin with Risk of Coronary Heart Disease and Death

E. Selvin[1], A. M. Rawlings[1], P. Lutsey[2], J. Pankow[2], L. Kao[1], M. Steffes[2], J. Coresh[1]. *[1]Johns Hopkins, Baltimore, MD, [2]University of Minnesota, Minneapolis, MN*

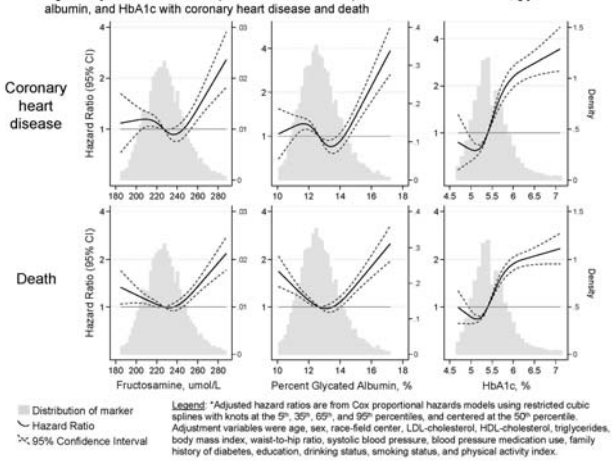
Background: HbA1c is the standard measure to monitor glucose control and is now used for diagnosis of diabetes. Fructosamine and glycated albumin are markers of short-term glycemic control that may add complementary information to HbA1c. However, the associations of fructosamine and glycated albumin with cardiovascular outcomes are uncharacterized.

Methods: measured glycated albumin and fructosamine in 11104 adult participants (792 with a history of diabetes) of the community-based ARIC Study without cardiovascular disease at baseline (1990-1992). We evaluated the associations of fructosamine and glycated albumin with incident coronary heart disease and total mortality. We compared these associations to those for HbA1c.

Results: Baseline HbA1c was highly correlated with fructosamine (Pearson's r =0.82) and glycated albumin (Pearson's r=0.86). During over two decades of follow-up there were 1,032 new cases of coronary heart disease and 2,594 deaths. In Cox proportional hazards models adjusted for traditional cardiovascular risk factors, elevated baseline levels of fructosamine and glycated albumin were significantly associated with coronary heart disease and total mortality (**Figure**). After additional adjustment for HbA1c, the associations were attenuated but remained significant, particularly at diabetic levels of fructosamine and glycated albumin. The associations with death were J-shaped, with an elevation of risk also apparent at the lowest levels of each biomarker, as has been previously observed for HbA1c.

Conclusion: The acceptance of new measures of hyperglycemia is partly dependent on establishing their association with long-term outcomes. We found that fructosamine and glycated albumin were associated with coronary heart disease and mortality and that these associations were similar to those observed for HbA1c. The elevated risk of death at very low levels of fructosamine, glycated albumin, and HbA1c deserves further examination.

Figure. Adjusted* hazard ratios (95% confidence interval) association of fructosamine, glycated albumin, and HbA1c with coronary heart disease and death



A-140

Study of the Association between Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Heart Failure Severity

d. I. hashad, S. Marzouk, M. sadaka, R. Zaghlool. *FACULTY OF MEDICINE, Alexandria, Egypt*

Objectives: The aim of the study was to investigate the association between endothelial nitric oxide synthase (eNOS) G894T gene polymorphism and severity of congestive heart failure (CHF) in a group of Egyptian patients.

Methods: This study was conducted on 30 consecutively selected Egyptian patients admitted to the Cardiology department at Alexandria Main University Hospital suffering from non valvular CHF which was documented clinically and by ECG. Thirty age & sex matched healthy individuals were also included in the study as a control group. eNOS G894T gene polymorphism was studied using polymerase chain reaction-Restriction fragment length polymorphism-(PCR-RFLP) **Results:**. Genotype distribution of eNOS G894T gene polymorphism among patients revealed 13 (43.3%) patients of GG genotype (wild type), 11 (36.7%) of GT genotype (heterozygote) and 6 (20%) were of the TT genotype (homozygote for the mutant allele). Among the controls, 16 (53.3%) were GG genotype, 12 (40%) were GT genotype and 2 (6.7%) were TT genotype. Thus, no statistically significant difference in genotype distribution between patients and controls was observed. In addition, the present study showed no statistically significant difference between different eNOS patients' genotypes as regards NYHA classes, ejection fraction and six-minute walk test. The prevalence of TT genotype was significantly higher in ischemic cardiomyopathy patients when compared to those who had dilated cardiomyopathy and also in hypertensive patients as compared to normotensive patients.

CONCLUSIONS: Although the mutant eNOS G894T allele is associated with both hypertension and dilated cardiomyopathy in Egyptian heart failure patients, it was not correlated to disease occurrence or severity. 1

A-141

Seasonal Variation of Vitamin D Deficiency in a Large Rural Health System: Effect of Assay Change on Deficiency Prevalence

J. B. Jones, H. H. Harrison, D. D. Sargent, J. Labarbera, M. Sneidman. *Geisinger Health System, Danville, PA*

25 hydroxy Vitamin D (25OH Vit D) continues to be a commonly ordered test to assess bone health in the large, rural Geisinger Health System outpatient population in central Pennsylvania USA (latitude 40-41 degrees N). It is well known that seasonal variation of 25OH Vit D levels exists in temperate latitudes as sunlight exposure varies. Our objective is to report practical impact of monthly surveillance of 25OH Vit D seasonal deficiency/sufficiency prevalence during 2009 to 2013 (N=228,714) using clinical cutoffs of <20, 20-30, and 30-100 ng/mL to judge Deficiency, Insufficiency, and Sufficiency, respectively.

Monthly test volumes increased dramatically in 2009 when the test was first performed in house (2300/month in July 2009 to 4000/month in Jan 2010). Since then the volume has steadily increased to 5500/month. Our osteoporosis management program reports that patients are uniformly monitored with 25OH Vit D and are at

steady state as far as population enrollment stands. Steady increases of 25OH Vit D test volumes (approximately 15%/year) may be attributed to generally increasing numbers of outpatients rather than significant changes in utilization.

Over the course of July 2009 to December 2013, cyclical seasonal variation was documented by monthly prevalence plots, with deficiency rates lowest in July-August-September (16.5%) and highest in Jan-Feb-March (27.7%). Inversely, sufficiency rates were highest in Jul-Aug- Sept (48.5%) and lowest in Jan-Feb-Mar (40.5%). Because deficiency rates repeatedly increase from summer to winter by an average of 68%, it is important to advise clinicians to interpret 25OH Vit D levels and prescribe supplementation taking the month of the year into account. Although common practice is to supplement 25OH Vit D in patients with deficient or insufficient levels (i.e. < 30 ng/mL), a systematic study of prescribing patterns has not yet been undertaken.

In November 2012, the 25OH Vit D immunoassay was changed from Diasorin to Roche. Correlation between the two assays using patient specimens (N=152) was acceptable; Deming slope= 0.963; correlation coefficient=0.8455. Ongoing monthly deficiency prevalence, although similar in amplitude and periodicity, changed somewhat with Roche Jan- Feb-Mar 2013 deficiency prevalence trending 11% higher and Roche Jul-Aug-Sept 2013 deficiency prevalence trending 24% lower than prior comparable monthly Diasorin results. It is too early to tell if this difference is physiological , analytical, or coincidental. Although the population surveillance data are unsorted by patient clinical status, the assay change per se may have an effect on shifting population outcome data, and should be more extensively studied along with more traditional specimen correlation.

We conclude that seasonal variation of 25OH Vit D significantly affects deficiency prevalence and has the potential to change treatment on a population basis. The season of testing should be factored into treatment and other clinical considerations based on limited duration cross-sectional studies. Additional studies are needed to determine if different assays perform differently in populations exposed to different amounts of sunlight and if these differences may be due to levels of Vit D precursor or binding protein.

A-142

Evaluation of circulating levels of inflammatory and bone formation markers in Axial Spondyloarthritis

T. S. Frode, K. R. de Andrade, G. R. W. de Castro, G. Vicente, J. S. da Rosa, M. Nader, I. A. Pereira. *University Federal of Santa Catarina, Brasil, Brazil*

Background: Studies have demonstrated the important role of bone remodelling and osteoimmunology in the progression of inflammatory lesions in Axial Spondyloarthritis (SpA) disease.

Objective: This study was conducted to evaluate the inflammatory response by analysis of the serum levels of pro-inflammatory and new bone formation markers in patients with SpA who were treated or not treated with anti-tumor necrosis factor-α (anti-TNF-α) or non-steroidal drugs (NSAIDs) and to correlate these markers with the clinical evaluation scores of the disease activity.

Methodology: The serum levels of myeloperoxidase (MPO), adenosine deaminase (ADA), nitric oxide metabolites (NOx), bone alkaline phosphatase (BAP), Dickkopf-1 (DKK-1), and osteoprotegerin (OP) were measured in 52 SpA patients who were treated or not with anti-TNF-α or NSAIDs and in 26 healthy controls using colourimetric and enzyme immunoassays. The activity and the severity of illness in patients with SpA were assessed using questionnaires (BASMI, Bath Ankylosing Spondylitis Metrology Index; BASFI, Bath Ankylosing Spondylitis Functional Index; and BASDAI, and Bath Ankylosing Spondylitis Disease Activity Index). The data were expressed as the mean ± standard deviation of the mean (SD) for symmetric distribution or in percentage. Comparison of clinical parameters and bone biomarkers between all groups were done with analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparisons among the groups. $P < 0.05$ was considered to be statistically significant. Results: A significant difference between the controls and the patients without medication was observed in relation to NOx, BAP, and OP ($p < 0.01$). When the patients were compared with regard to their treatment, there were no clinically significant differences between the groups ($p > 0.05$).

Conclusion: The NOx, BAP, and OP are emerging as an important inflammatory pathway in Axial Spondyloarthritis. Also the anti-TNF-α or non-steroidal drugs reduce the inflammation and destruction, however these treatment does not modify the serum levels of these biomarkers.

A-143

Suspected Lassa Fever (LF) Case Outcomes: A Comparison to a Non-Febrile Population in Sierra Leone

B. L. Brown[1], M. L. Boisen[1], L. M. Moses[2], J. S. Schieffelin[2], A. Goba[3], M. Momoh[3], M. Fullah[3], F. K. Kamara[3], L. Kanneh[3], S. H. Khan[3], D. Oottamasathien[1], D. S. Grant[4], D. Beckham[5], K. R. Pitts[1], J. Barnett[5], R. F. Garry[2]. *[1]Corgenix, Inc., Broomfield, CO, [2]Tulane University, New Orleans, LA, [3]Kenema Government Hospital, Kenema, Sierra Leone, [4]Ministry of Health and Sanitation, Kenema, Sierra Leone, [5]University of Colorado Anschutz Medical Campus, Denver, CO*

Background: Lassa virus (LASV) is the causative agent for Lassa fever (LF), causing an estimated 100,000 - 300,000 cases annually. The precise factors resulting in fatal outcome in LF patients are still largely uncharacterized, although hypovolemic shock is thought to ultimately result in death of afflicted subjects. Additionally, signs of acute renal failure are consistently noted preceding fatal outcomes. Evaluating the difference in clinical and laboratory outcomes between LF cases and non-febrile controls is important to better characterize the clinical presentation of patients with LASV infection and potentially select patients with a higher pre-test probability of infection for diagnostic testing.

Methods: This is a case controlled study of patients suspected of LASV infection, identified in Sierra Leone, West Africa. Cases include both suspected and confirmed LF patients who had a temperature of ≥38°C for <3 weeks and displayed clinical signs and symptoms of a LASV infection upon examination. Controls include non-febrile Sierra Leoneans with a temperature of ≤37.5°C. We measured five outcomes and compared results between the confirmed LF case group (n = 57) and the non-febrile control group (n = 118). We measured BMI, Pulse, BUN, Cr, and BUN:Cr ratio and correlation of clinical parameters with diagnosis and other clinical parameters. Other outcomes such as the metabolic panel and hemoglobin were collected and evaluated as well.

Results: Using the Wilcoxon-Mann-Whitney nonparametric test, we found that confirmed LF cases exhibit elevated BUN (p<.0001) and Cr (p=.0002) measurements (BUN: 10.5 mM/L, Cr: 216. 3 μM/L) compared to non-febrile controls (BUN: 3.4mM/L, Cr: 84.6μM/L). Confirmed LF cases also had a significantly higher respiratory rate (p<.0001) than the non-febrile controls (39.6 breaths per minute versus 21.6).

Conclusion: Early evaluation of BUN, Cr, and respiratory rate in febrile patients may aid in selecting patient populations at high risk for LF and improve diagnostic accuracy.

BMI, Pulse, BUN, Cr, and BUN:Cr compared between confirmed LF cases and non-febrile controls.

	Confirmed LF Cases	Non-febrile Controls	p-value
BMI	18.6	21.9	0.0006
Pulse	93.8	81.4	0.0004
BUN mM/L	10.5	3.4	<0.0001
Cr μM/L	216.3	84.6	0.0002
BUN:Cr mg/dL	19.2	12.7	0.0008

A-144

A comparative study of the prevalence of Salmonella typhi infection in the wenchi municipality using the Widal and culture methods

C. NKRUMAH[1], M. AMOAH[2]. *[1]METHODIST HOSPITAL, WENCHI, Ghana, [2]COLLEGE OF HEALTH, KINTAMPO, Ghana*

Background: *Salmonella typhi* is a Gram negative bacterium that can cause typhoid fever. Worldwide, typhoid fever is a serious public health problem, with an estimated 22 million cases, resulting in 200,000 deaths. The burden of the disease lies mainly in developing countries including Ghana where the provision of sanitary conditions may be inadequate. However, not much research work has been done to explore the problem confronting citizens and ways to eliminate them. Yearly report from Methodist Hospital's Laboratory, Wenchi (2012), indicates that 45.8% and 47.5% of In-patients and Out-patients respectively reacted to the Widal test. The total percentage of reactive cases in 2012 was 47.1%.This study was then carried out to determine the prevalence of typhoid fever and propose recommendations on the appropriate way of diagnosing the disease in Wenchi municipality.

Methods: Non-interventional exploratory study using a purposive sampling for the selection of respondents in March and April 2013. The study comprised 100 participants who were suspected of having typhoid fever and referred to the laboratory for diagnosis. Informed consent was obtained from each participant and then blood and stool specimens were collected. The blood was centrifuged and *Salmonella*

typhi H and O antibody titre determined following serial dilution with the Widal kits (*Salmonella typhi* Antigens H and O). After emulsification with physiological saline, the stool samples were then cultured directly onto Desoxycholate Citrate Agar (DCA). The plates were then incubated at 37 °C in for 24 hours and observed for bacterial growth. Culture results were then compared with the Widal test for each participant.

Results: About 41% of participants had high antibody titre to *S. typhi* H antigen while 12% also reacted to *S. typhi* O.The majority of the respondents were female (61%) and had the lowest reaction to the Widal *Salmonella typhi* H (42.6%) and O (11.5%) test but with no bacterial growth on the Agar. The males (29%) had the highest reaction to the Widal Salmonella typhi H (51.7%) and O (17.2%) test and also with no bacterial growth on the agar. Irrespective of the antibody titre shown in the Widal test, there was no corresponding bacterial growth by culture.

Conclusion: The high antibody titres recorded by the Widal technique with no corresponding bacterial growth by culture may point to the existence of circulating antibodies established during previous exposure. Therefore relying solely on the Widal test may lead to over diagnosis of the infection and concomitant abuse of antibiotics which may contribute the emergency of resistant strains in our region. Proper diagnosis of Salmonella typhi should thus be based on bacteria culture and sensitivity testing.

A-145

Performance of REBA MTB-XDR to detect Extensively Drug-resistant Tuberculosis in a High burden country

Y. Lee[1], M. Kang[2], H. Jung[3], S. Choi[4], K. Jo[5], T. Shim[5]. *[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Busan Paik Hospital Inje University College of Medicine, Busan, Korea, Republic of, [2]YD R&D Center, YD Diagnostics, Yongin-Si, Gyeonggi-do, Korea, Republic of, [3]Deparment of Internal Medicine, Inje University School of Medicine, Ilsan Paik Hospital, Goyang, Gyeonggi-do, Korea, Republic of, [4]Department of Internal Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Korea, Republic of, [5]Department of Pulmonary and Critical Care Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea, Republic of*

Setting: Multidrug-resistant/extensively drug-resistant tuberculosis (MDR/XDR-TB) is a serious problem worldwide. The early diagnosis and treatment of MDR/XDR-TB is very important. The REBA MTB-XDR (REBA-XDR) line probe assay has been developed recently for the detection of resistance to ofloxacin, kanamycin, and streptomycin in Korea.

Objective: The aim of this study was to evaluate the diagnostic accuracy of the REBA-XDR in detecting XDR-TB in acid fast bacilli (AFB) smear-positive sputum specimens.

Design: We prospectively enrolled 104 patients with AFB smear-positive specimens between July 2010 and January 2013. Mycobacterium tuberculosis was cultured in all samples. The performance characteristics were compared between the REBA-XDR and the conventional drug susceptibility test (DST), GenoType MTBDR*sl* assay, and DNA sequencing analysis results. The conventional DST results were considered to be the gold standard.

Results: Among the 104 specimens, MDR-TB was found in 29.8% (31/104) and XDR-TB in 7.7% (8/104). The sensitivity of the REBA-XDR in detecting resistance to ofloxacin, kanamycin, and streptomycin was 66.7%, 90.9%, and 60.0%, and the specificity was 93.3%, 93.5%, and 85.4%, respectively. The positive predictive values were 62.5%, 62.5%, and 40.9%, and the negative predictive values were 94.3%, 98.9%, and 92.7%, respectively. The accuracy was 89.4%, 93.3%, and 81.7%, respectively. Discordant results between REBA-XDR and conventional DST were found for ofloxacin in 11 samples (10.6%), for kanamycin in 7 samples (6.7%), and for streptomycin in 19 samples (18.3%). Two of 10 (20%) discordant REBA-XDR results for ofloxacin resistance and all 6 (100%) discordant specimens for kanamycin resistance corresponded to the results of the gold standard DNA sequencing test. Five of 10 (50%) discordant results for ofloxacin resistance and 2 of 6 (33.3%) discordant results for kanamycin in the MTBDR*sl* test corresponded to the results of the DNA sequencing test.

Conclusion: REBA-XDR seems to be a useful kit to "rule in" XDR-TB in a high-risk group for drug resistance, especially for the detection of kanamycin resistance.

A-146

Circulating Presepsin (Soluble CD14 Subtype) in Patients with Severe Sepsis and Septic Shock. Data from the Albumin Italian Outcome Sepsis (ALBIOS) Study

S. Masson[1], P. Caironi[2], C. Fanizza[3], R. Bernasconi[1], L. Pirozzi[3], A. Noto[4], V. Parrini[5], G. Pasetti[6], G. Tognoni[3], L. Gattinoni[2], R. Latini[1]. *[1]IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy, [2]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy, [3]Fondazione Mario Negri Sud, Santa Maria Imbaro, Italy, [4]A.O. San Paolo - Polo Universitario, Milan, Italy, [5]Ospedale del Mugello, Borgo San Lorenzo, Italy, [6]Ospedale San Giovanni di Dio, Orbetello Scalo, Italy*

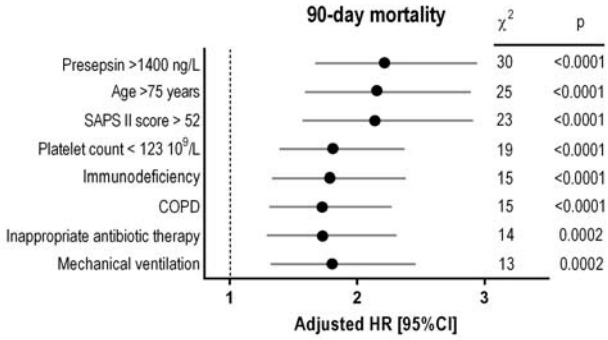
Background: The cornerstone of the emergency treatment of severe sepsis and septic shock is an early, goal- directed therapy. Presepsin, a soluble fragment of CD14 that participates in the innate recognition of pathogens, has been proposed as a novel diagnostic and prognostic marker in sepsis.

Aims: To validate presepsin as a prognostic marker in a large, representative cohort of septic patients.

Methods: Blood samples were collected 1, 2 and 7 days after enrolment in 997 patients with severe sepsis or septic shock enrolled in the multicenter Albumin Italian Outcome Sepsis (ALBIOS) trial (NCT00707122). Presepsin was measured in a central laboratory with a chemiluminescent enzyme immunoassay (PATHFAST Presepsin, Mitsubishi Chemical). The relation between presepsin concentration and mortality in Intensive Care Units (ICU) or at 90 days was assessed with Cox proportional hazards multivariable models. Prognostic discrimination was tested with reclassification metrics.

Results: Concentration of presepsin on day 1 was 946 [492-1887] ng/L (median [Q1-Q3]) and increased gradually with the number of organ dysfunctions and the number of newly developed organ failures in ICU. Circulating presepsin rose in decedents over 7 days in ICU while it markedly decreased in survivors ($p<0.0001$ for time-survival interaction). Presepsin on day 1 independently predicted ICU and 90-day mortality (Figure). Addition of presepsin measured on day 1 on top of all risk factors significantly improved prognostic discrimination and correctly reclassified the risk of 90-day mortality (c-statistics from 0.77 to 0.79, $p=0.004$; integrated discrimination improvement IDI = 0.03 [0.02-0.04], $p<0.0001$; continuous net reclassification index NRI = 0.53 [0.40-0.65], $p<0.0001$).

Conclusion: Presepsin is a robust predictor of mortality in patients with severe sepsis and septic shock. It provides incremental information on top of widespread clinical risk factors and may help in early risk stratification.



A-147

Neutrophil-Gelatinase -Lipocalin in adult cardiac surgery patients: beyond AKI

C. Cosma[1], M. Zaninotto[1], R. Bianco[2], M. Kosović[2], G. Gerosa[2], M. Plebani[1]. *[1]Department of Laboratory Medicine, University-Hospital, Padova, Italy, [2]Division of Cardiac Surgery, Department of Cardiac, Thoracic and Vascular Sciences, University, Padova, Italy*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) has been largely described as an early marker of acute kidney injury. We report the association of urinary NGAL (uNGAL) values with different adverse outcomes in adult cardiac surgery patients, particularly the need for continuous venous hemofiltration (CVVH), cardiac mechanical assist devices and low cardiac output syndrome

Materials and methods: fresh urine sample of 137 patients (34 females and 103 males; mean of age 64 y) undergoing cardiac surgery (coronary artery bypass, artificial heart valve, heart transplants, complex cardiac surgery) have been collected before, immediately after surgery and then 24 h and 48 h after. AKI was defined as an increase in plasma creatinine levels (more than 50% or more than 0.3 mg/dL (26.5 umol/L) in comparison to the preoperative value during the first 48 h after surgery. uNGAL has been evaluated using a chemiluminescent microparticle immunoassay (CMIA, Architect, Abbott Diagnostic) showing during the study a total imprecision (CV) ranging from 3.85 to 2.35% (20 ug/L and 1218 ug/L, respectively). The reference ranges adopted are 2-120 ug/L for uNGAL and 53-97 (females), 62-115 umol/L (males) for plasma creatinine respectively.

Results: Mean uNGAL levels peaked immediately after cardiac surgery (630 ± 890(SD) ug/L), and remained significantly higher 24 and 48 hours after surgery (565 ±842 and 293±455 respectively, p=0.0003). 24 patients (17%) developed AKI (mean of basal creatinine values and 48 hours after cardiac surgery 106±30.9 and 346.5±127.57 respectively, p<0.001)

Accuracy of u NGAL (AUC) as a predictor for AKI immediately after cardiac surgery and 24 and 48 hours later was 0.775 (95% confidence interval [CI], 0.660 to 0.890), 0.864 (95% CI, 0.788 to 0.940) and 0.838 (95% CI, 0.749 to 0.926), respectively. uNGAL levels correlated significantly with the need of CVVH, cardiac mechanical assist devices and low cardiac output syndrome being AUC 0.862 (95% CI, 0.779 to 0.945), 0.913 (95% CI; 0.840 to 986) and 0.910 (95% CI; 0.840 to 0.981), respectively.

Conclusions: Our study confirms the clinical usefulness of NGAL measurement for the early diagnosis of AKI but underlines that relevant clinical informations on adverse outcome of patients undergoing cardiac surgery may also be obtained.

A-148

Diabetes, pre-diabetes and incidence of subclinical myocardial injury

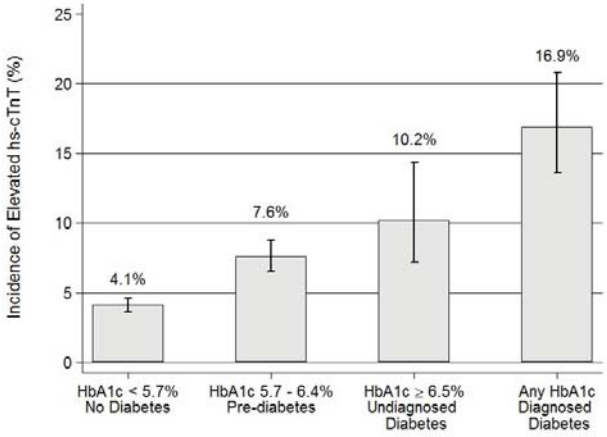
E. Selvin[1], M. Lazo[1], L. Shen[1], J. Rubin[1], J. McEvoy[1], R. Hoogeveen[2], C. Ballantyne[2], J. Coresh[1]. *[1]Johns Hopkins, Baltimore, MD, [2]Baylor College of Medicine, Houston, TX*

Background: Persons with pre-diabetes and diabetes are at high risk for cardiovascular events. However, the relationships of pre-diabetes and diabetes to development of subclinical myocardial damage are unclear. Our objective was to characterize the associations of pre-diabetes, undiagnosed diabetes, and diagnosed diabetes with 6-year incidence of subclinical myocardial injury, as assessed by a novel highly sensitive assay for cardiac troponin T (hs-cTnT).

Methods: We measured hs-cTnT at two time points, 6 years apart, among 8692 participants of the community-based Atherosclerosis Risk in Communities (ARIC) Study without a history of heart disease, silent MI by ECG, or stroke at baseline (1990-92). The primary outcome was incidence of elevated hs-cTnT (≥14 ng/L) at 6 years of follow-up.

Results: Cumulative probabilities of elevated hs-cTnT (≥14 ng/L) at 6 years among persons with no diabetes, pre-diabetes (HbA1c 5.7-6.4%), undiagnosed diabetes (HbA1c ≥6.5%), and diagnosed diabetes were 4.1%, 7.6%, 10.2%, and 16.9%, respectively. Across these same categories and compared to persons with no diabetes and HbA1c <5.7% (reference), the adjusted relative risks for incident elevated hs-cTnT were 1.40 (95%CI 1.13, 1.73), 1.85 (95%CI 1.24, 2.75), and 2.85 (95%CI 2.14, 3.75).

Conclusion: Pre-diabetes and diabetes were strongly associated with the future development of elevations in troponin T far below the threshold for a diagnosis of myocardial infarction. The results from this community-based prospective study provide evidence for a deleterious effect of hyperglycemia on the myocardium, possibly reflecting a microvascular etiology. Our findings underscore the importance preventing progression to early hyperglycemic states and development of diabetes.



A-149

Robust measurement of branched chain amino acids on the Vantera Clinical Analyzer and the clinical association of NMR-measured valine with type 2 diabetes

J. Wolak-Dinsmore, I. Shalaurova, S. P. Matyus, M. A. Connelly, J. D. Otvos. *LipoScience, Inc., Raleigh, NC*

Background: Metabolomic studies have shown that branched chain amino acid (BCAA) levels are independently associated with insulin resistance and type 2 diabetes (T2D). NMR technology has been employed for years to measure lipoprotein particle concentrations in a clinical laboratory setting. However, the information-rich nature of the NMR spectrum lends itself to the measurement of other clinically useful metabolites; therefore, assays were developed to quantify the BCAAs, valine, leucine and isoleucine.

Methods: Proton NMR spectra were collected on fasting serum samples using the Vantera Clinical Analyzer, a 400MHz NMR platform with automated fluidics sample handling, data processing and analysis. The NMR spectra were deconvoluted using proprietary software with models containing reference spectra from serum proteins and lipoproteins. For method comparison purposes, NMR-measured BCAAs were compared with mass spectrometry quantified BCAAs collected from the same serum samples. Valine concentrations were quantified from NMR spectra previously captured for participants in the Multi-Ethnic Study of Atherosclerosis (MESA) and multivariable logistic regression analyses were performed to interrogate the association of valine with the development of T2D.

Results: The levels of valine, leucine and isoleucine quantified in serum samples using NMR and mass spectrometry, were highly correlated (e.g. valine R^2=0.96). For the valine NMR assay, the coefficients of variation (CVs) for the inter-assay and intra-assay precision data were 2.7-5.9%. NMR-measured valine concentrations in MESA subjects (n=3309), who were non-diabetic and whose fasting plasma glucose was <110 mg/dL, were strongly associated with incident diabetes and made a statistically significant contribution to a logistic regression model containing age, gender, race and glucose (see Table).

Conclusions: Levels of the BCAAs valine, leucine and isoleucine can be obtained from the same NMR spectra acquired for lipoprotein particle concentrations. Similar to published BCAA literature, NMR-measured valine is strongly associated with incident diabetes.

	MESA (n=352/3309) (incident diabetes/total # subjects)	
	Wald [2]	p
Age	11.4	0.0007
Gender	20.8	<0.0001
Race	20.1	0.0002
Glucose	218.8	<0.0001
Valine	32.9	<0.0001

A-150

Different genotypes of a functional polymorphism of the TSHR gene are associated with the development and severity of Graves' and Hashimoto's diseases

M. WATANABE, N. Inoue, Y. Katsumata, Y. Hidaka, Y. Iwatani. *Osaka University Graduate School of Medicine, Suita, Japan*

Background: The disease severities of autoimmune thyroid diseases (AITDs), such as Hashimoto's disease (HD) and Graves' disease (GD), can vary among patients. ST4 is one of the splicing variants of thyrotropin receptor (TSHR). Increased ST4 transcription may enhance the generating of a shed A subunit, which results in the production of thyroid-stimulating antibody (TSAb). ST4 expression was higher in the AA genotype of *TSHR* rs179247 polymorphism compared to the GG genotype.

Methods: We genotyped this polymorphism in 98 HD patients including 44 patients developed hypothyroidism before the age of 50 years and were treated with thyroxine (severe HD) and 33 HD patients over the age of 50 years were left untreated and demonstrated euthyroid (mild HD), in 112 GD patients including 50 GD patients who had been treated with methimazole and were still positive for TRAb (intractable GD); and 33 GD patients in remission (GD in remission), and in 56 healthy volunteers.

Results: The AA genotype was more frequent in GD patients compared to control subjects (p=0.0201). In contrast, the frequency of the GG genotype was higher in HD patients compared to control subjects (p=0.0186). The A allele was more frequent in GD patients compared to the HD patients (p=0.0010). The AA genotype and A allele were more frequent in intractable GD patients compared to GD patients in remission (p=0.0024 and 0.0005, respectively). The GG genotype was more frequent in severe HD patients compared to control subjects (p=0.015). The proportion of patients who developed GD under 50 years of age was significantly higher in GD patients with the AA genotype compared to those with the AG/GG genotypes (P=0.0251).

Conclusion: The AA and GG genotypes of the rs179247 polymorphism of the *TSHR* gene were susceptible to GD and HD, respectively and were also associated with the intractability of GD and the severity of HD, respectively.

Summary of results		
Genotype	ST4 expression	Associated with
AA	HIGH	Development of **Graves' disease** Intractability of **Graves' disease**
GG	LOW	Development of **Hashimoto's disease** Severity of **Hashimoto's disease**

A-151

Tubular Damage is Ubiquitous in Newly-Diagnosed Patients with Multiple Myeloma: Comparison of Three Urinary and Two Serum Markers of Kidney Injury

I. Papassotiriou[1], D. Christoulas[2], E. Kastritis[2], M. Gkotzamanidou[2], A. Margeli[1], N. Kanellias[2], G. P. Papassotiriou[2], E. Eleutherakis-Papaiakovou[2], M. A. Dimopoulos[2], E. Terpos[2]. *[1]Department of Clinical Biochemistry, Aghia Sophia Children's Hospital, Athens, Greece, [2]Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece*

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein overproduced by proximal tubular cells in response to kidney injury, while kidney injury molecule-1 (KIM-1) is a type 1 transmembrane glycoprotein that is overexpressed in dedifferentiated proximal tubule epithelial cells after ischemic or toxic injury. Urinary NGAL and KIM-1 have never been evaluated in MM patients.

Patients and Methods: To assess the value of these molecules in MM, we measured urinary and serum NGAL, urinary KIM-1, urinary and serum cystatin-C (Cys-C) in 48 newly diagnosed symptomatic MM patients (27M/21F, median age 65 years). The estimated GFR (eGFR) was calculated using the CKD-EPI equation. (proposed by the CKD Epidemiology Collaboration and is widely accepted in renal impairment). Serum and urinary NGAL was evaluated using immunoturbidimetric assay (BioPorto Diagnostics A/S, Denmark) with a protocol applied in the Siemens Advia 1800 Clinical Chemistry System. Serum and urinary Cys-C was measured on the BN ProSpec analyzer (Siemens Healthcare Diagnostics, Liederbach, Germany), while urinary KIM-1 was also measured using an ELISA (R&D Systems, Minneapolis, MN, USA). For the urinary measurements, a 24h urine collection was used.

Results: The median values (range) for the studied markers in MM patients and in 120 healthy controls were: for urinary NGAL 36ng/ml (0.5-2512ng/ml) vs. 5.3ng/ml (0.7-9.8ng/ml), p<0.001; for serum NGAL 162ng/ml (53-576ng/ml) vs. 63ng/ml (37-106ng/ml), p<0.001; for urinary KIM-1 1.1ng/ml (0.13-4.87ng/ml) vs. 1.3ng/ml (0.1-5.3ng/ml), p=0.345; for urinary Cys-C 0.05mg/l (ND-13.9mg/l) vs. non-detectable, p<0.01; and for serum Cys-C 1.0mg/l (0.4-3.2mg/l) vs. 0.7mg/l (0.3-0.9mg/l), p<0.01. Almost all patients (93%) had higher levels of urinary NGAL than the higher value of the controls; the respective frequency for the other markers was: 68% for serum NGAL and serum Cys-C, 50% for urinary Cys-C and only 10% for urinary KIM-1. All studied markers correlated with eGFR: serum Cys-C (r=-0.758, p<0.001), serum NGAL (r=-0.627, p<0.001), urinary Cys-C (r=-0.498, p=0.008), urinary NGAL (r=-0.430, p=0.01) and urinary KIM-1 (r=-0.369, p=0.021). Only serum Cys-C strongly correlated with the involved serum free light chain (r=0.806, p<0.001). Urinary NGAL correlated also with urinary Cys-C (r=0.880, p<0.001), serum NGAL (r=0.503, p=0.002), 24-h proteinuria (r=0.431, p=0.01) and ISS stage (mean±SD values for ISS-1, ISS-2 and ISS-3 were: 31±29ng/mL, 47±52ng/mL and 408±695ng/mL, respectively; p=0.03). Serum Cys-C correlated also with ISS stage (the values for ISS-1, ISS-2 and ISS-3 were: 0.85±0.19mg/L, 0.94±0.24mg/L and 2.15±0.98mg/L, respectively; p=0.01), while urinary Cys-C correlated with 24-h proteinuria (r=0.564, p<0.001).

Conclusions: Our data suggest that almost all newly diagnosed symptomatic MM patients have tubular damage as assessed by elevated urinary NGAL suggesting that renal impairment is present very early in the disease course. Measurement of urinary NGAL and serum Cys-C offers valuable information for the kidney function of MM patients and their measurement may help in the identification of patients with high risk for the development of acute renal function. The value of KIM-1 seems to be very low in myeloma reflecting the differences in the pathogenesis of myeloma-related renal dysfunction than toxic acute renal injury of other etiology.

Tuesday, July 29, 2014

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

Endocrinology/Hormones

A-152

Assessing macroprolactin interference in prolactin assays after polyethylene glycol precipitation in two automatized platforms

R. R. Rodrigues, R. C. Basso, R. C. A. Sansão, P. Osorio, C. F. Pereira. *Diagnostico da America (DASA), Barueri, Brazil*

Background: Prolactin serum levels over 30 ng/ml in the absence of pregnancy and postpartum breastfeeding are indicators of hyperprolactinaemia which could impact in more complexes and expensive diagnostic protocols. Seric Prolactin are classified in three main forms: monomeric which is the predominant form; dimer, also known as big prolactin; and the high molecular weight form which is usually known as macroprolactin or big-big prolactin (bbPRL). It is known that macroprolactinaemia may correspond to approximately 20 - 25% of cases of hyperprolactinemia and it is a common disorder in a healthy population. Thus, the investigation of macroprolactinaemia as the main cause of hyperprolactinaemia would avoid clinical investigation of prolactinoma and other diseases. The reference test for detecting macroprolactin is gel filtration chromatography, but the test based on polyethylene glycol (PEG) is simpler and cheaper and has been validated in 1999 by Olokoga and Kane. All commercially available prolactin immunoassays have a cross-reactivity level with macroprolactin. The purpose of this study was to validate the PEG precipitation test using Siemens Healthcare Diagnostics ADVIA Centaur System when compared to Abbott Architect.

Methods: 46 patient samples presenting levels over 30 ng/mL previously dosed were re-tested in ADVIA Centaur and Abbott Architect after PEG treatment. Analysis of mean, standard deviation and correlation were calculated. Moreover, a cutoff of 60% was established to determine the presence of bbPRL.

Results: The samples were measured before extraction and the results were: 69.29 ng/mL average in ADVIA Centaur and 94.42 ng/mL in Architect with a standard deviation of 30.12 and 42.10 respectively and R2 of 0.88. 60% was regarded as cutoff for bbPRL screening. When comparing the ADVIA Centaur with Architect, a relative sensitivity of 100% and a Relative Specificity of 83.33% with positive predictive value of 97.56% and negative predictive value of 100% were obtained.

Conclusion: The Prolactin assay varies according to the elected methodology for Macroprolactin detection. Correlation results between compared instruments were satisfactory. Moreover, ADVIA Centaur is capable of dosing the three main forms of seric prolactin. Among 46 samples only one sample showed doubtful result for Architect and positive for ADVIA Centaur. As we can observe macroprolactin is a major interference source and may lead to diagnostic errors and processing errors involving patients with hyperprolactinemia. Samples present greater dispersion measurements prior to PEG precipitation for Architect results and the difference between the values of pure samples can be related to low interference that ADVIA Centaur system presents, being considered as a positive point once risk in releasing high results decreases. This provides a smaller number of high results which can generate inadequate diagnostic or request a further test to make the diagnosis. In conclusion, we can confirm that the evaluation of bbPRL methods, such as PEG precipitation is still necessary, even in trials that have low reactivity for macroprolactin as the ADVIA Centaur.

A-153

Vitamin D status in healthy and rheumatoid arthritis groups.

S. Kang, M. Lee, J. Yang, M. Kim, W. Lee. *KyungHee University Hospital at Gangdong, Seoul, Korea, Republic of*

Introduction: Vitamin D is important for maintenance of calcium homeostasis and bone metabolism. Its association with chronic and inflammatory diseases including rheumatoid arthritis (RA) has been suggested while consensus on the optimal level of vitamin D is yet to be made. The aims of this study are 1) to assess vitamin D status among healthy and RA groups from Korean population; 2) to evaluate biochemical markers of their relationship with vitamin D level; and 3) to determine an alternative cutoff level and assess the relationship with RA.

Materials and methods: The study includes 346 healthy individuals and 222 diagnosed RA patients during winter (January-February) and summer (July-August). C-reactive protein (CRP), adjusted calcium (aCa), erythrocyte sedimentation rate (ESR), parathyroid hormone (PTH), ß-isomerized C-terminal telopeptides (ß-CTx) and serum 25(OH)D levels were measured. Each groups were classified by vitamin D status and related markers into subgroups for comparison. Statistical analyses were made using t-test, ANOVA, chi-squared test and logistic regression analysis. Segmented linear regression analysis was used to determine an alternative cutoff. Statistical significance was determined at $P<0.05$.

Results: 25(OH)D level was lower in female, younger age group, high PTH subgroup and during winter. Vitamin D insufficiency/deficiency was highly prevalent among healthy group (95.7%) and RA group (98.2%) but the distribution of vitamin D status was not different. 25(OH)D andshowed a negative correlation with PTH ($P<0.01$, r=-0.29), however the increase of PTH level was mostly within reference range and increase of PTH beyond reference range level in vitamin D deficient subgroup was rare (2.3%). Despite vitamin D deficiency, ß-CTx and aCacalcium levels were not different among vitamin D status subgroups and no correlation with 25(OH)D or PTH was found. Comparison between healthy and RA group also revealed similar seasonal differences, RA group had lower 25(OH)D level during summer, although the distribution of vitamin D status was not different. The alternative cutoff 22.08 ng/mL, classified healthy and RA group as vitamin D insufficiency/deficiency in 85.54% and 90.54% respectively. The prevalence of vitamin D insufficiency/deficiecy was low but the difference between healthy and RA group was not found. Logistic regression analyses showed that 25(OH)D level was not associated with RA, and ESR and CRP as markers of disease activity.

Discussion: Most healthy individuals are categorized as vitamin D insufficiency/deficiency under the currently used conventional criteria for vitamin D status. The level of vitamin D deficiency from this study did not result in increment of PTH, ß-CTx or aCa greater than reference range. This finding is suggestive that the status of vitamin D deficiency using the current criteria may not be clinically practical. In contrast to previous reports on the relationship of vitamin D and chronic inflammatory diseases, application of lower cutoff in this study also did not exhibit association with RA and correlationor with markers of disease activity. Future investigation of vitamin D should be conducted through a large, randomized controlled trial and focus on deciding the optimal vitamin D level in correlation with clinically meaningful results, regarding calcium homeostasis and bone metabolism.

A-154

Evaluation of Hb A1c bias and precision across eight platforms in the presence of Hb AS and Hb AC

J. M. Rhea[1], M. Richter-Roche[1], A. Woodworth[2], N. Korpi-Steiner[3], J. Miller[4], D. Koch[5], R. Molinaro[1]. *[1]Emory University, Atlanta, GA, [2]Vanderbilt University, Nashville, TN, [3]University of North Carolina, Chapel Hill, NC, [4]University of Louisville, Louisville, KY, [5]Grady Memorial Hospital, Atlanta, GA*

Introduction: Changes in serial Hb A1c results are due to changes in the clinical condition or to inherent sources of biologic and analytic variation. Results of the 2012 CAP GH2-B proficiency survey which contained an Hb AS sample, suggest that samples containing an Hb variant may impact assay precision for some methods, and potentially reference change values (RCVs). Objective: To calculate the effect of Hb variants on RCV for Hb A1c by determining the precision of each method. Methods: Seven different NGSP-certified Hb A1c platforms were used to measure imprecision and bias using patient samples containing Hb AA, Hb AS, or Hb AC. Precision was determined following CLSI EP05-A2, and bias by calculating the percent difference between each test method and the comparative method (NGSP Secondary Reference Lab). RCVs were calculated using the standard formula. Results: Differences in imprecision and bias were observed, and were typically greater in samples containing an Hb variant. RCVs for all methods except two at were ≤0.5% Hb A1c; overall, RCVs were slightly increased in the presence of Hb AS and Hb AC at ≤0.8%. Conclusions: The total analytical error for the majority of assays was significantly greater in samples containing Hb AS or Hb AC, and may indicate a need for including proficiency samples containing the most common Hb variants, especially when assays are used to measure Hb A1c in populations with a high prevalence of Hb variants. In addition, while the clinical relevance of increased was beyond the scope of this study, the change in RCV suggests a difference in how serial Hb A1c results may be interpreted.

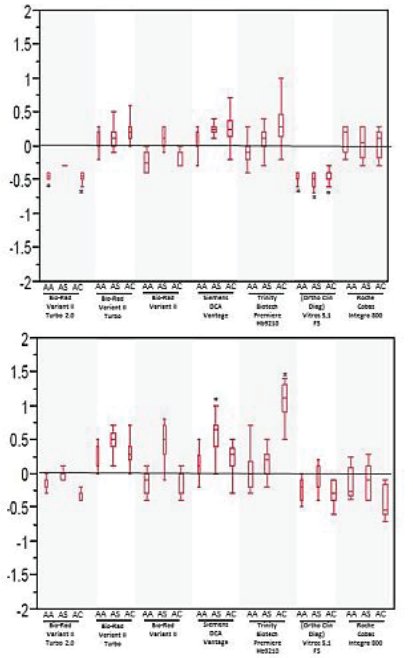


Figure 1. Box plots showing the absolute differences between each assay and the comparative method for each hemoglobin type at a low (A) and high (B) Hb A_{1c} concentration. The upper and lower limits of each box correspond to the 25th and 75th percentiles of the differences, respectively. The upper and lower bars represent the maximum and minimum differences between the comparative method. Clinically significant differences (±6%) are indicated with a (*).

A-155

Is it possible to make insulin tolerance test (ITT) better?

M. P. Campagnoli[1], A. Menna Barreto[1], M. Freire[2], M. Scharf[1]. *[1]DASA, Curitiba, Brazil., CURITIBA, Brazil, [2]DASA, Curitiba, Brazil., Rio de Janeiro, Brazil*

Background The diagnosis of growth hormone (GH) deficiency in children with growth retardation is complex. Because of the pattern of pulsatile secretion of GH, isolated determinations have no value. So, the functional stimulus tests for evaluation of GH secretory reserve are fundamental for the diagnosis. The Insulin Tolerance Test (ITT) is considered to be the gold standard test for diagnosis in children over 2 years of age. This test consists on the administration of intravenous insulin and sampling a multi amostral sequence of glucose and GH, and sometimes, cortisol.

The given insulin should be able to reduce 50% of fasting glucose or reaching below the normal range, promoting the stimulus needed to boost the production and release of GH and cortisol .

Bedside blood glucose control is done, during the lab test, through the measurement of plasma glucose with blood glucose strips. Decisions like, correction of blood glucose as well as administering another dose of insulin, are based on these values .

We observed that in many patients glucose measured at the bedside shows superior values to subsequently assayed by enzymatic method (hexokinase).

This study aims to determine the difference between plasma glucose measured by blood glucose strips (bedside) and enzymatic method and propose new targets for bedside blood glucose to increase the security of ITT.

Methods From March 2013 to January 2014 we conducted 423 ITT. In all these, measurements of plasma glucose were performed using blood glucose strips (bedside) and enzymatic method. The blood sampling was done at the same time for both methods. The results were matched and compared statistically.

Results: There is statistically significant difference between the plasma glucose levels measured by blood glucose strips and enzymatic method . The value assayed by the enzymatic method is smaller than the other (bedside). The median difference between all values is -9.624mg/dl (-10.11 to -9.137)

Regarding extreme glycaemia (under 60 mg / dL and greater than 99mg/dl) , the difference between the two methodologies is constant , statistically significant and even higher. The enzymatic method is -10.87 mg / dL (-11.0 to 10.64) lower than the equivalent sample at the bedside method (strips). At standard deviation (SD) we found a deviation of + / - 21.75mg/ dL.

Conclusion There are statistically significant and consistent difference between the two methods compared. In extreme glycaemia (outside normal range) this difference is even greater. Our data show that if the glucose levels at the bedside (strips) were held on average 10 mg / dl of hypoglycemia target to be achieved in the plasma assay by enzymatic method, we can increase exam security without compromising its reliability and possibly reduce clinical patient discomfort.

A-159

Laboratory process improvement through adjustments in calibrations of immunoassays

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Workflow optimization identifying the specific processes and improvements not only improves operational efficiency, it also reduces error and contains costs. Because system capabilities are not the only considerations when selecting the optimal system for the DASA laboratory, created customized workflow scenarios to help maximize your productivity.

Objective: The objective of this study is to show that continuous monitoring and standardization of quality system improved the flow in large laboratory routines, increase productivity and yield of reagents reducing the turnaround time on the pre-analytical (TAT), impacting on operational costs, reducing interfering in quality control.

Methods: Based on an extensive statistical analysis on the performance of systems ADVIA Centaur XP (Siemens Healthcare Diagnostics), were established changes in calibration routine for Progesterone test, setting 6 equipments in which the calibration for this analyte would be performed every seven days and for other six systems calibration would be performed every twenty-eight days, number of replicates of the calibrator was changed from 3 to 5 according to the instructions of reagents after a period of four months. The performance analysis of the test and the variation in assay constitute evaluated.

Results: The number of disparate control represented 15.4% with a confidence interval from 14.6 to 16.2%. In 8,862 processed control results, 1,363 were outside 2SD range. After implementation and improvement processes in the system, in between January and April 2012, the disparate controls number decreased to 7.8% with confidence interval of 7.2% to 8.4%. In 7,931 only 615 control results were outside 2SD range. The number of test calibrations decreased from 600 to 420 in the respective time periods and processed control number decrease 10%.

Conclusion: The preanalytical phase may cause the inaccuracy of the results when there is a long time for the release of the operating systems in laboratories of large routines. The results suggest that variations in the time interval between calibrations can impact the quality of the result, interfere with the performance of quality control and increase the release time of the equipment for routine, compromising the productivity of clinical laboratory routine.

A-160

Performance evaluation of novel C-peptide immunoassay reagent using a fully-automated immunoassay analyzer

Y. Watanabe, I. Sato, N. Hayashi, J. Saegusa, S. Kawano. *Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan*

Background: C-peptide is co-secreted with insulin in equimolar amounts from pancreatic β cells. Assessment of endogenous insulin production with C-peptide immunoassay requires sufficient sensitivity and high specificity. In this study, we evaluated the analytical performance of newly developed C-peptide reagent.

Methods: The ST AIA-PACK C-Peptide II reagent* on Tosoh AIA-2000 fully-automated immunoassay analyzer is an enzyme immunoassay which is performed entirely in a single cup. C-peptide in the sample is bound with monoclonal antibody immobilized on magnetic beads and alkaline phosphatase-labelled monoclonal antibody. After 10 minutes incubation at 37 ℃, the beads are washed to remove unbound materials and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labelled monoclonal antibody that binds to the beads is directly proportional to the C-peptide concentration in the sample. A standard curve is constructed using the Calibrator Set and unknown concentration of C-peptide is automatically calculated using this curve. In this study we evaluated the precision, functional sensitivity, interference, recovery and cross-reactivity of this new reagent toward human proinsulin. Method comparison, against ARCHITECT C-peptide immunoassay based on chemiluminescent immunoassay, was evaluated with clinical specimens from patients. Correlation of the C-peptide

concentrations between serum and EDTA plasma samples was also studied. Results: The standard curve extended from 0.02-30ng/mL for serum. Within-run and between-run coefficients of variation ranged from 2.5% to 3.5% and from 2.5% to 3.6%, respectively. Based on the imprecision profile, functional sensitivity (at 10% CV) of this reagent was 0.017ng/mL. Assay correlation with ARCHITECT C-peptide immunoassay was determined: y=0.97x+0.02, r=0.997, (n=50 x; ARCHITECT, y; AIA-2000). There was a good correlation between serum and EDTA plasma concentrations of C-peptide: y=0.96x-0.07, r=0.996, (n=220 x; EDTA plasma, y; serum). The cross-reactivity against purified human proinsulin was below 0.4%. Conclusion: ST AIA-PACK C-Peptide II reagent using a fully-automated analyzer, a novel enzyme immunoassay for detecting C-peptide, exhibited extremely low cross-reactivity with proinsulin, and showed high sensitivity for C-peptide. Our results demonstrated that this reagent is a reliable method for the rapid and accurate quantification of C-peptide in clinical laboratories, and turns out a useful tool for both the screening and the management of diabetic patients. *) This product has not been approved by the FDA yet.

A-161

Low testosterone concentrations: only mass spectrometry?

A. Fortunato[1], M. Caputo[2], P. Garofalo[3], C. Marchetti[1], R. Castello[4]. *[1]Laboratorio di Chimica Clinica ed Ematologia Ospedale "S. Bortolo", Vicenza, Italy, [2]Laboratorio Chimica Clinica e Microbiologia, Ospedale "Orlandi", Bussolengo (VR), Italy, [3]UOC Endocrinologia AOOR "Villa Sofia-Cervello", Palermo, Italy, [4]UOC Medicina generale indirizzo endocrinologico, AOUI, Verona, Italy*

Background: total testosterone level measurement is the most requested one among steroid hormones assays. Unfortunately, the diagnostic accuracy at low concentrations of the most common immunoassays proved to be insufficient. In 2007 the Endocrine Society recommended the determinations of testosterone in children and in women has to be done only with one reference method (extraction, chromatography and determination by mass spectrometry). Due the method related difficulties in most of the laboratories the testosterone determinations are still done by immunoassays.

Samples and methods: we measured testosterone with three different fully automated immunoassays present in most of the clinical labs and repeated the determinations both with a commercial RIA and LC-MS/MS method. The latter one, considered the reference method, has been done in the Perkin Elmer labs (Turku, Finland), with updated equipment, by skilled personnel and determinations carried out in replicated. The serum samples were collected from 70 patients, male and female in pediatric age. The obtained concentrations by LC-MS/MS, considered as reference, ranged from 11 to 110 ng/dL.

Results: the distribution of the concentrations obtained with the methods used should be noted that, although the averages and medians of the concentrations obtained with the LC-MS/MS method are less, the differences are not such as to distort the clinical information can be obtained: the 3 automated methods show values ranging from 10 to 134 ng/dL with correlations coefficients respectively to LC-MS/MS ranging from 0,829 to 0,934; whereas the RIA method shows a higher concentration's dispersal, values ranging from 20 to 149 ng/dL and a worse correlation to the reference method. (r=0,705).

Conclusions: the position of the scientific community on total blood testosterone measurement at low concentrations is critical to the use of direct immunoassays because without the necessary diagnostic accuracy, and recommends the use of methods that are not within the reach of general laboratories. Our results, although preliminary, open an interesting perspective on the possibility of arriving at a reasonable future to employ even the immunoassays, certainly more feasible, as an aid to diagnosis of common and important endocrine syndromes of the woman and the child.

A-162

Classification of Children with New-Onset Diabetes Mellitus Using an Auto-Antibody Algorithm

S. Prakash[1], V. Leung-Pineda[2], S. Suzuki[1], J. Radack[2], J. Dallas[2], P. S. Thornton[2], D. P. Wilson[2]. *[1]University of North Texas Health Science Center, Fort Worth, TX, [2]Cook Children's Medical Center, Fort Worth, TX*

Background: Historically the diagnosis of Type 2 diabetes mellitus (T2D) in children has relied on a typical clinical phenotype. The ability of experienced clinicians to correctly classify the type of DM based upon the clinical phenotype has, however, recently been challenged. Since the appropriate classification has important implications with regard to treatment options, expected outcomes and genetic counseling, a systematic, cost-effective algorithm to assist in the initial classification of DM is needed.

Objective: To evaluate the use of an auto-antibody algorithm to classify new onset diabetes patients and its use in curtailing testing costs

Methodology: Data from children (<18 yrs of age) hospitalized at CCMC from Jan 2010 - Oct 2012 with new-onset DM was analyzed. In contrast to T2D, T1D is an autoimmune disease (AD) characterized by the presence of ≥1 diabetes-related antibodies (DR-Ab). Historically the initial evaluation, including DR-Ab testing, has been left to the discretion of individual Pediatric Endocrinologists. Other Abs are often measured to assess concurrent autoimmune diseases that commonly occur in individuals with DM, such as Hashimoto's thyroiditis and celiac disease. Inclusion Criteria: 1) Age < 18 yrs at diagnosis; 2) New-onset DM; 3) Onset Jan 2010-Oct 2012.

Results & Conclusions: The American Diabetes Association classifies DM into T1D, T2D, gestational diabetes, and diabetes due to other causes. While the majority of those <18 yr of age have T1D, the number with T2D is increasing. Individuals with T2D are often obese. With the exponential increase in the number of children who have become overweight/obese, classifying DM based on a child's phenotype has become problematic. In children with overt signs/symptoms of DM the presence of ≥ 1 DR Ab is generally considered sufficient evidence of auto-immunity (i.e. T1D). In our study, subjects were routinely tested for 2 DR-Abs (GAD_{65} 97.9%; ICA 95.9%). Since 73.3% of subjects were positive for GAD_{65}, additional testing for ICA increased cost w/out additional benefit. While not included in the present study, additional screening tests are sometimes also requested for celiac and thyroid disease. For those whose initial screening was positive (celiac 12.5%; thyroid 16.1%), eliminating further testing would have helped reduce cost.

Ab testing to help classify children with new-onset DM may be enhanced with use of an algorithm, especially if it includes reflex testing. Reflex tests are tests automatically performed by the laboratory if the initial test requested fails to meet preset criteria. Subsequent tests can generally be performed w/out need for additional samples and may consist of ≥ 1 sequential tests. Although there is a charge for additional tests, if the likelihood of the criteria being met with the initial sample is high, reflex testing has the potential to reduce medical cost.

A-163

Quality control management improving immunoassays systems in the clinical laboratorial routine

D. Waltrick, A. L. N. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Automated analyzers provide several advantages on processing immunoassay methods. The literature demonstrates that laboratory errors can be associated with pre-analytical (30.6%), analytical (31.6%) and post-analytical (30.8%) processes or even due to combined processes (6%). Errors in the analytical phase are commonly related to lack of preventive maintenance, inappropriate Quality Control (QC) management, and improper handling of samples or reagents. Errors due to analytical problems have been significantly reduced over time, but there is evidence that this interference may have a serious impact on patient results, especially for immunoassays.

Objective: The aim of the study was to identify the potential causes for quality control variability in immunoassays to improve the laboratory routine productivity when adopting best quality control practices.

Methods: 32,760 quality control points of Immunoassay Plus QC Lot 40240 (BioRad) were collected during eight months using 14 ADVIA Centaur Systems (Siemens Helathcare Diagnostics), in the clinical laboratories. The statistics evaluated were: Coefficient of variation (CV), Standard deviation (SD), observed mean and outliers. Data was compared to those reported on Biorad International Quality Control Program. All assays that presented better or equal statistic results were considered acceptable. Assays with higher CV than the reference, Biorad worldwide report, were submitted to further technical investigation and corrective actions. After critical analysis of the first four months data, some improvements were implemented, such as: a new plan of the preventive maintenance, increasing periodicity from once per quarter to once each three months matching laboratory number of tests; a new definition of mean and standard deviation for each QC target level for each assay. Once improvements were implemented, quality control data was collected during the following four months.

Results: In the first four months period, before implementation of proposed improvements, the CV mean was 18.8%, 5,402 tests were spent in calibrations, 16,841 tests were used in QC material analysis and reagents profitability was 96.4%.

Thereafter, data of a new four months period were collected, showing CV mean decreased to 10.7%; 4,708 tests were spent in calibrations; 15,919 tests were used in QC material analysis and reagents profitability raised to 98.0%.

Conclusion: Through statistic analysis, it was possible to identify the importance on studying clinical lab routine in order to implement a best plan for preventive maintenance and appropriate rules of the quality control management. The new definition of a single target value for all ADVIA Centaur Systems was useful for reducing time to evaluate daily internal quality control and also to ensure the commutativity between the systems. This project brought financial and nonfinancial gains, such as lower consumption of reagents, reduced downtime and reported results confidence.

A-164

A Comparison of CVD risk in newly diagnosed hypothyroid and type 2 diabetes mellitus subjects using Framingham risk score sheet

P. Purohit, P. Sharma. *All India Institute of Medical Sciences, Jodhpur, India*

Background Thyroid disorders and type 2 diabetes are known for their association with CVDs owing to their effect on derangement of the lipid metabolism. However there are no studies to document a comparative CVD risk in these two disorders.

Aim We aimed to compare the various CVD risk parameters in thyroid disorder and type 2 DM subjects at the time of diagnosis.

Material and methods The study participants were 150 hypothyroid and 180 type 2 DM subjects reporting for the first time to our endocrinal clinics. The patients were selected on the basis of symptomatology, a TSH >5µIU/ml and a FBS > 126 mg/dl. All participants were evaluated for BMI, Blood Pressure, serum Insulin, HOMA - IR, Lipid profile, apo -B and A1. CVD risk was assessed using the Framingham risk score. Statistical analysis was done using the students -'t' test to assess significance.

Results At diagnosis the hypothyroid and T2DM subjects presented with raised BMI ($p>0.001$), hypertension (SBP 132.98 ±17.40 v/s 132.60± 12.18 [NS]; DBP 86.52± 9.82 v/s 88.79± 8.02 [NS]), insulin resistance (30.63±16.18 v/s 17.29 ± 15.61, $p<0.0001$), gross dyslipidemia, with the T2 diabetic subjects showing significantly raised total cholesterol (231.15± 22.19 v/s 213.50 ± 38.95, $p<0.0001$), triglycerides (197.35± 35.31 v/s 187.91 ± 39.12, $p<0.0001$), reduced HDLc (33.56± 2.67 v/s 42.99 ± 4.70, $p<0.0001$) and significantly reduced apo B (154.47± 12.87 v/s 175.58 ± 34.56, $p<0.0001$) and apo A1 (96.94± 8.55 v/s 139.76 ± 17.40, $p<0.0001$). The CVD risk ratios T.chol/HDLc 6.93 v/s 5.03 and apoB/apoA1 were 1.60 v/s 1.29. The ten year risk of CVD in the T2DM subjects was 25% and in the hypothyroid subjects was 13%.

Conclusion: The present study concludes a significantly raised CVD risk in T2DM as compared to hypothyroid subjects at diagnosis.

A-165

Serum ghrelin, obestatin and nesfatin1 levels in pregnant women with hyperemesis gravidarum

G. Ozturk[1], S. Erdinc[2], F. Ucar[1], Z. Ginis[1], G. Erden[1], N. Danisman[2]. *[1]Department of Clinical Biochemistry, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey, [2]Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey*

Backgrounds: Hyperemesis gravidarum, which affects 0.3-2.3% of pregnancies, is defined as excessive vomiting during pregnancy and usually starting at 4 to 5 weeks' gestation , which may lead to severe outcomes including weight loss, dehydration, ketonemia, ketonuria, fasting acidosis, alkalosis due to hydrochloric acid loss and hypokalemia. Although, their exact cause is unknown, various metabolic and neuromuscular factors have been implicated in the pathogenesis of Hyperemesis gravidarum. The aim of this study was to investigate the levels of prealbumin, total ghrelin , nesfatin 1, obestatin in Hyperemesis gravidarum.

Methods: A total of 40 pregnant women with Hyperemesis gravidarum and 38 pregnant women who were perfectly healthy and whose pregnancy had a normal course were included in this study. After an 8-12 hour overnight fast, blood samples were collected into plain tubes for obtaining serum. Blood samples were centrifuged at 2.500 g for 15 min at 4 oC within 30 min of collection, and serum samples were stored at -80oC until analysis. Measurements of ghrelin (Phoneix, USA) obestatin (Biovendor,Czech Republic) and nesfatin1 (Phoneix, USA) were per¬formed in an EPOCH system (BioTek Instruments, Inc, USA) using the com¬mercially available enzyme-linked immunosorbent assay kit in accordance with the manufactures' instructions. Prealbumin levels were measured by spectrophometric method. All statistical analyses were performed using the software SPSS 15.0 (SPSS inc., Chicago, Il, USA) program. For all statistical tests, two-tailed p value <0.05 indicated the statistical significance of the results.

Results: There were no differences in age, gestational week and BMIs between the patients with Hyperemesis gravidarum and control subjects. Serum ghrelin and prealbumin levels were significantly lower in patients with Hyperemesis gravidarum than the control group ($p<0.05$). Serum obestatin and nesfatin1 levels were not statistically different between the two groups.

Conclusions: Hyperemesis gravidarum is a disease of severe nausea, vomiting, and anorexia in early pregnancy resulting in dehydration and weight loss. Prealbumin is an indicator to assess nutritional status, so our data also suggests that prealbumin levels are decreased in patients with Hyperemesis gravidarum. Ghrelin is involved in stimulation of appetite, control of energy balance, and gastric motility. Ghrelin administration increases food intake through central mechanisms. One possible explanation might be that the decreased levels of ghrelin in Hyperemesis gravidarum may be a mechanism to lose of appetite and the energy balance of the Hyperemesis gravidarum pregnant women.

A-166

Evaluation of the Impact on IGF-I Control of Pharmacological Treatment with Octreotide LAR isolated compared to Association with Cabergoline in Patients with Acromegaly

L. F. A. Nery, C. M. Araújo, R. H. Jácomo, S. S. S. Costa, L. A. Naves. *Laboratório Sabin de Análises Clínicas, Brasília, Brazil*

Background: Discrepancies concerning GH and IGF-1 levels in acromegaly patients can occur in patients submitted to pharmacological treatment. Objectives: The aim of this study was to compare the efficacy in decrease and normalization of IGF-I values of Octreotide LAR treatment isolated and associated with cabergoline, based on IGF-1 determinations.

Methods: This is a case series study that enrolled 34 patients with confirmed diagnosis of acromegaly recruited from outpatient clinics of the Neuroendocrine Unit of the University Hospital of Brasilia. All of them received the diagnosis of acromegaly confirmed by clinical findings suggestive of the disease, elevated GH and age-matched IGF-I levels, GH not suppressible by the oral glucose load and evidence of pituitary adenoma on CT or MRI. The statistical analysis was performed using SPSS 17.0 software. Values are expressed as the mean ± standard deviation. The values of IGF-I are presented both as absolute values as percentage values of the upper normal limit normal range of IGF-I (% ULNV-IGF-I).

Results: The cohort was composed by 15 men and 19 women; mean age 54 (27-74) years old, divided in two groups, group 1, treated by Octreotide LAR (OC-LAR) 30 mg/month, and group 2 treated by Octreotide LAR (OC-LAR) 30 mg/month associated with cabergoline 2,0 mg/week (OC-LAR + CBG). Mean serum IGF-I and % ULNV-IGF-I pretreatment were significantly higher in the group OC-LAR + CBG. Those variables decreased 6 and 12 months after treatment in both groups, and the values of OC-LAR+CBG group became inferior to OC-LAR group. However, no significant difference was found between the OC-LAR and OC-LAR + CBG group neither 6 nor 12 months after treatment.

Conclusion: OC-LAR + CBG association resulted in a significantly higher decrease of IFG-I, both 6 and 12 months after treatment, compared to those treated with OC-LAR

A-167

ADVIA Centaur® Vitamin D Total Assay*: Expected Vitamin D Values in a Healthy Pediatric Population

H. Mindicino[1], J. Freeman[1], R. Christenson[2], B. Plouffe[1], A. Woods[3], M. Zgela[1]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]University of Maryland School of Medicine, Baltimore, MD, [3]Siemens Healthcare Diagnostics, Berkeley, CA*

Background: Vitamin D is a hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. It is a key regulator of bone metabolism. Vitamin D_3 is derived from skin exposure to sunlight while food supplements contain either Vitamins D_2 or D_3. Vitamin D deficiency is caused by a lack of exposure to sunlight and by insufficient dietary intake. Measurements of serum or plasma levels of the metabolite 25-hydroxyvitamin D (25[OH]D) are the best indicators of nutritional Vitamin D status.

Objective: The goal of the study was to test healthy pediatric donor specimens in order to establish pediatric observed values for the ADVIA Centaur Vitamin D Total assay*.

Methods: Serum samples were obtained from donors with ages ranging from 1 year up to 21 years. Donors resided in 8 regions, geographically distributed across the continental U.S. Donations occurred throughout one calendar year. All donors were free of chronic or active diseases, and were not receiving any prescription medications within 7 days of donation. All samples were assayed for iPTH and TSH on the Siemens IMMULITE® 2000 Immunoassay System. Samples with abnormal iPTH or TSH levels were excluded. The remaining samples were assayed for total vitamin D Total on the Siemens ADVIA Centaur. The ADVIA Centaur Vitamin D Assay used was aligned to the ID-LC/MS/MS 25(OH)vitamin D Reference Measurement Procedure (RMP), the reference procedure for the Vitamin D Standardization Program (VDSP).

Results: After all inclusion criteria were met, 227 samples were assayed for Vitamin D Total. Values were calculated for each age group, by season, and by geographic location. The lower and upper reference limits were estimated as the 2.5th and the 97.5th percentiles of the distribution of test results for each age group. The n, mean, median, 2.5th and the 97.5th percentiles for each population sub-group were:

Northern: n=119, mean=23.55, median=22.17, 25th% = 12.36, 75th = 38.92

Southern: n=108, mean=26.33, median=25.47, 25th%= 9.70, 75th% = 49.16

Summer: n=136, mean=26.64, median=24.86, 25th% = 12.46, 75th% = 46.58

Winter: n=91 , mean=22.23 , median=22.10 , 25th% =9.70 , 75th% = 32.38

1yr-3yr: n=22 , mean=23.34 , median=24.41 , 25th% = 13.98, 75th% =32.45

3yr-12yr: n=114 , mean=24.99 , median=23.10 , 25th% =12.46 , 75th% =45.96

12yr-21 yr: n=91 , mean=25.09 , median=23.95 , 25th% =8.16 , 75th% =45.83

All Donors: n=227 , mean=24.87 , median=23.37 , 25th% =11.36 , 75th% =45.83

Conclusion: Vitamin D levels were consistent across the 3 age groups with no apparent changes with age. There was no statistical difference between those receiving and not receiving vitamin supplements. Vitamin D levels were statistically higher for the southern region versus northern region, and for summer (maximum sunlight) versus winter (minimum sunlight). There was extensive overlap in ranges for all sub-populations and the expected range for the entire population can applied to any of the sub-populations.

* This version of the ADVIA Centaur Vitamin D Total assay is not available for sale in the U.S.

Product availability varies from country to country and is subject to local regulatory requirements.

A-168

Enigma behind Thyroid Function Tests: Harmonization Efforts

B. Das. *Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, Maharashtra, India*

Background: Thyroid function tests (TFTs) form a very important set of tests in a pathology laboratory; a tool that clinicians and patients alike depend on to pin down the symptoms for treatment and relief However, it is this very set of tests that have come in question. First, what is the normal and acceptable range (upper & lower limits) of TSH in a particular population has been debated in different scientific fora A second problem which the doctors and technicians have to grapple with is the variability of test-results in itself; even a broadly similar set of instruments and methods can give up to 40% more or less values in TSH levels. In our laboratory, we validated thyroid function test and established reference range of TSH for Indian population.

Methods: In our laboratory, validation of thyroid function test were done [with particular reference to Thyroid Stimulating Hormone (TSH)] by verifying analytical accuracy and precision, and Analytical measurement range (AMR) as well as sigma metrics. We have also verified the reference range for Indian Population. We have screened 800 subjects. 630 healthy subjects were chosen in the study group for reference interval verification.

Results: In our laboratory, we have seen, high degree of analytical accuracy between two instruments (r2 = 0.985). Within Run (Repeatability) Precision and Within Laboratory Precision were comparable with the manufacturer's claim. Our obtained reference range (0.62 - 4.22 micro IU/ml) was within that of the manufacturer's (0.35 - 4.94 micro IU/ml). AMR was also verified with C.V. 1.70%, 1.89% and 2.51%, for control sera. Our obtained reference range (0.33 - 4.90 micro IU/ml) was correlating with that of the manufacturer's (0.35 - 4.94 micro IU/ml).

Conclusion: In our laboratory, we have verified thyroid function tests in our hospital set up. However, standardization of TSH and other thyroid function test is still a formidable challenge, due to the lack of proper reference intervals and standardized measurement procedures.

Our laboratory validation protocol will help any laboratory personnel from any part of the world to validate & establish reference interval based on their own population demographic variation.

A-169

Glutamate Decarboxylase Antibody Positivity in Diabetics

L. Ong, M. Tan, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore*

Background: Glutamate decarboxylase antibody(GAD) testing is useful in identifying patients with latent autoimmune diabetes in adults (LADA), and some studies showed that higher antibody titres were associated with specific phenotypes. We looked at patients with positive GAD and reviewed their laboratory and clinical features.

Methods: All GAD tests performed in 2013 were included, and positive results were analyzed with respect to demographic parameters, indication for testing (evaluation of diabetes mellitus or neurological signs or symptoms), presence of other autoantibodies or autoimmune diseases. GAD was performed using radioimmunoassay using the CentAK® kit and a positive result was defined as ≥0.9U/L. Statistical analysis was done using SPSS Version 17.0.

Results: There were 454 GAD requests in 2013, with 75(16.5%) positive cases. The median age was 39.3 years old, with female: male ratio of 0.55.

In patients with positive GAD, the median age was 43.4 years old, with female: male ratio of 1.06. There were 45 Chinese, 10 Indians, 9 Malays and 10 from other ethnic groups. 94.6% were requests to exclude LADA, and 21.3% had concurrent requests for anti-islet cell antibody, with 50% positivity. 4 patients had co-existing autoimmune diseases (myasthenia gravis, thyroid disease with positive thyroid peroxidase antibody, vitiligo and pernicious anemia).

GAD titres showed a Gaussian distribution with a left skew and peak at 0-25U/L. There was no association between GAD titres and age or C peptide levels.

Conclusion: GAD levels were predominantly ordered to evaluate diabetes mellitus in younger patients and there was an association with anti-islet cell antibody and other autoimmune diseases. Further studies may be performed to determine the clinical significance of high GAD titres in diabetics.

A-170

New therapeutic effect of metformin due to increased levels of FGF21 ?

D. Stejskal[1], R. Ochmanova[2], M. Uvirova[3]. *[1]Agel Research and Training Institute, branch Novy Jicin, Laboratories Agel, Novy Jicin, Czech Republic, [2]Agel Research and Training Institute, branch Prostejov, Central Moravian Hospital Trust, Department of laboratory medicine, Prostejov, Czech Republic, [3]Agel Research and Training Institute, branch Ostrava, CGB Laboratory, Ostrava, Czech Republic*

Background:: Fibroblast growth factor 21 (FGF21) is an endocrine hormone that exhibits anti-obesity and anti-diabetes effects. Recently was presented that metformin in patients with type 2 diabetes modulates FGF21 expression and blood concentration. Results indicate that metformin induced expression of FGF21 through an ATF4-dependent mechanism by inhibiting mitochondrial respiration independently of AMPK and it´s concentration in blood. AIM: Studying the effect of metformin on the concentration of FGF21 in serum in type 2 diabetes patients.

Methods: The study was approved by the Ethics Commission of the Hospital Šternberk. Study was monocentric, prospective and randomized. A total of 108 individuals were recruited for our study (18 healthy controls (HC), 18 T2D individuals without anti-diabetes therapy (W), 18 T2D individuals with diabetes monotherapy with derivate of sullfonylurea (SU),18 T2D individuals with diabetes monotherapy 500 mg metformin/day (M5), 18 T2D individuals with diabetes therapy 1000 mg metformin/day (M10), 18 T2D individuals with diabetes therapy 1500 mg metformin/day (M15). Anthropometric (height, weight, BMI, waist circumference (WC)), clinical (systolic and diastolic pressures) and laboratory fasting analyses were performed. Serum samples were separated in a cooled centrifuge and immediatelly analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, high sensitivity CRP, creatinine, uric acid, AST, ALT (all Siemens, Advia 1800). FGF21 serum level was determined by a commercially available ELISA kit (Biovendor, DS2, Dynex) in serum samples stored at -80o C.

Results: The study analysed 108 subjects, of which 18 were in good health while 100 probands suffered from T2D. In-defined subgroups, we found no significant differences by gender in FGF21 concentration. Healthy individuals had the lowest

values of FGF21, in other subjects are increased by the value of the diagnosis, the type of therapy and dose (HC 84.2 ng/l vs W 111.6 vs. SU 158.6 vs. M5 269.7 vs. M10 404.1 vs. M15 558.7, P <0.01). Changes remained significant after adjustment for age, sex and BMI. Serum glucose levels fluctuated in subgroups below 8 mmol/l.

Conclusion:: in a randomized prospective study, we for the first time confirmed the recently presented hypothesis that metformin leads to the rise of FGF21. The new finding was the fact that this happens regardless of gender, weight and age of probands. FGF21 induction by metformin might explain a portion of the beneficial metabolic effects of metformin

A-171

Pre-analytical assessment of AMH stability in human serum using a well-characterized midpro-mature immunoassay

A. Kumar[1], B. Kalra[1], A. S. Patel[1], G. Savjani[1], E. E. Eklund[2], G. Lambert-Messerlian[2]. *[1]Ansh Labs, Webster, TX, [2]Women and Infants Hospital, Providence, RI*

Background: The aim of the study was to assess the stability of AMH in human serum using a well-characterized midpro-mature immunoassay.

Relevance: AMH is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulfide bridges. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. To date, no publication has clearly stated if the AMH variability is related to process (pre-analytical) or the assay. The AMH in female serum is mostly pro-mature associated form. The kinetics of association of pro and mature is rapid. Assay design that includes stable epitope antibodies and is not impacted by molecule association or complements will generate reproducible results.

Methods: A prospective study (n=16) was designed in which serum samples were tested within 3 hrs of draw, aliquoted and stored at room temperature (RT), -20°C, 2-8°C and re-assayed at 7, 10, 24, 48 and 168 hours. Multiple samples were thawed up to 4 cycles and measured at two independent sites. A well-characterized two-step, ELISA (Ansh Labs, US AMH, AL-105) was used to measure AMH levels in 25 µL of sample in < 3 hours. The assay is specific for human and measures pro-mature AMH complex. The assay is calibrated (0.09-19.4 ng/mL) against standardized recombinant human AMH.

Results: No significant changes were observed when samples were stored at RT, 2-8°C and -20°C. The median AMH concentration (16 serum samples, range 0.34-20 ng/mL) measured at 7, 10, 24, 48 and 168 hrs were 5.2, 4.9, 5.1, 4.9, 5.0 ng/mL at RT, 5.0, 5.0, 4.7, 4.3, 4.4 ng/mL at -20°C and 4.8, 4.8, 5.0, 4.7, 4.9 ng/mL at 2-8°C. The average CV on multiple runs at RT, 2-8°C, -20°C was 8.7%, 6.9%, 9.3%, respectively. Total imprecision (all data points) on stored samples and two controls were 9.3%, 4.8% and 3.1%, respectively. Freeze thaw analysis on two independent sample sets showed that AMH levels were stable over 4 thaw cycles, with median levels of 4.5, 4.7, 4.8, 4.7 ng/mL obtained at site 1 (n=4) and 1.2, 1.2, 1.3, 1.3 ng/mL, respectively at site 2 (n=5).

Conclusion: AMH as a biomolecule is very stable. This finding helps to resolve the uncertainty related to AMH sample stability and reliability of AMH measurements that have been debated. This study demonstrates that well-characterized assays and good pre-analytical methods will produce reliable and reproducible results.

A-172

Performance of Thyroid Hormone Assays on Mindray CL-2000i Chemiluminescence Immunoassay System

Y. Qiu[1], J. Hao[2], C. Kang[1], P. Feng[1], C. Yang[1], S. Yuan[1], Y. Zhang[1], F. Xia[3]. *[1]Nanfang Hospital, Southern Medical Univeristy, Guangzhou, China, [2]Nanshan Hospital, Huazhong University of Science and Technology, Shenzhen, China, [3]Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China*

Background: Thyroid hormones are among the first-line tests subject to international collaborative investigations, aiming at assessing the key performances and comparability of results between the available tests. Mindray CL-2000i Chemiluminescence Immunoassay System (CL-2000i) is a recently launched automatic immunoassay system. We have evaluated the performance of the system with serum thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), and total triiodothyronine (TT3).

Methods: Imprecision studies were performed according to the CLSI EP5-A2 guideline, and have been evaluated using two samples with low and high concentration. The within run imprecision was performed by measuring each sample for 20 times. The total imprecision was evaluated by measuring each sample continuously for 20 days with the same lot of reagent. The imprecision was expressed as coefficient of variation (CV%). Patient samples from healthy subjects, hypo and hyperthyroidism were freshly collected from the clinical laboratory of our hospital. The comparison studies were performed using CL 2000i and the reference methods in our laboratory, Siemens ADVIA Centaur and Beckman Coulter DxI 800.

Results: The CVs of TSH, FT4, FT3, TT4, and TT3 are in a range from 1.09 - 6.16% for within run, and from 3.22 - 9.16% for total imprecision. TT4 shows relative higher CVs for both within run and total imprecision comparing to other parameters, but within the manufacturer's claim (≤ 10%). The comparison studies indicated slopes for the five thyroid hormone parameters ranged from 0.748 to 1.051 and the intercepts from -0.48 to 0.6. All of the five assays displayed high interassay correlation (r^2 > 0.92). TSH displayed the highest correlation between CL 2000i system and Centaur XP system (slope = 0.993; r^2 = 0.971), while TT4 showed the lowest agreement between CL 2000i system and the Centaur XP (slope = 1.051; r^2 = 0.921).

Conclusion: The imprecision was highly acceptable for all the five parameters of thyroid hormones tested. The method comparison between CL-2000i system and the reference methods evidenced high concordance. Therefore, the parameters of CL-2000i system are well suited for the detection of thyroid hormones in clinical laboratories.

A-173

Linearity study of fertility assays assuring the quality control requirements

D. Waltrick, A. L. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background:In the fertility trials, each step in the reproduction process is evaluated to monitor gynecological health. There are basic exams requested for each phase of the process. To certify released results accuracy, it is important to ensure the linearity of the tests.A Brazilian laboratory implemented an Easy Linearity Curve (ELC) tool to monitor the immune-hormone analytical systems, establishing a self-inspection program to verify the efficiency and accuracy of procedures and results. The use of a tool that checks assay linearity provides additional safety and reliability of the results. This study aims to use the statistic tool to monitor the AMR of prolactin, progesterone, LH, FSH, testosterone and estradiol.

Materials and methods: We selected samples from the laboratory routine, with concentrations within assay linearity range for each test. Samples were tested in Advia Centaur® XP (Siemens Healthcare Diagnostics) using a chemiluminescent method and analyzed with the ELC tool.

Results: AMR studies were carried out according to CLSI Guideline EP6-A. Results are demonstrated in the table below.

Discussion: The tests showed satisfactory linearity results with different samples. In this study, the sample pool was not used, because all the results from this dilution test presented high percentage of recovery, above the manufacturer's reference, due to a matrix effect. Because of that, we established the utilization of different samples, respecting the expected concentrations of the sample pool, if diluted samples were prepared. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level.

Conclusion: The fertility trials tested in this study presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target. Thus tests were approved by the Quality Control Management in the laboratory.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Target %	Obtained TE %	TE max Target %	Linear Regression	R2
Prolactin	0.3 - 200 ng/ml	4.9	5.75	12.65	21.1	1.013x + (-4.058)	0.99505
Progesterone	0.21 - 60 ng/ml	3.1	8.71	15.47	25	1.038x + (-0.67)	0.99821
LH	0.07 - 200 mUI/ml	3.2	4.78	10.48	19.8	0.988x + (-0.947)	0.99699
FSH	0.3 - 200 mUI/ml	4.5	5.5	8.9	17.1	1x + (-0.835)	0.99934
Estradiol	11.8 - 3000 pg/ml	3.5	4.53	12.6	21.6	1.029x + (15.194)	0.99797
Testosterone	10 - 1500 ng/dl	3.2	8.3	16.54	25	1.086x + (-30.634)	0.99356

A-174

Calcitriol and its free and bioavailable fractions are better markers than 25 hydroxy vitamin D for monitoring vitamin D status during Pregnancy

R. Pandian[1], J. Pandian[1], Z. Seres[2], A. Elias[1]. *[1]Pan Laboratories, IRVINE, CA, [2]Immunodiagnostic system LTD, Bolden, United Kingdom*

Objective To compare bioavailable 25-hydroxy vitamin D(25OH-D)concentrations with those of bioavailable calcitriol

(1, 25 di Hydroxy vitamin D) in sera of pregnant women.

Relevance Pregnancy is associated with major changes in calcium homeostasis with critical roles played by vitamin D and its metabolites.Current practice favors monitoring of vitamin D status in pregnancy using 25OH-D concentrations in serum. Calcitriol is the active form of vitamin D.The level of 25OH-D and calcitriol vary due to changes in maternal serum vitamin D binding protein(DBP).Serum concentration of bioavailable and free vitamin D are not influenced by DBP. Therefore, we measured total, bioavailable and free fractions of 25OH-D or calcitriol in serum samples of pregnant and non-pregnant women. Correlation between total 25OH-D and its fractions (bioavailable and free) and calcitriol and its fractions with PTH and CTX(C-terminal collagen degradation product) was explored in order to determine which compound or compounds were optimal markers of vitamin D status in pregnancy.

Methods Bioavailable 25OH-D or bioavailable calcitriol are fractions, not bound to DBP.They are the combined fractions of albumin bound and free fractions of 25OH-D or calcitriol.To obtain the bioavailable fraction, total vitamin D

(25OH-D or calcitriol)was quantitated using immunoassays(IDS, Phoenix,AZ). DBP was quantitated by an ELISA using reagents from R&D systems. Albumin was quantitated by a calorimetric method.Using the affinity constants of 25OH-D and calcitriol for DBP, and the affinity constants of 25OH-D or calcitriol for serum albumin, bioavailable 25OH-D,bioavailable calcitriol,free 25OH-D and free calcitriol were calculated.PTH and CTX assays were performed in pregnancy serum samples using IDS kits.Pregnant serum samples (n=54) were collected between 27 to 38 weeks of pregnancy.

Results Total 25OH-D was significantly lower in pregnant women despite a significant increase in DBP (276 ± 15 Vs 410 ± 30ug/ml).Bioavailable and free 25OH-D levels were lower than normal(n=55),and were in the deficiency or insufficiency range although the levels of PTH and CTX were in the normal range.The correlation between PTH with total 25OH-D was poor(r2= 0.3).There was also poor correlation between PTH and bioavailable or free 25OH-D (r2= <0.5). Calcitriol was high in the pregnancy samples (127.5 ± 15.5 pg/ml)compared to non-pregnant samples (36.2 ± 5.6 pg/ml) and the DBP-corrected bioavailable and free calcitriol was twofold higher than non-pregnant controls.Calcitriol and its fractions (bioavailable and free of calcitriol) correlated well with serum PTH and CTX (r2 = >0.9).

Conclusion Current assessment of vitamin D status in pregnant women by measurement of 25OH-D does not adequately reflect calcium homeostasis in pregnancy.25OH-D or its fractions do not correlate with PTH or CTX. On the other hand, calcitriol correlates well with PTH and CTX in pregnancy when determined either as total, bioavailable or free calcitriol.The data indicate that bioavailable or free calcitriol are the best markers for determining vitamin D status in pregnant women.

A-175

Analytical Measurement Range (AMR) Monitoring for Immune-hormone System

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Linearity tests verify if a method is able to provide linear results, proportionally to the analyte's concentration. It is extremely important to ensure the accuracy of the results released. A Brazilian laboratory established a self-inspection program to verify the efficiency and accuracy of procedures and results through the implementation of the Easy Linearity Curve (ELC) statistic tool to monitor an immune-hormone system. ELC checks assay linearity and provides additional safety and reliability to their results. This study aims to use the statistic tool to monitor the AMR of ten immune-hormone parameters.

Materials and methods: Samples were selected from the laboratory routine, electing concentrations within assay linearity range for each test. Then, samples were tested for Insulin-like Growth Factor 1 (IGF-1), Insulin-like Growth Factor Binding Protein 3 (IGFBP-3), Growth Hormone (GRH), Prolactin (PRL), Sex Hormone Binding Globulin (SHBG), C-Peptide (Pep-C), Homocysteine (HCY), Adrenocorticotropic Hormone (ACTH) and Intact Parathyroid Hormone (iPTH) on IMMULITE 2000® System (Siemens Diagnostics Healthcare) and results analyzed with the ELC tool.

Results and Discussion: AMR studies were carried out according to CLSI Guideline EP6-A. Results are shown in the following table bellow.Tested Samples showed satisfactory linearity results for each parameter. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level. All results analyzed by ELC, presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target.

Conclusion: We conclude that the implementation of a self-verification program as ELC can increase efficiency and accuracy of procedures and results; aiding laboratory on accomplishing Quality Control Program requirements and guaranteeing safety and reliability of their released results.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
IGFBP3	0.1 - 16 ug/mL	1.40	6.3	14.6	17.50	1.09x + (-0.236)	0.99377
IGF1	20 - 1600 ug/dL	2.80	4.7	8.8	14.90	0.985x + (2.037)	0.99995
GRH	0.05 - 40 ng/mL	3.20	4.6	12.6	20.00	0.982x + (-0.246)	0.99634
PRL	0.5 150 ng/mL	2.50	5.9	14.7	21.10	0.93x + (3.362)	0.99509
SHBG	2 - 180 nmol/L	3.20	6.2	13.2	21.10	0.946x + (1.952)	0.99837
Pep C	0.1 - 20 ng/mL	2.40	8.3	11.2	20.80	1.03x + (-0.044)	0.99956
HCY	2 - 50 umol/L	7.70	6.4	15.3	17.70	1.053x + (-0.145)	0.99986
ACTH	5 - 1250 pg/mL	3.94	4.6	9.1	11.82	0.986x + (3.936)	0.99844
IPTH	3 - 2500 pg/mL	6.73	13.0	20.4	30.20	1.042x + (51.56)	0.98744

A-176

Clinical Applications Of Adiponectin Measurements In Type 2 Diabetes Mellitus - Screening, Diagnosis and Marker of Diabetes Control

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

Background: Adipose tissue-derived adiponectin has pleiotropic protective effects with suppression of inflammatory and metabolic derangements that may result in insulin resistance, metabolic syndrome, Type-2 diabetes (T2DM) and cardiovascular disease. No study has evaluated the potential clinical significance of adiponectin measurements that may be useful in routine practice. The aim of this study was to evaluate adiponectin as a screening tool and diagnostic marker of T2DM and diabetic control.

Methods: Fasting adiponectin, insulin and glucose and HbA1c were determined in 575 subjects with undiagnosed diabetes but with family history of T2DM. To evaluate adiponectin as a marker of DM control, we studied 376 patients with known T2DM duration of 12.4 ± 8.1 years. Clinical and anthropometric data were recorded and subjects were classified on the basis of the degree of adiposity, insulin resistance (IR) using the homeostasis model assessment, and achievement of target HbA1c levels < 53mmol/mol. Using standard cut off values for glucose and HbA1c, receiver operating characteristic curve (ROC) analysis was used to examine the diagnostic performance characteristics for undiagnosed DM.

Results: In undiagnosed subjects, adiponectin was significantly lower in subjects with IR (7.0 vs 8.5 µg/mL) and diabetic subjects (7.4 vs 8.6 µg/mL) compared with those without. 73 of 575 subjects were found to have T2DM. Binary logistic regression showed that the odds ratio of T2DM as predicted by adiponectin was 0.88 [95% confidence interval 0.80-0.96; p = 0.007]. At cut-off points of 7.5 µg/mL, the diagnostic sensitivity and specificity of adiponectin for T2DM were 88% and 51% respectively. Using the ADA glucose and HbA1c diagnostic criteria as reference, the area under the adiponectin ROC curve for diagnosis of DM was 0.740 (95% CI 0.570 - 0.910). In known T2DM subjects, those with good control (HbA1c < 53mmol/mol) had significantly higher adiponectin (8.5 vs 7.1 µg/mL) compared to subjects with poor control.

Conclusions: Adiponectin levels are associated with better glycemic control and could be useful adjunct for screening for IR and T2DM. Therapeutic measures that increase adiponectin levels might be valuable targets for improving diabetes control and decreasing complications.

A-177

Performance of GH stimulation tests at a private laboratory in Brazil: Is insulin tolerance test still the best?

P. B. M. C. Araujo, K. Marca, L. Santos, J. Duarte, A. C. Bispo, M. Freire. *Diagnósticos da America S.A., Rio de janeiro, Brazil*

Background: Growth hormone deficiency (GHD) is the most important endocrine cause of short stature in childhood. Once growth hormone (GH) secretion is pulsatile, diagnosis of GHD is made with a combination of clinical assessment, IGF1 and

IGFBP3 levels, and GH stimulation tests. In Brazil, the most common GH stimulation tests are insulin tolerance test (ITT), clonidine and glucagon. The aim of this study was to determine the performance of those GH provocative tests and to make comparisons among them and with IGF1 levels.

Methods: Retrospective assessment of GH stimulation tests, performed during the year of 2013 in children between 4 to 18 years old, including 141 ITT (mean age 11,72 2,58 yrs, 72,3% male), 285 clonidine tests (mean age 10,41 2,91 yrs, 76,5% male) and 42 glucagon tests (mean age 7,00 3,4 yrs, 66,7% male). The mean dose of each medication administered was respectively 0,074UI/kg, 0,138 mg and 0,568 mg and the mean glucose nadir at ITT was 27,95 8,02. Comparison among tests showed statistical difference regarding peak stimulated GH (GH > 5,0 ng/mL) between ITT and glucagon (p<0,01) and ITT and clonidine (p<0,01), but not between glucagon and clonidine tests (p=0,1014). Peak stimulated GH happened at 26,24% (mean GH 10,62 5,78), 82,10% (mean GH 11,88 5,79) and 71,43% (mean GH 11,63 7,38) from ITT, clonidine and glucagon tests, respectively. GH peaks concentration was at time of hypoglycemia at ITT (70,6%), at 60 minutes after stimulation with clonidine (60,3%) and at 2h after stimulation with glucagon (63,3%). Levels of IGF1 did not correlate with GH answer to the stimulation at insulin (p=0,6165), clonidine (p=0,4914) and glucagon tests (p=0,5551).

Conclusion: Our finding that clonidine and glucagon tests presented a better rate of response compared with ITT represents a new scenario for GH provocative tests, once ITT has been considered the gold standard test for GHD investigation. Maybe it could be explained by the fact that in a private laboratory environment, we can not let the patient recover spontaneously, which could increase side effects. Instead, the recovery from hypoglycemia is done with oral glucose, which may impair the GH peak, finding reported by Yeste and cols. Hence, clonidine and glucagon tests emerge as a reliable and safer alternative to ITT. Surprisingly, IGF1 levels did not correlate with rates of GH response to the tests, which highlights the importance of a clinical/laboratory combined evaluation.

A-180

A Fully-Automated 1,25-Dihydroxy Vitamin DXp Assay on the IDS-iSYS Automated System

J. Tran[1], D. Bautista[2], Z. Seres[3], L. Cornaut[4], T. Gundlach[3], A. Rousseau[4], H. Griesser[3]. *[1]Immunodiagnostic Systems Ltd, Westminster, CA, [2]Immunodiagnostic Systems Ltd, San Clemente, CA, [3]Immunodiagnostic Systems Ltd, Boldon, United Kingdom, [4]Immunodiagnostic Systems Ltd, Pouilly, France*

1,25-Dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. Due to its lipophilic nature and low circulating concentration, the measurement of 1,25D concentration levels has been labour intensive and technique dependent in addition to multiple equipments required for the sample purification procedure. We reported the results of fully automated IDS-iSYS 1,25 VitD^{Xp} assay.

IDS-iSYS 1,25 VitD^{Xp} assay purifies 1,25D in human sera utilising the anti-1,25D antibody coated magnetic particles in cuvette 1. After incubation, the magnetic particles are washed and 1,25D is eluted. The eluate is transferred to cuvette 2 where the immunoassay procedure is performed utilising the IDS-iSYS 1,25-Dihydroxy Vitamin D test. The purified 1,25D competes with 1,25D-Acridinium (1,25D-ACR) for a limited amount of biotinylated anti-1,25D antibody sites. Bound complexes are captured via streptavidin-coated magnetic particles. Following washing, the bound 1,25D-ACR is measured where signal generated is inversely proportional to the 1,25D concentration in the sample. Below are the preliminary analytical performance of the IDS-iSYS 1,25 VitD^{Xp} (iSYS XP125) assay:

Performances	Results
Functional sensitivity	<12.0pg/mL
Inter-assay precision	16.0% (23.5pg/mL)
	8.6% (59.4pg/mL)
	9.6% (79.3pg/ml)
	6.9% (141pg/mL)
Linearity	92-109%
Method comparison against IDS-iSYS 125D (n = 81)	iSYS XP125 = 0.87 x (iSYS 125D) +6.4pg/mL r = 0.95

Combining the innovative on-board sample purification procedure with the already proven IDS-iSYS 1,25 Dihydroxy Vitamin D test, the IDS-iSYS 1,25 VitD^{Xp} delivers accurate results for patient care while enhancing the clinical laboratory 1,25D testing efficiency.

A-181

POST-FIRE STRESS REACTIVITY IN VOLUNTEER FIREFIGHTERS

M. A. LEGUIZAMON[1], S. UCA-Villarrica[2], A. Lird[3], C. Mora[2], M. Cabral[4]. *[1]Laboratorio Central del Hospital de Clínicas. Facultad de Ciencias Médicas. Universidad Nacional de Asunción. San Lorenzo-Paraguay, ASUNCION, Paraguay, [2]Universidad Católica "Nuestra Señora de la Asunción", Campus Guaira. Facultad de Medicina. Cátedra de Bioquímica. Guairá-Paraguay, VILLARRICA, Paraguay, [3]Laboratorio Central del Hospital de Clínicas. Facultad de Ciencias Médicas. Universidad Nacional de Asunción. San Lorenzo-Paraguay, SAN LORENZO, Paraguay, [4]Instituto de Investigaciones en Ciencias de la Salud., ASUNCION, Paraguay*

Firefighters belong to a population especially sensitive to stress factors. The responsibility for "people" sharpen the stress feeling due to the need of pushing oneself, sometimes more that one can. The physical and emotional stress, just like diseases, could increase the levels of cortisol because during the normal response to stress, the hypophysis secretes more corticotropin. **Objective:** To determine the stress level, the analysis of the level of cortisol in serum in Volunteer Firefighters of Caaguazú, before and after a 12-hour duty is presented. **Material and methods**: observational and longitudinal design. Subjects: Male and female volunteer firefighters of any rank and in activity from Caaguazú-Paraguay that accepted blood drawing and do not present disease of the suprarenal glands like Cushing Disease. The determinations performed were levels of cortisol before and after a 12-hour duty. **Results**: Thirty two firefighters participated, 81% were men and 34% were aspirants. Age range was 18 to 33 years. In the first simple, there was a mean of 10.58±5.2 ug/dl and in the second 12.42±5.3 ug/dl. (p=0.0001). There was an increase of 15.43% in the aspirants, 22.29% in the firefighters and 4.34% in the instructors. In the others positions, such as driver and members of the board of directors there was an increase in the level of cortisol of 18.39%. **Conclusions:** the rank of the firefighters as well as the years of experience in the community service influence considerably in the stress level as the aspirants presented more variation in the level of cortisol in serum after the stress situation.

Keywords: cortisol, stress, firefighters.

A-182

Evaluation of a direct HbA1c Assay on a fully automated Chemistry Analyzer versus two common HPLC methods.

D. Rampersaud[1], H. Lee[1], W. Walters[2]. *[1]Clinitox Diagnostix Inc, Mississauga, ON, Canada, [2]Pointe Scientific, Canton, MI*

Pointe Scientific, Inc. (now part of MedTest) has developed a latex-enhanced immunoturbidimetric assay that directly measures the % HbA1c in whole blood. This new method was adapted to a Roche Hitachi 917 clinical chemistry analyzer in a fashion that offline sample pretreatment was not required. The objective of this study was to evaluate the performance of this direct HbA1c assay on the Hitachi 917 chemistry analyzer versus a Tosoh G8 and a Bio Rad Variant II (HPLC methods) at a large commercial lab.

Samples with normal hemoglobin that were run on the Tosoh G8 versus the Hitachi 917 showed a correlation of R=0.987, with a regression equation of y = 1.18x – 0.80. (n=172). Additional samples containing various hemoglobin variants were compared to the results from the Biorad and Tosoh systems. Utilizing all samples (normal and variants) resulted in a correlation of R=0.958, with a regression equation of y = 1.15x – 0.77. (n=256). High and low Biorad QC materials were run for Day-to-Day and Within Run precision. Results are shown below:

Day to Day Precision				Within Run Precision		
Average	4.83	9.21		Average	4.75	9.27
Std Dev	0.144	0.166		Std Dev	0.165	0.082
%CV	2.97	1.80		%CV	3.47	0.89

The data shows that for normal samples, performance of the Pointe Scientific HbA1c assay is comparable to the Tosoh HPLC system. When Biorad and Tosoh variant sample results were included, the correlation was minimally affected. These results demonstrate that this assay can be a viable solution for large volume testing environments where HPLC systems may not be capable of handling a very high volume of samples.

A-183

Analytical evaluation of a glycated protein method on the Siemens ADVIA 1800

T. Higgins, K. Rodriguez Capote, K. Tovell, D. Holmes, J. Dayton. *DynaLIFEDx,, Edmonton, AB, Canada*

Background HbA1c has become the gold standard for assessment of glycemic control in individuals with diabetes mellitus. However the test cannot be used if the individual has a homozygous hemoglobin variant or a condition in which there is a rapid turnover of red blood cells. In these cases glycated protein has been proposed as an alternative assay. We wished to evaluate the analytical performance of the Diazyme Glycated Protein assay, establish a reference interval and to compare the glycated protein results against HbA1c results.

Methods The Diazyme Glycated Protein assay was programmed onto a Siemens ADVIA 1800 analyzer using a program supplied by the reagent manufacturer. Glycated protein was analyzed on samples that had serum albumin, total protein and HbA1c results available. Samples with a bisalbumin were also analyzed for glycated protein by 2 methods.

Results The within run imprecision was calculated at 1.2% and 0.4% respectively at glycated protein concentrations of 181.2umol/L and 684.2 umol/L. At the same concentrations the between run imprecision was 2.3% and 1.2% respectively. Correlation of glycated protein against HbA1c gave a regression equation of y (glycated protein) = 56.41(HbA1c)-35.72, (n=155, r^2=0.91) The reference range was calculated on 42 samples that had both glucose and HbA1c within the respective reference intervals and was 165 (90% CI 145 to 186) to 367 (90%CI 340 to 386) umol/L. This compares with the manufacturer's suggested reference interval of 100 to 295 umol/L. Using the ratio glycated albumin /albumin the reference interval was 10.5 (90%CI 9.8 to 11.4) to 18.5(90%CI 17.5 to 19.4). Linearity was established from 10 to 1150 umol/L. Comparison of Diazyme Glycated Protein results on bisalbumin samples with a glycated albumin method, Lucica GA-L, gave a correlation equation of y(Diazyme)=-0.5 (Lucica GA-L) +6.2 (n=25,) r^2=1, two tailed T test p=0.14).

Conclusion The analytical performance of the Diazyme Glycated Protein was satisfactory although the reference interval obtained was higher than that suggested by the manufacturer. Comparison against a glycated albumin method for bisalbunemia samples was good although the absolute values are different. The effect of albumin variants needs to be further evaluated.

A-184

Hypercalcemia with Normal PTH: A Diagnostic Puzzle?

O. Krasnozhen-Ratush, L. Bilello, S. Weinerman, L. K. Bjornson. *Hofstra-NSLIJ Scool of Medicine/North Shore-LIJ Health System, Manhasset, NY*

Introduction: The two major causes of hypercalcemia are primary hyperparathyroidism and malignancy, where patients generally have elevated PTH results in the former and suppressed PTH in the latter condition. However, there is a smaller group of patients who present with mild hypercalcemia and normal PTH that can be a diagnostic puzzle if clinical history and condition are unremarkable.

Objective: To determine the prevalence of hypercalcemia with normal PTH in a large endocrinology practice in an integrated health system and to determine what effect correcting (adjusting) the calcium concentration for albumin would have on the classification of hypercalcemia.

Results/Discussion: 3,958 calcium results were retrieved by a computer search of the data base from the endocrinology faculty practice at North Shore-LIJ Health System in Long Island, NY from January 2013 through January 2014, where 155 (3.9%) of these results have been classified as hypercalcemic, i.e., above 10.5 mg/dL. This study focused on calcium, PTH and albumin results without any patient history or other clinical information. Within this original hypercalcemic group a subgroup of 42 (27.1%) patients were identified with normal PTH results. For this subgroup albumin results were also retrieved and a corrected calcium concentration was calculated according to a standard textbook equation; Ca (corrected) = Ca (measured) + 0.8 x (4.0 – Albumin), where the calcium and albumin units of measure are mg/dL and g/dL, respectively. From the original subgroup of 42 patients, 18 (42.9%) were reclassified to normocalcemia based on corrected calcium results whereas 24 (57.1%) remained in the hypercalcemia classification. While the correction of calcium for albumin is not routinely performed or reported in most laboratories, its' primary application has been for patients with hypoalbuminemia and not for patients with normal or high normal albumin as in this subgroup. However, the fact that a corrected calcium concentration reclassifies nearly half of these patients as normocalcemic gives rise to questions regarding the original classification of hypercalcemia based on total calcium concentrations. While measurement of free (ionized) calcium is considered a more accurate assessment of calcium status than total calcium, most laboratories primarily perform total calcium measurements for outpatients since this assay is easily automated and provides rapid, cost effective results. Outreach physicians do not often order free calcium because it is more expensive, has special requirements for blood collection and is usually not required for diagnosing calcium abnormalities. However, corrected calcium results are an estimation and free calcium measurements should be performed if the total calcium measurement is in question.

Conclusions: We have investigated a subgroup of hypercalcemic patients that have normal PTH results and have found that nearly half (42.9%) are reclassified as normocalcemic when the total calcium concentration is corrected for albumin. Since corrected calcium concentrations are an estimation it is suggested that measurement of free calcium may provide a more accurate assessment of calcium status for some patients in this subgroup.

A-185

Validation of a productive platform for HbA1c testing

N. Z. Maluf, A. L. N. Camilo, C. Rosin, C. F. d. Pereira, O. V. P. Denardin. *DASA, Barueri, Brazil*

Background: The current guidelines for diabetes have recommended the use of HbA1c testing for diagnosis as well for monitoring of diabetes mellitus type 2. In HbA1c testing has the important role of diagnosis, there is the need for accurate and precise methods for quantification as recommended by the National Glycated Hemoglobin Standardization Program (NGSP). The purpose of exceeding the expectations of customers makes the labs have to consolidate their growth strategies and strengthen its activities in order to deliver results quickly, with quality and updated methodologies. Large routines demand faster methods which are efficient and have unquestionable quality results. DASA's routine faces an average of 917,000 tests per month where we need to ensure that the time spent for tests processing do not generate a negative impact on laboratory routine, while guaranteeing that the integrity of the samples and the results of high quality promote the positive impacts within established time to deliver results. This study aims to validate a platform which improves productivity, reduces the time for analysis of samples and the number of installed equipments, enables the allocation of resources and maintains acceptable correlation with the methodology currently used for HbA1c testing.

Methods: HbA1c for Siemens ADVIA 2400 Chemistry ® is a turbidimetric assay with range from 2.9% to 15.4%. To assess the assay's correlation, we analyzed 100 whole blood samples collected in EDTA K2 tubes. The samples were divided according to the three following ranges: 4 to 6%, 6 to 14% and >15%. The results were compared with results obtained from Tosoh G7 ® platform. To compare the productivity, time to analyze the samples and TAT (Turn Around Time) were used 5 days of LIS data for routine performed at Tosoh G7 ® and a second period held on ADVIA 2400 Chemistry®.

Results: The comparative analysis of the results revealed a correlation coefficient (r) of 0.98 for HbA1c in ADVIA ® 2400 Chemistry and a linear regression equation y = 0.9808 x + 0.2838 and R2 = 0.9579. When we verified LIS data analysis, the total processing time was 10.6 hours for a routine of 7,422 samples on five platforms Tosoh G7 ® and 4 hours for a routine of 8,550 samples on one platform ADVIA 2400 Chemistry ®. The TAT for ADVIA Chemistry 2400 ® was 06 hours and 33 minutes and the Tosoh G7 ® was 20 hours and 32 minutes.

Conclusion: In the present study, Siemens HbA1c assay for the ADVIA Chemistry System has a proper correlation with the results of the Tosoh G7 ®. Thus, it ensures that the migration of this assay to tested platform causes no significant difference in results and clinical conduct. Furthermore, ADVIA Chemistry ® 2400 presented higher throughput and a 68% reduction of TAT compared to the Tosoh G7 ®.

A-186

The correlation of Fasting and Post Prandial Plasma Glucose with Estimated Average Glucose Levels

S. Ozdemir, N. Ozcan, M. Yavuz Taslipinar, F. Ucar, Z. Ginis, A. Guneyk, A. E. Arzuhal, E. Bulut, G. Erden. *Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey*

Background: Association of fasting plasma glucose (FPG) and post prandial plasma glucose (PPG) on hemoglobin glycation is still controversial. Estimated average glucose (eAG) is a value calculated by hemoglobin A1c (HbA1c) that represents the integrated values for glucose over the preceding 8-12 weeks. The 'A1C-Derived Average Glucose (ADAG)' study showed a linear relationship between HbA1c and

eAG in both diabetic and non-diabetic patient populations. The aim of the present study was to investigate the correlation of fasting and post prandial plasma glucose with estimated average glucose levels.

Methods: This retrospective study includes 7887 subjects with HbA1c data(within a 4 months period). Three subgroups were created: group 1 with FPG (n=4596; 2218 diabetic, 2478 non-diabetic), group 2 with PPG (n=2775, 1683 diabetic, 1092 non-diabetic), and group 3 with FPG and PPG (n=516; 280 diabetic, 236 non-diabetic). The equation published by the ADAG study was used to obtain eAG with the following formula: eAG mg/dL = 28.7x HbA1c(NGSP,%)-46.7 [eAGmmol/L=1.59x HbA1c(NGSP,%)-2.59]. HbA1c values were measured by boronate affinity chromatography methods (Trinity Biotech, Premier Hb9210, Ireland). Glucose was measured by glucose oxidase method (ADVIA® 2400 Chemistry System, Siemens Healthcare Diagnostics Inc.,Tarrytown USA). The correlation coefficients and their significance were calculated using the Pearson test.

Results: In all subjects for group 1 and 2, FPG and PPG showed a strong positive correlation with eAG (r=0.817, p<0.01 and r=0.853, p<0.01 respectively). There was a positive correlation between FPG and eAG in group 1(diabetic subgroup r=0.643; p< 0.01 and nondiabetic subgroup r=0.397; p<0.01). A positive correlation was observed between postprandial glucose and eAG in group 2 (diabetic subgroup r=0.762; p< 0.01 and nondiabetic subgroup r=0.357; p<0.01). It was also found out that eAG had positive correlations with FPG and postprandial glucose in group 3 (diabetic subgroup r=0.725; p< 0.01 and nondiabetic subgroup r=0.581; p<0.01; diabetic subgroup r=0.632; p< 0.01 and nondiabetic subgroup r= 0.255; p<0.01 respectively).

Conclusion: eAG values obtained from HbA1c were highly correlated with FPG and PPG values. Thus, we may suggest that blood glucose expressed as eAG improves the understanding of blood glucose monitoring.

A-187

Development of quantitative Estradiol assay for fully automated analyzer LUMIPULS™ G1200

Y. Kitamura, Y. Ishii, H. Murakami, S. Kojima, M. Tomita, S. Sato, K. Aoyagi. *Fujirebio.Inc, Tokyo, Japan*

Background: Estradiol (E2) is one of a female steroid hormone which is produced in ovarian tissue. Mainly it is used for monitoring of ovarian hypo-function or infertility treatment. In this time, we developed new reagent (Lumipulse G E2-III) which has excellent correlation with ID-GC/MS and reference materials (IRMM) and improved cross reactivity to some drugs or E2 derivative. Lumipulse G E2-III is one-step immunoassay, and E2 in specimen samples and ALP-labeled E2 competitively react with anti E2 monoclonal antibody coated on the micro particles. It is finally detected based on CLEIA technology. Here we show the excellent fundamental performance with fully automated chemiluminescence analyzer LUMIPULSE G1200.

Methods: The sample types used for this study were serum or Heparin-Li. Correlation with ID-GC/MS, commercial competitive kit, matched pair correlation between serum and plasma, cross-reactivity to drugs, within-run precision, limit of detection (LoD) and limit of quantification (LoQ) were evaluated following recommendation from CLSI documents EP-5, EP-7, EP-12, EP-14 and EP-17. All evaluations were executed with LUMIPULSE G1200 (FUJIREBIO INC.).

Results: Correlation with ID-GC/MS with 25 specimen samples was excellent (slope: 1.04, regression: 1.00) and the measurement value in Lumipulse G E2-III calibrators was traceable to BCR577 reference materials. The significant correlation with the commercial available kit with 79 specimen samples was observed (Cobas, slope: 0.93, regression: 1.00, Centaur, slope: 1.06, regression: 1.00). Correlation between serum and heparin-Li with 56 matched pair samples was excellent (slope: 1.03, regression: 1.00). Within-run precision % CVs for our assay ranged from 1 to 3% when 3 different conc. of samples were tested, the calculated LoD is at 15 pg/mL and the LoQ ranged from 13 pg/mL to 17 pg/mL. As a result of evaluation with total 43 kinds of drugs and E2 derivatives, cross reactivity with almost all cross reactants were < 0.1 %.

Conclusion: These results demonstrated that improved Lumipulse G E2-III is a precise and highly useful for measuring E2 in serum and heparin-Li. Also this assay is perfectly traceable to ID-GC/MS and reference materials.

A-188

Plasma total homocysteine, high - sensitivity C- reactive protein and thyroid function in metabolic syndrome patients in Port Harcourt, Nigeria.

C. G. Orluwene[1], M. O. Mommoh[2]. *[1]University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria, [2]NNPC Zonal Industrial Hospital, Port Harcourt, Nigeria*

Aims and Objectives: Metabolic syndrome is assuming epidemic proportions in most populations of the world and so are its co-morbidities. Metabolic syndrome, hypothyroidism, total plasma homocysteine and C-reactive protein are independent risk factors for cardiovascular disease. There could be an association between these risk factors. The aim of our study was to examine the levels of thyroid hormones, total plasma homocysteine and C-reactive protein in metabolic syndrome patients and assess the possibility of an association between these four risk factors for cardiovascular disease.

Patients and Methods: A total of 93 subjects were recruited for this study. (48 with metabolic syndrome and 45 as the control group). Basic demographic data, components of the metabolic syndrome, thyroid hormones, high-sensitivity C-reactive protein and total plasma homocysteine were estimated for all subjects using standards methods. Appropriate statistic was used for data analysis.

Results: Components of the metabolic syndrome, thyrotropin, total plasma homocysteine and high sensitivity C-reactive protein were significantly higher in the study group (P<0.05) while free thyroxine and high density lipoprotein cholesterol were significantly lower in the study group (P<0.05). The predominant type of thyroid dysfunction in the study group was sub-clinical hypothyroidism (87.5% in the study group compared to 18% in the control; P<0.05). Logistic regression showed significant association between sub-clinical hypothyroidism, total plasma homocysteine and high-sensitivity C-reactive protein in the study group.

Conclusion: There was a strong association between metabolic syndrome, sub-clinical hypopthyroidism, total plasma homocysteine and high-sensitivity C-reactive protein. Females are at an increased risk of this association.

Keywords: Metabolic syndrome, Thyrotropin, Sub-clinical hypothyroidism, total plasma homocysteine, high-sensitivity C-reactive protein.

A-189

An Evaluation of Three Novel Biomarkers: Total-sLHCGR, LH-sLHCGR and hCG-sLHCGR.

M. Jansen[1], C. F. Rosmalen[1], S. Banerjee[2], A. E. Chambers[2]. *[1]NBCL B.V., Nijmegen, Netherlands, [2]Origin Biomarkers, Lanarkshire, United Kingdom*

Background: Researchers in the field of human reproduction need better research tools for the care of pregnant woman (e.g., prenatal diagnosis) and women who wish to become pregnant (e.g., fertility treatment). The assays for the soluble receptor for both human chorionic gonadotropin (hCG) and human luteinizing hormone (hLH) and the same soluble receptor bound to either hCG or hLH could be used as such research tools. The receptor is called the soluble LH/hCG receptor or sLHCGR. Three different ELISA assays have been developed and based on the type of HRP-labeled detector antibody employed, the user can quantitate Total sLHCGR, a complex of LH with sLHCGR or a complex of hCG with sLHCGR in a standard microtiter plate format. **Methods:** The levels of Total-sLHCGR, LH-sLHCGR or hCG-sLHCGR were measured using sandwich ELISA. The three ELISA formats are the same; samples were diluted 5-fold, incubated for 10 minutes in a microtiter plate coated with a monoclonal capture antibody directed against the receptor. Next the HRP-labeled conjugate was added and incubated for 1.5 hour to generate the sandwich. After a wash step the substrate was added and after 20 minutes the reaction was stopped and read at 450 nm using a microplate reader. The optical densities from the reader are directly proportional to the amount of Total-sLHCGR, hCG-sLHCGR or LH-sLHCGR present in the sample. **Results:** The analytical sensitivity for the assays are 0.01, 0.004, and 0.02 Pmol/mL for the Total-sLHCGR, hCG-sLHCGR and LH-sLHCGR, respectively. The within assay precision was done at three different levels for the three assays (n=16). For hCG-sLHCGR the CV% are 4.4, 3.5, and 3.5% at 2.1, 11.7 and 18.0 Pmol/mL, respectively. For LH-sLHCGR the CV% are 3.2, 3.7, and 3.5% at 2.1, 11.2 and 28.9 Pmol/mL, respectively. For Total-sLHCGR the CV% are 7.4, 6.5, and 9.0 % at 3.5, 4.3 and 11.5 Pmol/mL, respectively. Inter-assay precision was determined for the three assays (n=4) and were for the hCG-sLHCGR 2.7, 7.7 and 4.2% at 2.2, 12.7 and 19.1 Pmol/mL, respectively. For LH-sLHCGR the CV% were 3.5, 3.6, and 2.9% at 2.1, 11.6 and 30.3 Pmol/mL, respectively. For Total-sLHCGR the CV% were 8.9, 8.6, and 11.0% at 3.7, 5.2, and 11.8 Pmol/mL, respectively. Sample recoveries for the three assays are between 80.6 and 101.7% **Conclusion:** Three

simple, fast ELISA assays for the detection of Total-sLHCGR, hCG-sLHCGR and LH-sLHCGR in serum or plasma were evaluated and were shown to be precise and sensitive for the detection of these novel biomarkers.

A-190

Iatrogenic Vitamin D Toxicity in an Infant: Clinical Relevance of Vitamin D Metabolic Profiling

H. Ketha, H. Wadams, M. Fahse, A. Lteif, S. Grebe, R. J. Singh. *Mayo Clinic, Rochester, MN*

Background Public concern over vitamin D (vitD) deficiency has led to widespread use of over the counter (OTC) vitD supplements, containing up to 10,000 IU (400IU=10µg). Overzealous use of such supplements can result in vitD toxicity. Infants are particularly vulnerable to toxicity associated with vitD overdose. Mutations in the CYP24A1 gene have been shown to cause reduced serum 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) to 25-hydroxyvitamin D 25(OH)D ratio (<0.02), elevated serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), hypercalcemia, hypercalciuria and nephrolithiasis. Additionally, studies in infants have shown that C3 epimer of 25(OH) D can contribute upto 9-61.1% of the total 25-OH)D. Therefore, measurements of parathyroid hormone (PTH) and vitD metabolites 25(OH)D, $1,25(OH)_2D$, 3-Epi-25-hydroxyvitamin D (3EPI-25(OH)D) and $24,25(OH)_2D$ are useful to diagnose and manage hypercalcemia due to vitD overdose or due to CYP24A1 mutations.

Relevance to Clinical Laboratories Measurement of vitD metabolites 25(OH)D, $1,25(OH)_2D$, 3EPI-25(OH)D and $24,25(OH)_2D$ is of clinical value for differentiating between genetic vs iatrogenic causes of hypercalcemia.

Case A significantly underweight four month old female presented with a three day history of emesis, diarrhea, lethargy and dehydration. The medical work-up revealed hypercalcemia, hypercalciuria and nephrocalcinosis. She had been exclusively breast-fed and had been given OTC vitD supplementation at a higher dose than recommended on the supplement's label. 25(OH)D, $1,25(OH)_2D$, 3EPI-25(OH)D, $24,25(OH)_2D$ and the vitD content of the OTC preparation were measured by LC-MS/MS.

Results Nephrocalcinosis was confirmed by ultrasound studies. Serum cacium (SCa) was 18.7 mg/dL (ref range: 9-11 mg/dL) and PTH was < 6pg/mL (ref range: 15-65 pg/mL) at presentation. Urine calcium was 157 mg/dL with a calcium to creatinine (Cr) ratio of 2618 mg/g of Cr (ref range <2100 mg/g). The vitD content of the supplement was threefold higher (6000 IU of D/drop) than listed on the label (2000IU). Combined with the gross overdosing, this was estimated to have resulted in a daily vitD dose of 50,000 IU for two months. The SCa gradually decreased upon calcitonin injection on day 3, but trended upward again (15.5, 13.3, 12.1, 10.4, 9.8, 10.7, 10.9, 10.3 and 11.6 mg/dL on days 1, 3, 5, 7, 11, 14, 20, 25 and 40 respectively). The 25(OH)D decreased slowly from 294 ng/mL on day 1 to 257, 227, 197, 189, 138, 124 and 84 ng/mL on days 3, 5, 7, 11, 14, 20, 25, and 40 respectively. Serum 3EPI-25(OH)D levels were 44 % to 29 % of the 25(OH)D levels(126-36 ng/mL). Serum $1,25(OH)_2D$ levels were elevated. The ratio of $24,25(OH)_2D$ to 25(OH)D was 0.11-0.14 (ref range: 0.07-0.18).

Discussion Genetic cause of hypercalcemia, hypercalciuria and nephrocalcinosis could be ruled out on the basis of normal $24,25(OH)_2D$ to 25(OH)D ratio. Clear warning labels regarding maximum allowable doses of OTC vitD supplements are of value from a public health perspective.

Conclusion Infants receiving high dose OTC vitD supplementation are vulnerable to vitD toxicity. Vitamin D metabolic profiling is of value for evaluating such cases and to exclude certain genetic causes. Our study also emphasizes the need for stricter regulation of vitD content in OTC supplements and prominent warning labels regarding maximum allowable daily doses.

A-192

Insulin resistance and secretory functions in Pre-diabetics and newly diagnosed diabetics of north-west India: role of adipocyte mediators

P. Sharma, P. Purohit. *All India Institute of Medical Sciences, Jodhpur, India*

Background: Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. There has been a dearth of studies evaluating the natural course of insulin secretion in pre-diabetics progressing to diabetes in north western India.

Aim: To examine the natural history of insulin secretory dysfunction and insulin resistance during the development of diabetes and to examine the role of adipocyte mediators non-esterified fatty acids (NEFA) in development of type 2 DM.

Material and Method: The study was conducted on newly diagnosed, untreated hyperglycaemic patients attending our diabetic clinic. The study subjects were grouped as-Group I: Healthy control (n = 56), Group II: Pre-diabetics (n=39), Group III: Diabetics (n= 124). All subjects were evaluated for waist to hip ratio (W: H), body mass index (BMI), fasting blood glucose, insulin, HOMA-IR, HOMA-beta, NEFA and lipid profile. Statistical analysis was done using ANOVA and Multiple logistic regression analysis.

Results: The diabetic subjects had significantly raised W: H and BMI as compared to the pre-diabetics (0.88±0.06; 27.44±4.62) and controls (0.87±0.06; 24.2±4.34) with the F value 14.64 ($p<0.001$) and 5.98 ($p=0.003$) respectively for W: H and BMI. Age adjusted base line characteristics according to BMI and W: H quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for total cholesterol (TCH), triglycerides (TG), HOMA-IR and HOMA-beta. Similarly Age adjusted base line characteristics according to NEFA quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for BMI, W: H, lipid profile, insulin, HOMA-IR and HOMA-beta. Finally Multiple logistic regression analysis in newly diagnosed type 2 diabetic subjects with family history (Negative v/s Positive) as a dependent variable showed the strongest risk due to raised NEFA (OR 3.83), followed by HOMA-IR (OR 1.38), TCH (OR 1.35), WC (OR 1.7) and TG (OR 1.13).

Conclusion: We conclude that the insulin secretory rates and IR in pre-diabetics and newly diagnosed type 2 diabetics are associated with BMI, W: H and NEFA. W: H and NEFA can prove to be a strong predictor of type 2 DM even with a negative family history.

A-193

Comparison of Aldosterone and Renin Determination by Conventional Radioimmunoassay and by Automated Chemiluminescente Immunoassay

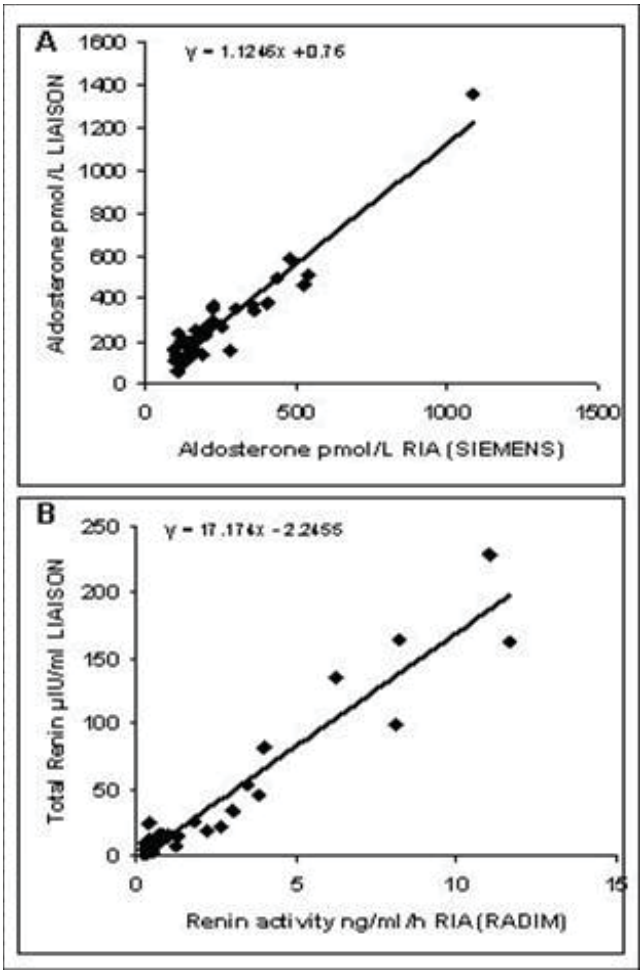
E. Zeruya, Y. Sharabi, H. Kanety, R. Hemi. *Sheba Medical Center, RAMAT-GAN, Israel*

Background: Renin-angiotensin-aldosterone system plays a paramount role in water homeostasis and electrolyte balance and in the regulation of arterial pressure; Aldosterone, major mineralcorticoid secreted by adrenal cortex, keeps water and salt balance helping in maintaining blood pressure. Renin, a proteolytic enzyme synthesized primarily by the juxtaglomerular cells of the kidneys converts angiotensinogen to angiotensin I, which is then cleaved by angiotensin converting enzyme to form angiotensin II. Angiotensin II increases blood pressure directly through vasoconstriction and indirectly by stimulating secretion of aldosterone. Measurement of plasma renin and aldosterone provides a marker of renin-angiotensin-aldosterone system activity.

Objective: To evaluate analytical performances of commercially available automated immunoassays for Aldosterone and direct renin (DiaSorin LIAISON®) and to compare LIAISON results to currently used radioimmunoassay (RIA).

Results: 43 plasma samples were measured for Renin activity (PRA) and Renin concentration (PRC) by RIA (RADIM) and LIAISON, respectively. In addition, 57 serum samples were analyzed for Aldosterone levels by RIA (SIEMENS) and LIAISON. High correlation was found between the two systems both for Aldosterone and Renin (R=0.95 and R=0.96, respectively, Figure 1 A&B). Satisfying clinical concordance of 95% and 100% was found between both LIAISON Aldosterone and Renin assays vs. RIA, respectively. Assays performance (precision and linearity) was tested and found compatible with the manufacturer's declaration.

Conclusion: LIAISON Aldosterone and Direct Renin assays are precise, accurate and reliable and can be used as an alternative to the conventional RIA which is technically demanding and laborious. Our data suggests that the LIAISON Direct Renin assay can be a good diagnostic and practice replacement for the RIA PRA assay, although biochemically, they measure different parameters. Setting normal ranges for the Renin/Aldoseterone ratio on the LIAISON is required in order to diagnose and monitor hypertensive patients.



A-196

Linearity study to define the Analytical Measurement Range (AMR) for immunoassays

A. L. Camilo, D. Waltrick, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: Linearity studies are crucial to assure analytical range accuracy and to validate reagents performance in clinical laboratory. Due to difficulties to evaluate linearity results, a Brazilian laboratory implemented a new tool (Easy Linearity Curve, ELC).ELC established self-inspection program, immune-hormone analytical systems monitoring and quality control requirements accomplishment. We described linearity data for nine different immunoassays: VB12, Folato(FOL), Ferritin(FER), BNP, Cortisol (Cor), C-Peptide(Pep C) and Insulin(Ins).

Materials and methods: Samples were selected from laboratory routine, with concentrations within assay linearity range for each test, performed by chemiluminescence on Advia Centaur® XP (Siemens Healthcare Diagnostics). Linearity was evaluated by calculating the recovery of repetition performed. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level. In addition, they were analyzed with ELC tool to validate immunoassay's AMR according to CLSI Guideline EP6-A.

Results and Discussion: Tests showed satisfactory linearity results for different samples. They are summarized in the table below.The sample pools were not used, because all the results from this dilution test presented high percentage of recovery, higher than manufacturer's references, due to a matrix effect (data not showed).The main difficulty in linearity studies is to obtain samples in the ideal analytical range. ELC uses an algorithm that suggests samples' dilutions to contemplate the whole AMR for each parameter. In this study, for each analyte, five different samples were selected to simulate ideal concentrations covering the linear range.

Conclusion: Based on our results, we do not recommend the use of sample pools in linearity studies of hormones.The presented protocol permits laboratory autonomy to AMR self-management, solving linearity studies issues, ensuring additional safety and reliability of released results, and Laboratory's Quality Control Improvement.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
VB12	45 - 2000 ng/mL	4.13	7.50	14.35	30.00	0.958x + (50.874)	0.99759
FOL	0.35 - 24 ng/mL	2.43	12.00	23.43	39.00	0.854x + (1.557)	0.96698
FER	0.5 - 1650 ng/mL	5.92	7.10	5.38	16.90	1.109x + (-32.411)	0.99532
BNP	2 - 5000 pg/mL	2.63	2.67	9.59	12.43	1.038x + (-76.888)	0.99672
Cor	0.2 - 75 ug/dL	3.97	10.50	13.76	29.80	1.057x + (-1.134)	0.99941
Pep C	0.05 - 30 ng/dL	5.14	8.30	12.70	20.80	0.985x + (-0.173)	0.99360
Ins	0.5 - 300 mU/L	4.19	10.60	12.74	32.90	0.993x + (4.202)	0.98842

A-197

Analytical performance of the Insulin-like growth factor I (IGF-I) assayin two Immunoassays Systems that used different standardization procedures

M. L. Moreira, M. D. C. Freire. *Diagnósticos da América, Duque de Caxias, Brazil*

Background: Insulin-like growth factor I (IGF-I) is intended for use in the diagnosis and monitoring of children who have growth-related disorders and adults with acromegaly.Large variability exists among different IGF-I assays owing to differences in calibration, antibody specificity, isoform recognition. The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) established criteria for standardization and evaluation of IGF-1 assays(Recombinant IGF-1, coded 02/254) and for the content monitoring of therapeutic products.

Objective:This study intends to evaluate the analytical performance of the IGF-I assay on Siemens IMMULITE 2000 IGF-I (Recombinant IGF-1, coded 87/518) and Liaison Analyser IGF-I Diasorin (Recombinant IGF-1, coded 02/254).

Methods:We tested59 patient's serum samples from DASA, Rio de Janeiro, with concentrations within the range of 25 ng/mL to 1039 ng/mL, in both analytical platforms.The IMMULITE 2000 IGF-I is a 2-cycle, sequential immunometric assay withcalibration range of 20-1600 ng/mL.In the assay procedure, prediluted patient sample is needed to reduce interference from binding proteins before analysis. The photon output is proportional to theconcentration of the analyte.The Liaison Analyser IGF-I is a 1-cycle, immunometric assay with calibration range of IGF-I 10-1500 ng/mL.

Results:Comparison between IMMULITE 2000 and Liaison assaysyielded a correlation coefficient of 0.97, with linear regression of x(IMMULITE 2000)=0.792(Liaison) + 30.48ng/mL. Moreover, means were 313 and 278 ng/mLfor IMMULITE 2000 and Liaison Analyser, respectively. Test t-student was 1.049 (expected is 1.98) and estimated total error (TE) was 20.2% which is lower than the allowed TE (22.35%) in all levels of decision-making practice.

Conclusion:Preliminary assessment of these clinical evaluation results indicates that the IMMULITE 2000 IGF-I immunoassay is a precise method for measuring IGF-I in serum across a wide range of clinically relevant concentrations and shows good correlation to the Liaison IGF-I assaydespiteof using different standardization.

A-198

Diiodothyropropionic acid interferes with TT3 and FT3 measurements on common Immunoassay Platforms for Thyroid Function Panel.

X. Yi, S. Refetoff, E. K. Y. Leung, K. T. J. Yeo. *The University of Chicago, Chicago, IL*

Background: Monocarboxylate transporter 8 (MCT8) is a thyroid hormone-specific cell membrane transporter. MCT8 deficiency produces in young males an unusual pattern of thyroid hormone abnormality with elevated serum triiodothyronine (TT3) and causes severe neuropsychiatric defect. A thyroid hormone analogue, diiodothyropropionic acid (DITPA) was found to enter cells independently of MCT8. Therefore, this compound was tested in children with MCT8 deficiency and found to normalize their thyroid function tests and improve their nutritional status. A problem in the follow up of DITPA treatment is the interference of DITPA in the routine laboratory measurement of TT3. This necessitates the measurement of DITPA by LC-MS/MS to correct for its interference in TT3 determination by immunoassay. The objective of this study was to evaluate the possible interference of DITPA on commercial thyroid assays available from four in-vitro diagnostic companies. **Method**: Pooled human serum was collected and stock DITPA (1mg/ml) was added to create a serum set containing 3 -75 µg/dL DITPA. This sample set was then assayed for TT3, TT4, free T3, free T4, and TSH on the Roche Elecsys, Siemens IMMULITE, Siemens ADVIA Centaur, Siemens Dimension EXL, Siemens Dimension RXL,

Beckman Access, and Abbott Architect platforms, where available, respectively. All samples were analyzed in duplicate and the respective assay values in the DITPA-treated samples were expressed as the difference above the baseline sample. In addition, to investigate if the interference of DITPA could be overcome by excess TT3, serum samples at a constant concentration of 20 µg/dL DITPA were spiked with increasing amount of TT3 and measured on Roche Elecsys TT3 method. **Results**: At 75 µg/dL DITPA, the overestimation above the baseline endogenous values were: Elecsys: TT3, 193 ng/dL, FT3, >3000 pg/dL; Access: TT3, 745 ng/dL, FT3, 2405 pg/dL; IMMULITE: TT3, 146 ng/dL; Architect: TT3, 33 ng/dL, FT3, 96 pg/dL, respectively. Minimal interference was observed for ADVIA Centaur TT3 assay at all concentration of DITPA tested. TT4, FT4, and TSH tests were not affected by DITPA on all the immunoassay platforms tested. It is also interesting that with excess TT3, the DITPA interference on the Elecsys TT3 declined from 208% to 114% above baseline. **Conclusion:** DITPA significantly interferes with several commercial TT3 and FT3 assays with the exception of the Siemens ADVIA Centaur TT3 assay. This suggests that the TT3 antibody reagents used in the Elecsys, Access, Immulite and Architect cross-react significantly with DITPA. For patients undergoing treatment, the ADVIA Centaur TT3 assay is suitable for monitoring response to DITPA therapy.

A-199

Gender Differences in the interactions between Adipokines and the Insulin-Like Growth Factor-I System in a Metabolically High Risk Population

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

Background: Accumulating evidence indicate important roles for the insulin-like growth factor (IGF)/IGF-binding protein (IGFBP) system in metabolic homeostasis. Despite potential molecular mechanisms that link obesity and insulin resistance with the IGF system, the pathophysiological metabolic interactions between adipose tissue derived adipokines and the IGF-I system remain unknown due to conflicting reports in the literature. In this study, we test the hypothesis that gender differences could be responsible in part for the conflicting reports on the associations of some adipokines with the IGF system.

Methods: Fasting adiponectin, resistin, leptin, leptin receptor (sOB-R), insulin, glucose, total IGF-I, IGFBP-3 and full lipid profile were determined in 590 (238M and 352F) first-degree relatives of patients with Type 2 Diabetes Mellitus. Sex hormone binding globulin (SHBG), oestradiol (E2), testosterone (T), were also measured. Free androgen index (FAI), Free leptin index (FLI), bioavailable IGF-1 (BIGF1), beta-cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) (Homeostasis Model Assessment) were calculated. The data were analysed using simple and multivariate regression analyses.

Results: There are significant differences in mean (SEM) BIGF1 between males (87.6 (9.1)) and females (67.7 (4.6)). There were also significant gender differences in adiponectin, leptin, sOB-R, FLI, %S and IR. There were no gender differences in resistin and IGFBP3. Significant gender differences were found in the correlations of BIGF1. The following showed significant correlations with BIGF1 in females but not in males: adiponectin, sOB-R, FLI, SHBG, glucose, insulin, %S, IR, waist circumference, BMI, Apo B, total cholesterol, triglycerides and LDL-cholesterol. Males and females showed similar correlations of all other variables with BIGF1. Correlations with sex hormones (E2, T, SHBG, FAI) were not significant in males and females. Multivariate linear regression analysis showed that age, BMI, WC, adiponectin, FLI were significant determinants of BIGF1 in females but not in males. Age was the only significant determinant of BIGF1 in males.

Conclusions: There are significant gender differences in the metabolic interaction between adipokines and the IGF-1 system. Despite the putative links with obesity, sex steroids do not play a role in the gender differences.

A-200

LC-MS/MS detection of increased Androstenedione levels in patients receiving Danazol therapy

V. Gounden[1], D. El-Maouche[2], B. R. Stolze[1], R. Muniyappa[3], S. J. Soldin[4]. *[1]National Institutes of Health (Clinical Center), Bethesda, MD, [2]National Institutes of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, [4]National Institutes of Health (Clinical Center) andDepartment of Medicine, Georgetown University, Washington, District of Columbia, Bethesda, MD*

Background : Danazol is a 17-ethinyl testosterone derivative. Danazol has long been used in the management of endometriosis, however its reported immunomodulatory effects such as reducing interleukin-1 and TNF-α have led to the use of danazol in management of immune related conditions such as aplastic anemia. The side effects associated with danazol are largely due to its androgenic effects. Danazol has been reported to act as an interference in the immunoassay measurement of various androgens (including androstenedione), resulting in falsely elevated values for these hormones. As a consequence measurement of these hormones in patients receiving danazol is best performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Here we report eight cases of significantly elevated androstendione (AND) levels following LC-MS/MS measurement in patients receiving danazaol for aplastic anemia.

Method: LC-MS/MS measurement: AND is measured as part of a steroid hormone panel. Samples were prepared as per previously published method (Guo T et al. Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-photospray ionization tandem mass spectrometry. Clin Chim Acta 2006;372:76-82 and Mendu DR et al Clin Chem 2011 Abstract E-57, pA212). An Agilent 6460 triple quadropole mass spectrometer (Agilent, USA) equipped with an atmospheric pressure photoionization was used, employing isotope dilution with deuterium labeled internal standard for each analyte. Quantitation by multiple reaction monitoring (MRM) was performed in the positive ion mode. The quantifier MRM transition for androstenedione used was 287.2> 97.1 and the qualifier MRM of 287.2 >109 was used to confirm. The ratio of response of the two MRMs used, ranged from 56-58%. Retention times (RT) for AND were 6.540 -6.556 minutes for patient samples and 6.529- 6.554 minutes for the accompanying internal standard (IS). The ratio of androstenedione RT to internal standard RT for each specimen run was 1.00, thus confirming the identity of the peak.

Results: A total of 8 adult patients (female n=5; male n=3) were identified with increased AND values at either 6 or 12 month follow- up post danazol initiation. Baseline AND values for the female patients ranged from 38 - 176 ng/dL (reference interval 17- 175) and at 6 or 12 month follow up values increased markedly and ranged from 8128-33703 ng/dL. For the male patients baseline AND values ranged from 105- 240 ng/dL (reference interval 25-125) and at follow up values increased to range from 5609 to 17325 ng/dL. Similar increases were not observed for the other androgens measured. Of interest two of the three males had elevated LC-MS/MS estradiol levels above the reference interval on follow-up while all the female patients had estradiol concentrations that remained within the appropriate estradiol reference interval during therapy. Whilst on therapy patients responded well and side effects of therapy were reported to be minimal.

Conclusion: One of the important advantages of MS analysis is the greater specificity of over immunoassay based testing In the above described cases use of two MRM transitions (quantifier and qualifier) enabled the laboratory to confirm the presence of elevated AND and exclude the likelihood of an interference.

A-201

Evaluation of the effect of elevated Fetal Hemoglobin (HbF) on three HbA1c Assays Methods in Marshfield Clinic system.

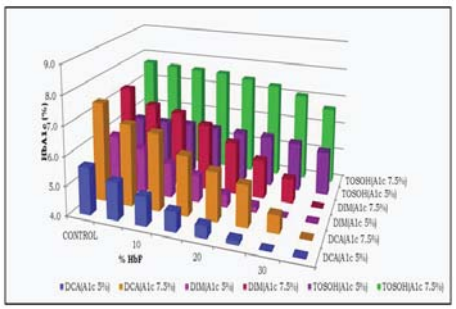
A. KHAJURIA, B. Scheibe. *Marshfield Clinic, Marshfield, WI*

Background: Accurate measurement of HbA1c is crucial for decision making in diabetic control and diagnosis. Elevated levels of HbF are reported to falsely decrease the HbA1c results. There are many clinical conditions presenting with elevated HbF and prevalence of elevated HbF can be as high as 7 to 8% in a diabetic population and Clinicians may be unaware of potential interference with HbA1c results. At a glycemic control target of 6.5% the critical difference between two results within a subject should not exceed ~0.4%. It is therefore, crucial that laboratories are aware, to what extent HbF interference affects HbA1c results.

Methodology: Following commercial assays were evaluated; TOSOHG8 (HPLC), Dimension EXL 200 & DCA 2000 (Immunoassay). Two whole blood EDTA patient pools as Normal (5 to 6%) and Abnormal (7 to 8%) were prepared and incubated with varying concentration of HbF (5 to 40%) by mixing umbilical cord blood with known HbF levels (estimated by G8). The effect of HbF interference was then evaluated relative to control pools. Percent decrease in HbA1c greater than 5% was considered a significant change.

Results: TOSOH G8 did not show any interference with up to 25% HbF concentration. Dimension & DCA 2000, however exhibited a dose dependent interference with HbF (>5%) at 5% HbF concentration.

Conclusions: Elevated HbF can be identified in Ion-exchange HPLC but not in Immunoassays. Laboratory professionals should make clinicians aware of potential interference from elevated HbF levels on a particular method that could adversely affect HbA1c results. Clinicians can then make informed decisions if HbA1c results appear discrepant related to patient history and glucose hemostasis.



A-202

VALIDITY OF A SEMI-QUANTITATIVE METHOD FOR MICROALBUMINURIA SCREENING IN DIABETES MELLITUS

J. D. Santotoribio, C. Cañavate-Solano, A. García de la Torre, F. Arce-Matute, S. Pérez-Ramos. *Puerto Real University Hospital, Cádiz, Spain*

INTRODUCTION Urine Albumin is an early marker of chronic kidney disease (CKD) in diabetic patients. The excretion of recent urine albumin greater than 20 mg/L (microalbuminuria) is considered as a predictor of diabetic CKD. The aim of this study is to analyse the validity of a semi-quantitative method for microalbuminuria screening in diabetes mellitus.

METHODS Recent urine microalbuminuria of diabetic patients were determined by two methods: 1. Semi-quantitative: Colorimetric method using the strip H13 in DIRUY H-800 PLUS (RAL®). The content of microalbuminuria is inversely proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000.

2. Quantitative: microalbuminuria was measured by immunoturbidity in COBAS C311 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the quantification of microalbuminuria: positive (microalbuminuria > 20 mg/L) and negative (microalbuminuria < 20 mg/L). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

RESULTS We analyzed 469 diabetic patients between 27 and 85 y.o. (mean age = 56.3), 82 patients (17.5%) had a positive microalbuminuria and 387 patients (82.5%) were negative. The AUC was 0.985 ($p < 0.0001$). With a cut-off color scale less than 1305 determined by the test strip, we obtained a sensitivity of 100% and a specificity of 86.3%. With these results, it would only be necessary the quantification by immunoturbidimetry the samples with a value lower than 1305. In this case it would not have been necessary to measure microalbuminuria in 334 samples of the 469 studied, getting a saving of 71%.

CONCLUSIONS The semi-quantitative method by test strip, can be used as screening for microalbuminuria in diabetic patients with a sensitivity of 100%. Microalbuminuria would only be measured in samples with positive test strip.

A-203

Procollagen of type-1 N-terminal propeptide levels by Elecsys assay correlates with bone formation rate in Chronic Kidney Disease

S. Cremers, D. Dempster, H. Zhou, E. Dworakowski, M. Kamanda-Kosseh, T. Nickolas. *Columbia University Medical Center, New York City, NY*

Background: Renal osteodystrophy is a common metabolic bone disorder due to chronic kidney disease (CKD) that is associated with high risk of bone loss, fracture and death. It is characterized principally by a spectrum of abnormal bone turnover ranging from extremely low (adynamic bone disease) to high (osteitis fibrosa cystica). Bone turnover assessment is an absolute required for treatment. However, bone turnover is currently determined by tetracycline double-labeled transiliac crest bone biopsy with histomorphometry, an invasive, and not widely available procedure. Non-invasive turnover determination in CKD is controversial because most bone turnover markers (BTMs) are renally cleared; thus, in CKD they may be non-specifically elevated and not reflect accurately remodeling activity. Procollagen of type-1 N-terminal propeptide (P1NP) is a biochemical marker of bone formation, it circulates in mono- and multi-meric forms and multimeric P1NP is cleared non-renally. Our group reported that higher circulating total levels of P1NP predicted incident bone loss among pre-dialysis and dialysis-dependent CKD patients. We hypothesized that in CKD high levels of total serum P1NP would correlate with high bone formation rate (BFR) measured by biopsy.

Methods: In 22 patients (male=8; female=14; mean age±SD 68±11 years,) with CKD stages 2-5D, fasting morning blood was collected within 6-months of double tetracycline double-labeled transiliac bone biopsy. Estimated glomerular filtration rate (eGFR) was by MDRD formula. Total serum PINP (mono- and multi-meric) was measured by Electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics, Indianapolis, IN). Intra- and inter-assay precision were 1.1% and 5.5% respectively (Reference range = 20-100 ug/L). BFR (µm3/ µm2/day) was determined histomorphometrically in trabecular, endocortical and intra-cortical bone from biopsy using American Society for Bone and Mineral Research (ASBMR) criteria. Data are presented as mean±SD. P1NP and BFR were log transformed prior to analyses and relationships were determined by Pearson correlations.

Results: Five patients were on hemodialysis and mean eGFR in pre-dialysis patients was 36±17 mL/min. Mean BFR at trabecular, endocortical and intracortical regions were 0.018±0.031, 0.019±0.035 and 0.030±0.038 respectively and there were no significant differences in BFR between pre-dialysis and hemodialysis patients. Mean PINP for the total, pre-dialysis and hemodialysis cohorts were 332±585ug/L, 98±51ug/L and 1125±878ug/L respectively. P1NP levels were significantly greater in hemodialysis compared to pre-dialysis ($p=0.004$) and there were no significant relationships between P1NP and eGFR among pre-dialysis patients. There were significant, moderate and direct associations between PINP and BFR in the three envelopes (R2 0.41, 0.34 and 0.34, all $p<0.05$ for trabecular, endocortical and intracortical bone, respectively).

Conclusion: These data suggest that measurement of total serum PINP by the Elecsys assay correlates well with BFR in CKD. Larger studies are needed in CKD populations to validate these data, and to determine whether P1NP predicts future fracture and can be used to guide treatment to protect against bone loss and fracture.

A-204

The contribution of angiotensin II-dependent oxidative stress to megalin expression in the renal cortex during the normoalbuminuric stage of diabetes mellitus in the rat.

Y. Kurosaki[1], Y. Yamada[2], P. K. Carmines[3], T. Tsukushi[4], A. Imoto[1], T. Ichikawa[2], M. Yokoba[1], S. Obata[4], H. Ikenaga[5], Y. Aoki[6], T. Takenaka[7], T. Ichikawa[1], M. Katagiri[1], N. Ishii[1]. *[1]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [2]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [3]University of Nebraska College of Medicine, Omaha, NE, [4]Kitasato University Hospital, Sagamihara, Japan, [5]Ikenaga Clinic, Ohtawara, Japan, [6]Kanagawa Health Service Association, Yokohama, Japan, [7]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: Renal albuminuria can result from impaired albumin handling by the glomerulus or the renal tubules. Megalin plays a critical role in proximal tubular albumin reabsorption, and altered megalin expression or function can contribute to renal tubular albuminuria. Elevated glucose reabsorption during acute hyperglycemia results in impaired tubular handling of protein and proximal tubular damage (*Enzyme Protein* 8:243-50, 1994-1995). Moreover, renal albuminuria during diabetes mellitus (DM) is associated with decreased megalin expression in the proximal tubule (*Diabetes* 56:380-388, 2007). During the early stage of diabetes mellitus (DM) in rats, prior to development of albuminuria, the renal cortex exhibits oxidative stress that can be suppressed by renin-angiotensin system (RAS) inhibition (*Clin Sci* 24:543-52, 2013); however, it is not known whether these events arising during the normoalbumuric stage of DM influence megalin expression and protein excretion.

Objective: The goal of this study was to evaluate impact of oxidative stress suppression, achieved by angiotensin II receptor blocker (ARB) treatment, on proximal tubular megalin expression during the normoalbuminuric stage of DM in rats.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg,*i.p.*), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ+TLM group (n=4): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure, glomerular filtration rate (GFR) were measured, as well as urinary albumin levels and activity of N-acetyl-β-D-glucosaminidase (NAG; a proximal tubule-derived enzyme) in urine. Further, we measured renal cortical 3-nitrotyrosine (3-NT) production (oxidative stress marker) by HPLC and megalin expression (Western blot analysis).

Results: Blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups ($P<0.05$), confirming development of DM; however, blood pressure and urine albumin level did not differ among groups. GFR and urinary NAG activity (an index of proximal tubule damage) were increased in the STZ group compared with Sham (each $P<0.05$), and these changes were prevented by TLM-treatment (each $P<0.05$ STZ vs. STZ+TLM). Renal cortical 3-NT production in the STZ group was 70% greater than in the Sham group (Sham, 34.0±3.0 pmol/mg protein; STZ, 58.4±2.1 pmol/mg protein; $P<0.05$ vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 35.5±3.5 pmol/mg protein; $P<0.05$ vs. STZ). Renal cortical megalin expression was elevated in the STZ group (303±89% of Sham; $P<0.05$); however, the enhanced expression of megalin in the STZ group was not evident in the STZ+TLM group (149±61% of Sham; $P<0.05$ STZ vs. STZ+TLM).

Conclusions: These observations demonstrate that increased renal cortical megalin expression accompanies oxidative stress during the early stage of DM, prior to development of albuminuria. The ability of ARB treatment to prevent the DM-induced elevation of megalin implicates the renin-angiotensin system in this phenomenon, perhaps through an oxidative stress-dependent mechanism.

A-205

Analytical validation of the new Roche Thyroglobulin II eletrochemiluminescent immunoassay.

M. C. Batista, P. R. S. Ferreira, C. E. S. Ferreira, A. C. L. Faulhaber, C. L. P. Mangueira. *Albert Einstein Hospital, São Paulo - SP, Brazil*

Background: Thyroglobulin (Tg) is a serum marker of thyroid cancer. It is thus critical to measure it accurately in the low range of 0.1 to 1.0 ng/mL level. Unfortunately, this is not possible with most commercial kits, including the traditional Roche eletrochemiluminescent Tg assay. One exception is the Beckman-Coulter Tg chemiluminescent assay (Brea, USA), which has a limit of quantitation (LOQ) of 0.1 ng/mL and, for this reason, is considered by many the reference method. In this study, we evaluated the analytical specifications of the new Roche Tg II eletrochemiluminescent immunoassay (Mannheim, Germany).

.Methods: All serum samples selected for this protocol were routine clinical specimens previously assayed for Tg in our lab using the Beckman-Coulter Access II® assay. They were all rerun within one week in Roche E170 Modular Analytics® using the new Tg II assay. This is an eletrochemiluminescent immunometric assay using 2 monoclonal antibodies to form a sandwich complex with Tg. Three samples with low, normal or high Tg levels were run in Modular E170, all in duplicate in the morning and afternoon for 5 days, and then used to calculate intra and interassay variation and LOQ (lowest concentration with CV ≤ 20%). Linearity and analytical measuring range (AMR) were evaluated in Modular E170 by mixing a low and high Tg sample in different proportions. Correlation studies were performed in 46 samples assayed in both Access II and Modular E170. Results were analyzed with EP Evaluator 11.0 software (Data Innovations, South Burlington, USA).

Results: Intra and interassay CV were 2.3% and 5.1% at 0.28 ng/mL; 1.5% and 2.7% at 2.2 ng/mL; and 0.7% and 2.3% at 45.9 ng/mL, respectively. LOD was set at ≤ 0.2 ng/mL, based on the low CV found at this level. The assay was linear at 0.21-493.3 ng/mL, which was defined as the AMR. Modular E170 results were compared with Access II using Deming regression over a range of 0.20 to 34.4 ng/mL. The correlation coefficient was 0.96, average error index 1.50 (range -3.71 to 6.41), slope 1.47, intercept -0.46 and standard error of estimate 3.17. The methods were not considered equivalent within allowable total error of 21.9% (biologic variation database, Ricos C 2012).

Conclusion: The new Roche Tg II assay exhibited an excellent precision and linearity down to 0.2 ng/mL. Although results correlated well with the Beckman-Coulter Tg assay, they were not considered equivalent to this reference method.

A-206

Prediabetic Importance of Serum Zinc Alpha Glycoprotein and Ghrelin Levels in Subjects Classified According to Oral Glucose Loading Test and Fasting Glucose Levels

E. Kurtuluş, D. Konukoglu. *Istanbul University, Cerrahpasa Medical Faculty,Biochemistry Department, Istanbul, Turkey*

Background: Increasing evidence suggests that the postprandial state and fasting hyperglycemia are a contributing factor to the development of Diabetes Mellitus. It has been recently suggested that an adipokine, zinc-α2-glycoprotein (ZAG), may also have a protective role in the prevention of obesity and its associated disorders. ZAG has been proposed to play a role in the pathogenesis of insulin resistance and suspected to be related with Type 2 Diabetes. Ghrelin a peptide hormone secreted mainly by the stomach, increases appetite and food intake. It has been suggested that Ghrelin hormone plays role in insulin secretion and glucose metabolism. In the present study we determined serum ZAG and Ghrelin levels, and evaluated whether the relationship between serum ZAG and ghrelin levels in prediabetic stages. **Methods**: Subjects were categorized according to WHO criteria as Controls (n:23, women:13, men:10, mean ages:55,6 ± 7,7 years) , Impaired Fasting Glucose (IFG; women:29, men:23, mean ages: 55,1 ± 7,0 years), Impaired Glucose Tolerance (IGT; women:26, men: 20,mean ages: 59,1 ± 8,4 years) and Diabetic Glucose Tolerance (DGT; women:15,men: 15, mean age 59,9 ± 11,1 years) in our study. There was no any difference in Body Mass Index and plasma lipids levels (total cholesterol, triglyceride, HDL and LDL -cholesterol) between groups. Subject's patients did not use any medication or vitamin pills. Baseline serum ZAG and Ghrelin levels were determined by ELISA. Serum insülin levels were determined by chemiluminesans assay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Results: Serum ZAG levels in Control group were found to be significantly higher than in DGT, IGT and IFG groups (p<0,005, p<0,001 and p<0,001 respectively). IFG group have significantly lower serum ZAG levels than both DGT and IGT groups (p<0,005 and p<0,001). Serum Ghrelin levels in IGT was significantly higher than in IFG, DGT and Controls (p<0,001, p<0,001 and p<0,001 respectively). Subjects with IFG have significantly higher serum ghrelin levels than Controls (p<0, 05). There was a significant negative correlation between ZAG and HOMA-IR (r:-0,321, p<0,001), as well as Ghrelin levels.(r=-0,530,p< 0,001). A positive correlation were obtained from serum ZAG and 2-hours post challenged plasma glucose levels (r=0,187, p<0, 05). Conclusion: The results of our study suggest that ZAG and Ghrelin are involved prediabetic stages and their levels can be important in the regulation of glucose metabolism. ZAG and ghrelin was found to be effective in the opposite direction. Also, we thought that basal ZAG levels can be a predictive marker for the 2-hours post challenged glucose levels. The present workwas supported by the Research Fund of Istanbul University. Project No. 29822

A-207

Angiotensin II-dependent oxidative stress and increased hypoxia-inducible factor-1α expression in the renal cortex during the normoalbuminuric stage of diabetic mellitus in the rat.

Y. Yamada[1], Y. Kurosaki[2], P. K. Carmines[3], T. Tsukushi[4], A. Imoto[2], T. Ichikawa[1], M. Yokoba[2], H. Ikenaga[5], Y. Aoki[6], T. Takenaka[7], T. Ichikawa[2], M. Katagiri[2], N. Ishii[2]. *[1]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [2]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [3]University of Nebraska College of Medicine, Omaha, NE, [4]Kitasato University Hospital, Sagamihara, Japan, [5]Ikenaga Clinic, Otawara, Japan, [6]Kanagawa Health Service Association, Yokohama, Japan, [7]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: Hypoxia-inducible factor-1 (HIF-1) is composed of α and β subunits, with HIF-1α considered to be a master regulator of the hypoxic response. HIF-1α and oxidative stress associate with the progression of diabetic nephropathy (*Clin Exp Pharmacol Physiol* 33:997-1001, 2006). We previously reported that renal cortical oxidant production is already increased during the early stage of diabetes mellitus (DM), prior to development of albuminuria, and that renin-angiotensin system (RAS) inhibition suppresses development of renal oxidative stress under these conditions (*Clin Sci* 124:543-52,2013). However, the relationship between oxidative stress and the hypoxic response in the kidney during normoalbuminuric stage of DM has not been established.

Objective: The goal of this study was to determine the effect of oxidative stress suppression by RAS inhibition (treatment with an angiotensin II receptor blocker; ARB) on HIF-1α expression in the renal cortex during the normoalbuminuric stage of DM.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, *i.p.*), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ+TLM group (n=5): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. Production of 3-nitrotyrosine (3-NT; an oxidative stress marker) in the renal cortex was measured by HPLC, and HIF-1α expression was quantified by the Western blot analysis.

Results: Blood glucose levels were significantly higher in STZ rats than in Sham rats, and was unaffected by TLM (similar to other treatments that suppress the RAS). Blood pressure did not differ among groups. Compared with the Sham group, GFR

was increased in the STZ group (P<0.05), and this was prevented by TLM treatment (P<0.05 STZ vs. STZ+TLM). Renal cortical 3-NT production in the STZ group was 70% greater than in the Sham group (Sham, 35.2±3.4 pmol/mg protein; STZ, 59.6±1.4 pmol/mg protein; P<0.05 vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 37.3±3.5 pmol/mg protein; P<0.05 vs. STZ). The STZ group also displayed an increase in renal cortical HIF-1α expression (257±26% of Sham; P<0.05); however, the DM-induced increase in HIF-1α expression was not evident in the STZ+TLM group (157±11% of Sham; P<0.05 STZ vs. STZ+TLM).

Conclusions: An increase in renal HIF-1α expression accompanies oxidative stress during the normoalbuminuric stage of DM in the rat, and both of these phenomena are prevented by ARB. These observations indicate that the hypoxic response arises in the renal cortex early during the course of DM, and that this occurs either directly or indirectly under the influence of the RAS, possibly secondary to the presence of oxidative stress.

A-208

Evaluation of TSH Levels in Rio de Janeiro State/Brazil Neonatal Screening.

M. F. M. C. Pinheiro, C. M. M. Oliveira, S. V. L. Argolo, O. F. Silva Filho. *DASA, Rio de Janeiro, Brazil*

Background: Congenital Hypothyroidism (CH) is the most common congenital endocrine disorder and it is a leading preventable mental retardation. Its incidence ranges from 1:2000 to 1:4000 live births in iodine-sufficient countries. In Brazil, the screening for CH is mandatory by law and usually done by TSH determination on dried blood spot on filter paper samples collected by heel puncture. Diagnostic confirmation is required dosing TSH and free T4 in serum. The objective of this study was to evaluate the distribution of TSH levels in newborn´s blood samples from Rio de Janeiro state and the frequency of CH confirmed patients. We also compared our results to those described by the manufacturer.

Methods: We evaluated 18,609 dried blood spots on filter paper samples for TSH of newborns from Rio de Janeiro state over one year period (2013). The range of age was 3 to 30 days of life. We used an automatic immunofluorimetric system, GSP Neonatal hTSH kit (Wallac Oy, Turku, Finland). The cutoff value for TSH was 10.0 mUI/L, children with levels above these limits were recalled for confirmation with serum TSH and FT4.

Results: The most of TSH levels, 85.3%, were less than 2.0 mUI/L. The percentiles 95 and 99 were 3.0 and 4.7 mUI/L, respectively. Comparing to the percentiles described by the manufacturer, 7.9 and 10.7mUI/L, our results were much lower. We found 9 samples (0.05%) above the TSH cutoff. All these patients underwent measurement of serum TSH and FT4. Congenital Hypothyroidism was confirmed in 7 (1:2658) patients. Their initial TSH filter paper level ranged from 12.8 to 279.0 mUI/L, with mean 115.0 mUI/L and median 42.8 mUI/L.

Conclusion: Our data are indicative that, in this Brazilian population, the distribution of TSH levels in newborn's blood filter samples were lower to those presented by the manufacturer, based on European individuals studies. This reinforces the need for each laboratory to evaluate the TSH levels in its specific population. Using 10.0 mUI/L as the TSH cutoff we found, in this Brazilian population, a CH incidence (1:2658) similar to others iodine-sufficient regions.

A-209

Linearity study of thyroid assays assuring the quality control requirements

D. Waltrick, A. L. Camilo, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background:The ability to quantitate circulating levels of thyroid hormones is important in evaluating thyroid function. It is especially useful in the differential diagnosis of primary (thyroid) from secondary (pituitary) and tertiary (hypothalamus) hypothyroidism.To certify released results accuracy, it is important to ensure the linearity of the tests.A Brazilian laboratory implemented an Easy Linearity Curve(ELC) tool to monitor the immune-hormone analytical systems, establishing a self-inspection program to verify the efficiency and accuracy of procedures and results. The use of a tool that checks assay linearity provides additional safety and reliability of the results. This study aims to use the statistic tool to monitor the AMR of TSH,T4, FT4, T3 and FT3.

Materials and methods: We selected samples from the laboratory routine, with concentrations within assay linearity range for each test. Samples were tested in Advia Centaur® XP (Siemens Healthcare Diagnostics) using a chemiluminescent method and analyzed with the ELC tool.

Results:AMR studies were carried out according to CLSI Guideline EP6-A.Results are demonstrated in the table below.

Discussion: The tests showed satisfactory linearity results with different samples. In this study, the sample pool was not used, because all the results from this dilution test presented high percentage of recovery, above the manufacturer's reference, due to a matrix effect. Because of that, we established the utilization of different samples, respecting the expected concentrations of the sample pool,if diluted samples were prepared.The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level.

Conclusion: The thyroid trials tested in this study presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory's target.Thus, tests were approved by the Quality Control Management in the laboratory.

Table 1 -

Assay	Assay Linearity Range	Obtained CV %	CV Lab max Target %	Obtained TE %	TE max Target %	Linear Regression	R2
TSH3UL	0.008 - 150 uIU/mL	4.92	9.70	6.93	23.70	0.990x + (0.312)	0.99973
T4	0.3 - 30 ug/dL	2.38	5.10	8.98	10.55	1.003x + (-0.017)	0.99983
FT4	0.1 – 12 ug/dL	9.68	5.26	11.79	15.00	0.975x + (0.057)	0.99684
T3	10 – 800 ng/dL	6.15	5.95	13.10	20.00	1.028x + (-9.808)	0.99778
FT3	0.2 – 2 ug/dL	5.72	4.00	14.00	30.00	1.003x+ (0.031)	0.99074

A-210

A REVIEW OF 312 GROWTH HORMONE STIMULATION TESTS PERFORMED AT A REFERENCE LABORATORY (DASA-RJ) IN RIO DE JANEIRO - BRAZIL

Y. Schrank, M. C. Freire, R. Fontes, D. M. V. Gomes, O. F. Silva. *DASA - laboratory medicine, rio de janeiro, Brazil*

Background: There is still no consensus on the stimulation test considered the "gold standard" for the diagnosis of GH deficiency. The optimal criteria for a definitive test of growth hormone function are not met by any single stimulus. Lack of standardization of GH response to each type of stimulus, poor reproducibility and lack of correlation between the response to the test and "growth" are some of the various limitations of these tests. The aim of our study was to examine the main tests of GH stimulus applied in our environment so as the response to these tests.

Materials and Methods: We did a retrospective review of 312 patients submitted to GH stimulation test in a period of 12 months. A test was considered responsive when peak GH >5ng/mL.

Results: GH stimulus with Clonidine was the most requested test .The mean age of our patients was 10,2 years, and male:female ratio was 2,4:1. Most of patients were therefore male. Interestingly, however, among patients submitted do the Glucagon simulation test, the majority were female. The greatest GH peak was seen with Glucagon stimulus. No significant complications were observed with the applied tests.

STIMULUS	N (%)	Mean AGE (years)	GENDER (M/F ratio)	RESPON-SIVE (%)	mean GH peak (ng/mL)	GH peak (time)
CLONIDINE	202 (63,7%)	10,1	2,9	73	9,5	60' and 90'
INSULIN	85 (26,8%)	11,6	2,1	50	7,0	60'
GLUCAGON	28 (8,8%)	6,5	1,2	68	10,7	120'
PIRIDOSTIGMIN	2 (0,7%)	11,5	1	0	0,05	-

Conclusions: The high number of tested patients so as the high rate of GH response to Clonidine suggests that this is the preferred screening test by test prescribers. On the other hand, the Insulin stimulation seems to be preferably reserved to confirm the diagnosis of GH deficiency, since the number of patients submitted to these test was significantly smaller and the percentage of responders was only 50%. The use of Insulin as a second-line test in the investigation of GH deficiency is easily explained by the justified fear of potential complications associated with this test. Moreover, Glucagon stimulation is the preferred screening test in children under 6 years.

A-211

Strategy to Improve Diabetes diagnosis in Primary Care: Preliminary Results and Evaluation.

M. Salinas[1], M. Lopez-Garrigos[1], A. Asencio[2], M. Gutierrez[1], J. Lugo[1], C. Leiva-Salinas[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]CAP Mutxamel, Mutxamel, Spain, [3]University of Virginia, Charlottesville, VA*

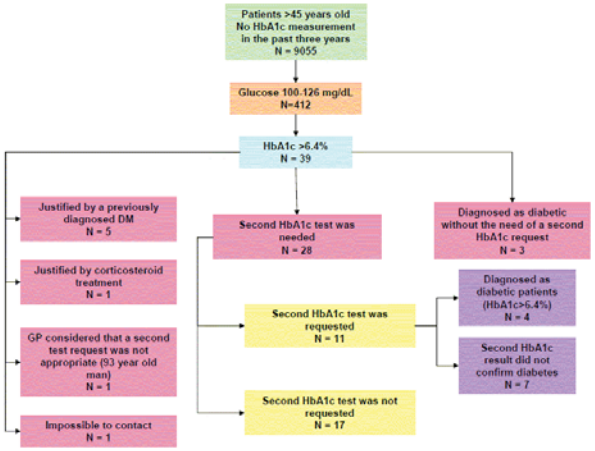
BACKGROUND: With the introduction of HbA1c as a tool to diagnose diabetes, a strategy was designed, established and evaluated in consensus with general

practitioners (GPs) to detect diabetic patients through an opportunistic study to improve HbA1c requesting, and to ascertain if previous HbA1c demand was appropriate to detect diabetes.

METHODS: The laboratory decided to approach the GPs to design a strategy that would improve the diabetes diagnosis efficiency: Laboratory Information System (LIS) automatically would add HbA1c to every sample from primary care patients older than 45 years, without an HbA1c request in the previous three years and glucose results between 100 and 126 mg/dl. If results were above 6.4% , LIS recommended a second request in 3-6 month period. In a last meeting the strategy was approved, established March 1st 2013 and evaluated after a 6 month period. HbA1c was measured using a Variant™ II Turbo Hemoglobin Testing System (Bio-Rad Laboratories, Madrid, Spain).

RESULTS: 412 HbA1c were added automatically, causing 39 HbA1c values above 6.4%. After medical record review, 6 HbA1c results above 6.4% were justified. In one case it was impossible to contact because a change of residency. 3 patients were diagnosed as diabetic without the need of a second request. To eleven patients a second HbA1c was requested, being 4 diagnosed as diabetic patients (HbA1c >6.4%) and in seven patients the second HbA1c result did not confirm diabetes. Despite their abnormal HbA1c results, until now, to 17 patients has not been requested a second HbA1c to confirm/discard the illness. Results are showed in figure.The cost of adding the 412 HbA1c was 535.6 US dollars. At this moment, each case of the seven diabetes diagnosed represented a cost of 76.5 US dollars.

CONCLUSION: Our proposed opportunistic screening to discover diabetes seems cost-effective. Hba1c was previously under requested.



A-212

Measurement of serum testosterone, androstenedione and dehydroepiandrosterone (DHEA) levels using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS)

R. Büttler[1], F. Martens[1], M. Ackermans[2], M. Blankenstein[1], A. Heijboer[1]. *[1]VU university medical center, Amsterdam, Netherlands, [2]Academic Medical Center, Amsterdam, Netherlands*

Background: The adrenal and gonadal androgens testosterone, androstenedione and dehydroepiandro-sterone (DHEA) play an important role in sexual development and fertility as well as in several other processes.

Methods: We developed a method to assess serum testosterone, androstenedione and DHEA levels in one run using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS). Sample preparation consisted of addition of internal standards ($^{13}C_3$-testosterone, $^{13}C_3$- androstenedione and $^{2}H_6$-DHEA) and a liquid-liquid extraction using hexane-ether. The samples were analyzed on an Acquity 2D UPLC system (Waters), equipped with a C4 column (Waters) and a Kinetex Fluorophenyl column (Phenomenex), and a Xevo TQ-S tandem mass spectrometer (Waters). The three analytes were baseline separated in a total run time of 9 minutes. The calibration curves ranged from 0.10 to 26 nmol/L for testosterone and androstenedione, and from 0.96 to 78 nmol/L for DHEA.

Results: The intra-assay CVs were <4.0%, <4.6% and <7.0% for testosterone, androstenedione and DHEA, respectively. The inter-assay CVs were <6% for testosterone and <8% for androstenedione and DHEA. At the lower concentrations inter-assay CVs were 15%, 7.0% and 9.3%, for testosterone (0.08 nM), androstenedione (0.47 nM) and DHEA (1.18 nM), respectively. Recoveries of spiked analytes were 101-107%, 99-106% and 92-104% for testosterone, androstenedione and DHEA, respectively. Linearity was shown in dilution series (mean R^2 was >0.999 for all analytes). This method tested negative for interference from DHEA-sulphate, estrone, 17β-estradiol, androsterone, 17-hydroxy progesterone, dihydrotestosterone, epi-testosterone, cortisone and cortisol and did not show ion suppression. The method was shown to be suitable for serum as well as EDTA and heparin plasma.

The present testosterone method compared well (y = 1.000 x + 0.035 nmol/L; r = 0,9982) to another ID-LC-MS/MS method for testosterone concordant with a published reference method (Bui et al. 2013). In the near future, the present method will also be compared to another LC-MS/MS method for androstenedione and DHEA.

Conclusion: We developed a sensitive and accurate method to measure serum testosterone, androstenedione and DHEA levels in one run.

A-213

Glucagon Quantification: Comparison of Radioimmunoassay and Sandwich ELISA methods

M. Raines, C. King, S. Christensen, P. Amin, D. Schranz. *Pacific Biomarkers, Seattle, WA*

Background: Accurate and robust measurement of glucagon is important in understanding glucagon's role in glucose metabolism and homeostasis as well as its role in the pathology of type 2 Diabetes and other metabolic diseases. It is also commonly used in clinical studies as a surrogate marker of drug efficacy. Competitive RIA methods, like the ALPCO Glucagon RIA (A), have been the gold standard for measuring glucagon but can be limiting due to the short shelf-life, long ordering lead times, long assay times, and large sample volume required (≥1 mL). The objective of this study is to evaluate the performance of the R&D Systems Glucagon Quantikine ELISA (R) and the Mercodia Glucagon ELISA (M) relative to the FDA cleared ALPCO Glucagon RIA.

Methods: All three assays were validated for precision, linearity, recovery, sensitivity, and normal glucagon ranges using fasting plasma samples collected in either K2EDTA or P800 tubes, frozen and stored at -70 ºC. A set of fasting and nonfasting P800 plasma samples were also used to directly compare glucagon results of all three assays.

Results: Precision for all three methods was acceptable with the intra-assay precision being less than 5% for all three assays and inter-assay precision being 3.8 - 11.9% (A), 4.9 - 9.2% (R), and 4.3-8.1% (M). Dilutional linearity was acceptable up to 40-fold (A), 16-fold (R), and 16,000-fold (M) dilutions for the three assays. The Mercodia ELISA was the most sensitive with a lower limit of quantitation (where the % CV is equal to 20%) of 1.5 pmol/L (M) versus 8.6 (A) and 9 pmol/L (R) for the two other kits. The most striking difference in the three assays was in the glucagon values observed in apparently healthy donor samples. The mean glucagon values for normal samples analyzed using the ALPCO RIA method were much higher (39.3 pmol/L) than the R&D systems (28.8 pmol/L) and Mercodia (9.1 pmol/L). Although the R&D systems ELISA had mean normal glucagon values that were more in line with those obtained for the ALPCO RIA, Deming Regression Analysis using the same set of P800 plasma samples yielded a correlation coefficient of 0.6445 and slope of 2.174, while the Mercodia ELISA had a correlation of 0.9093 and a slope of 0.606 when compared with the ALPCO RIA. The majority of the twenty samples analyzed with the R&D systems ELISA yielded glucagon values 30-50% lower than the ALPCO RIA method, but there were three samples that had glucagon values that were higher in the R&D Systems ELISA than the ALPCO RIA which resulted in poor correlation. The Mercodia ELISA glucagon values were consistently lower than the ALPCO RIA values with greater biases observed for samples less than 10.0 pmol/L. Potential cross-reactivity with other glucagon-related molecules is speculated and currently being investigated as it may account for some of the differences observed between the three assays.

Conclusion: The Mercodia Glucagon ELISA may be a suitable alternative to the ALPCO Glucagon RIA method especially when sample volumes are limiting and better sensitivity is required.

A-214

Associations of Leukocyte Telomere Length with Cardiometabolic Risk Factors and Circulating Biomarkers of Inflammation and Oxidative Stress

R. M. AlKhaldi, O. Mojiminiyi, F. Al Mulla, N. Abdella. *Kuwait University, Kuwait, Kuwait*

Background: Telomeres are TTAGGG sequences at the end of chromosomes necessary for and chromosomal integrity which upon reaching critical length,cell become senescent or otherwise dysfunctional. However, telomerase a reverse

transcriptase enzyme prevents telomere exhaustion and chromosomal instability. Telomeres and telomerase were linked to aging & associated diseases namely obesity, diabetes type 2 [T2DM] and cancer. We hypothesize that shortened telomere length would be associated with cardio-metabolic risk factors, and that this relationship might be mediated by obesity related metabolic changes.

Methods: Indices of obesity (Body Mass Index [BMI], Waist Circumference [WC], Waist to height Ratio [WHtR]), glycated Hemoglobin [HbA1c%], lipid profile, fasting glucose, serum human Telomerase Reverse Transcriptase [hTERT], total adiponectin, Insulin, Myeloperoxidase [MPO], Malondialdehyde [MDA], Total Oxidative stress status [TOS] and Leukocyte Telomere Length [LTL] were measured in 225 T2DM patients and 245 age and sex matched controls. Insulin resistance [IR] was estimated using Homeostasis Model Assessment [HOMA] calculator.

Results: T2DM patients had significantly ($p<0.0001$) lower LTL compared to controls [(Mean±SD:2.1±0.2) vs. (Mean±SD:4.1±0.1)] respectively. Levels of hTERT were higher in controls compared to T2DM patients [(Mean±SD: 32.9±8.9 ng/mL) vs. (Mean±SD: 21.4±4.7 ng/mL)]. Spearman's rank correlation coefficients showed that LTL correlated negatively with age [r = -0.2, p=0.009], BMI [r = -0.3, p=0.006], WC [r = -0.3, p<0.0001], and Insulin [r = -0.2, p=0.03]. The significance of these correlations disappeared after adjusting BMI but not age and/or sex. Additionally, LTL correlated negatively and strongly with WHtR [r = -0.5, p=0.004], and HbA1c% [r = -0.6, p=0.003]. These significant correlations were not affected by BMI, age or sex. Multivariate regression analysis showed that LTL negatively associated with BMI [β=-0.7, p=0.005], WC [β=-5.7, p=0.004], HOMA-IR [β=-1.1, p=0.003], MPO [β=-0.6, p<0.0001], MDA [β=-0.1, p=0.04], TOS= [β=-2.2, p<0.0001]. hTERT showed similar trends in relation to BMI [β=-0.2, p=0.004], WC [β=-1.4, p=0.006], HOMA-IR [β=-1.3, p=0.007], MPO [β=-0.6, p<0.0001], MDA [β=-0.42, p=0.002], TOS[β=-0.3, p=0.007]. On the other hand, LTL and hTERT were associated significantly and positively associated with adiponectin [β=3.1, p=0.02; β=1.5, p=0.003] respectively. Using binary logistic regression analysis, higher BMI was associated with higher risk of telomeres' shortening [OR=2.4, p=0.008]. Higher WC and WHtR were associated with higher risk of telomere shortening [(OR=2.4, p=0.001); (OR=1.9, p=0.002)] respectively. Other obesity related factors such as IR, hyper-insulinemia and hyper-triglyridemia [(OR=7.7, p<0.0001), (OR=1.2, p<0.0001), (OR=1.3, p=0.01)] were also associated with higher risk of short telomeres. Higher levels of adiponectin were associated with lower risk of telomere shortening [OR=0.7, p=0.004]. Additionally, shorter telomere length were associated significantly with higher risk of T2DM [OR=7.5, p=0.003]. Higher hTERT levels though were associated with lower risk of T2DM [OR=0.8, p=0.008].

Conclusions: Our results demonstrate the link between telomere biology, cardiometabolic risk factors, and T2DM in the Kuwaiti population which has not been studied before. Metabolic changes such as the dys-regulation of adipokines (such as adiponectin), dys-lipidemia, hyper-insulinemia, IR and obesity associated inflammatory process, could play a role in mediating telomere shortening. Since, obesity and T2DM are increasing at epidemic pace in Kuwait; telomere attrition & telomerase levels could be potential cardio-metabolic risk markers of obesity and T2DM.

A-215

Associations of Common TERC Single Nucleotide Polymorphisms with Telomere Length, Human Telomerase Reverse Transcriptase and Obesity Related Factors

R. M. AlKhaldi[1], O. Mojiminiyi[1], F. Al Mulla[1], N. Abdella[2]. *[1]Pathology Department, College of Medicine, Kuwait University, Kuwait, Kuwait, [2]Medicine Department, College of Medicine, Kuwait University, Kuwait, Kuwait*

Background: Quantitative trait locus studies have mapped putative loci that probably be involved in the regulation of leukocyte telomere length [LTL] to human chromosomes 3p26.1, 10q26.13, 12q12.22 and 14q23.2. The strongest associations with LTL were reported for SNP rs12696304 and rs16847897 near TERC on 3q26. It is unclear though whether this locus identified in Europeans, American, and Chinese exerts a similar effect on LTL in other populations. Additionally, the effect of such SNPs on serum levels of human telomerase reverse transcriptase (hTERT) has not been explored before in any population. The aim of this research to: study the influence of TERC SNPs on LTL, levels of [hTERT], indices of obesity and explore the potential associations with type 2 diabetes mellitus [T2DM].

Methods: In a study on 225 T2DM patients and 245 age and sex matched controls, we used Allelic Discrimination (AD) genotyping to determine near TERC SNPs (rs12696304 and rs16847897). Fasting [hTERT], adiponectin, Insulin, Myeloperoxidase [MPO], and [LTL] were also measured. Body Mass Index (BMI), and waist circumference (WC) were also recoded and subjects were classified on the basis of the degree of obesity. Body fat percentage (BF%] was measured using Bioimpedance analysis [BIA]. Insulin resistance [IR] was assesed using [HOMA-IR] calculator.

Results: [C/C] genotype of SNP rs16847897 was significantly associated with telomere shortening [OR=1.6, p=0.004] and lower levels of hTERT [OR=0.4, p=0.006]. Nevertheless, [C/C] genotype was significantly associated with higher BMI [OR=2.2, p=0.006], WC [OR=23.4, p=0.007] and BF% [OR=2.0, p=0.005]. However, [C/C] genotype SNP rs16847897 was associated with hypo-adiponectemia [OR=0.6, p=0.006]. We found that [G/G] genotype of SNP rs12696304 was significantly associated with shorter telomeres [OR=1.5, p=0.004], lower levels of hTERT [OR=0.7, p=0.006] and hypo-adiponectemia [OR=0.5, p=0.008]. [G/G] genotype of SNP rs12696304 was associated with higher anthropometric measures such as BMI [OR=1.2, P=0.006], WC [OR=5.3, P=0.004] and BF% [OR=1.9, p=0.003]. Binary logistic regression showed that; [C/C] genotype of SNP rs16847897 and [G/G] genotype of SNP rs12696304 were significantly associated with higher T2DM risj [OR=1.7, p=0.004]. Carriers of haplotype [CG] had significantly higher ($p<0.0.0001$) BMI compared to the other two identified haplotypes [CC] and [GG] [BMI_{CG} 30.8±8.2 Kg/m^2 vs. BMI_{CC} 26.9±8.4 Kg/m^2 and BMI_{GG} 28.7±5.3 Kg/m^2]. Similar trends were observed for WC and BF%. Additionally, telomere lengths were significantly the shortest and hTERT levels were the lowest in([CG], LTL: 0.8±.01; hTERT: 21.8±5.5 ng/mL])haplotypes compared to the other haplotypes([CC], LTL: 1.03±0.1; hTERT): 23.7±6.9 ng/mL] and ([GG], LTL: 1.5±0.1; hTERT: 28.1±5.4 ng/mL]). On the other hand, levels of MPO were significantly higher in haplotype ([CG], MPO: 6.6±1.7 ng/mL) compared to other two haplotypes [CC],(MPO: 3.9±0.4 ng/mL]) and ([GG], MPO: 4.1±0.4 ng/mL]). We also found that [CG] haplotype was associated significantly with higher risk of T2DM [OR=1.5, p=0.006] and IR [OR=2.6, p=0.03].

Conclusions: We provide insights into genetic determination of a structure that is critically involved in genomic stability. Given the importance of telomeres in nuclear and cellular function and the central role of telomere length in determining telomere function; our findings could have broad relevance for both normal and pathological age associated processes.

A-216

Do anesthesia provider personnel working indoors have lower Vitamin D levels?

G. Erden[1], S. Ozdemir[1], G. Ozturk[1], A. I. Erden[2], D. Kara[2], S. Isik[3], J. Ergil[4], C. Vural[5], A. E. Arzuhal[1]. *[1]Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Clinical Biochemistry Ankara, Turkey, [2]Hacettepe University Faculty of Medicine, Department of Anesthesiology and Reanimation, Turkey, [3]Ankara Numune Research and Training Hospital, Department of Endocrinology Ankara, Turkey, [4]Ankara Diskapi Yildirim Beyazit Research and Training Hospital, Department of Anesthesiology and ReanimationAnkara, Turkey, [5]Ankara Numune Research and Training Hospital, Department of Anesthesiology and Reanimation Ank, Turkey*

Background: There has been an increasing awareness to vitamin D deficiency. Some of the causes of vitamin D deficiency are reduced skin synthesis (sunscreen, skin pigment etc), decreased absorption, increased catabolism, heritable disorders, decreased synthesis of 1,25 dihydroxyvitamin D, acquired disorders. One approach for clinicians to decide which patients demand screening laboratory testing is to consider serum testing in patients at high risk for vitamin D deficiency. As the most well-known source of vitamin D is known as sun exposure, anesthesia providers and anesthesia support personnel working indoors (in operating rooms) might be considered at increased risk of vitamin D deficiency due to limited sun exposure. This study aimed to investigate whether there was a higher vitamin D insufficiency or deficiency rate among anesthesia personnel working indoors when compared with personnel working outdoors.

Methods: 125 volunteered anesthesia (provider and support) personnel and 55 control (outdoor workers in marketplace) subjects were included in this study. All of the individuals were apparently healthy Turkish citizens of Ankara, Turkey (39 ° North, 32 ° East).The study was performed at the end of the winter (February 15-March 15 2013). Socioeconomic status, daily diet, vitamin D supplementation, periods of exposure to sunlight, the use of sunscreen, regular physical activities, family history of bone fractures and osteoporosis, and the clothing style in all of the individuals were asked about in a questionnaire. People with high BMI, chronic disease such as asthma, type 1 diabetes mellitus, hypertension, history of cardiac, kidney or liver disease, those taking calcium, vitamin D or multivitamin supplements were excluded from the study. Serum levels of total 25 hydroxyvitamin D (25-OHD) were measured by a chemiluminescent immunoassay (CLIA) method using an autoanalyzer (LIAISON DiaSorin, Italy). 25-OHD levels were categorized as follows: Deficient: <10 ng/

mL ; Insufficient 11-29 ng/mL and Adequate: >30 ng/mL. Data were tabulated and subjected to analysis using the Statistical Package for Social Science (SPSS) version 17.0.

Results: 74.4% of indoor anesthesia personnel and 76.6% of outdoor workers had serum 25-OHD concentrations <10 ng/mL. 20.8% of anesthesia personnel and 23.4 % of outdoor workers had serum 25-OHD concentration levels 10-20 ng/mL. 4.8 of % anesthesia personnel had serum 25-OHD concentration levels 21-30 ng/mL. There was no significant difference in the mean serum 25-OHD level between two groups (Anesthesia group: 8.98±4.89 ng/mL, Control group: 8.21±2.64 ng/mL) (p>0.05).

Conclusion: This study in Ankara suggests that significant proportions of the study populations had very low vitamin D levels at the end of winter. Vitamin D deficiency/ insufficiency is common among indoor and outdoor workers. Anesthesia personnel do not have a significant higher Vitamin D deficiency/insufficiency risk. As we have seen, UV irradiance is not the only determinant of vitamin D status. Individuals living at lower latitudes in relatively sunny environments are also at risk of vitamin D insufficiency. Vitamin D supplementation may be suggested in all groups in Ankara, including those with the highest sun exposure.

A-217

Increased Cortisol and NADPH Production in Magnesium Deficient Hepatocytes: Implicated in the Onset of Insulin Resistance and Obesity.

C. Voma[1], A. Romani[2]. *[1]Cleveland State University, Cleveland, OH, [2]Case Western Reserve University, Cleveland, OH*

Most of the clinically quantifiable liver functions take place within the hepatocytes (80% of liver cells). The current western diet is approximately 35% deficient in magnesium (Mg^{2+}). Subnormal Mg^{2+} concentrations have been reported in both diabetes and obesity, but no clear-cut cause-effect mechanism has been stated to elucidate the onset of these pathological conditions in Mg^{2+} deficiency. At the cellular level, Mg^{2+} is highly concentrated within organelles including the endoplasmic reticulum (ER), in which 15-20 mM $[Mg^{2+}]_{Total}$ has been measured. Hexose 6-phosphate dehydrogenase (H6PD), the reticular counterpart of the cytosolic G6PD, is the main NADPH generating enzyme within the ER of the hepatocyte and is regarded as an ancillary enzyme in pre-receptor glucocorticoid activation. In the present study, we report that by modulating glucose 6-phosphate entry into the ER of HepG2 cells, Mg^{2+} also regulates the oxidation of this substrate via H6PD. This regulatory effect is dynamic as glucose 6-phosphate entry and oxidation can be rapidly down-regulated by the addition of exogenous Mg^{2+}. In addition, HepG2 cells growing in low Mg^{2+} show a marked increase in H6PD mRNA and protein expression. Metabolically, these effects on H6PD are important as this enzyme increases intra-reticular NADPH production, which favors fatty acid and cholesterol synthesis. Under Mg^{2+} deficient conditions, exposure of HepG2 cells to cortisone results in a marked production of cortisol via the NADPH-dependent 11β-HSD1, thus eliciting high intra-hepatic active glucocorticoid concentrations, which in turn affects hepatocyte metabolism. Obesity is a known risk factor for type 2 diabetes. However, the degree of obesity varies greatly in people with type 2 diabetes. Not all type 2 diabetic patients are overweight or obese, and not everyone who is overweight or obese will necessarily develop type 2 diabetes, suggesting the involvement of other mechanisms in the development of the pathology. 11-β-OHSD1 has been implicated as one of these auxiliary mechanisms, as it would lead to cortisol-based insulin resistance at least in certain patients.

HepG2 cells were grown in the presence of 0.6 (deficient) or 1.0 mM (physiological) $[Mg^{2+}]o$ and analyzed for NADPH and 11β-HSD1-mediated cortisol production. The mRNA expression level of H6PD, G6Pase, and 11β-HSD1 were analyzed by RT-PCR while protein expression was assessed by Western Blot analysis. Under our experimental conditions, insulin responsiveness - assessed as pAKT level by Western Blot analysis – was decreased by approximately 25% while cortisol production was increased and associated with an increased expression of PEPCK, a key enzyme in gluconeogenesis activation. Taken together, our results indicate that the ~60% increase in NADPH production via H6PD in Mg^{2+} deficient cells resulted in increased cortisol production and a decreased insulin responsiveness. In addition, these Mg^{2+} deficient cells showed 3 to 4 fold increase in H6PD and 11β-HSD1 mRNA and protein expression.

Our results support the hypothesis that Mg^{2+} deficiency increases H6PD activity and expression, setting the conditions for increased production of cortisol and decreased hepatic insulin responsiveness.

A-218

Comparison of IFA and RIA based assays for measuring adrenal autoantibody response

O. Zhukov, J. Popov, R. Vergara, D. Keno, L. Liu, J. Nakamoto, S. J. Naides. *Quest Diagnostics Nichols Institute, San Juan Capistrano, CA*

Background: The diagnosis of autoimmune Addison's disease (ADD), a primary adrenal insufficiency, depends on demonstrating inappropriately low cortisol production and high titers of adrenal cortex autoantibodies (ACAs) or 21-hydroxylase (21-OH) autoantibodies. ACA titers are determined using an immunofluorescence assay (IFA), while 21-OH autoantibodies are detected with a radioimmunoassay (RIA). In IFAs, response against 21-hydroxylase (21-OH) accounts for majority of the immunoreactivity, but antibodies against two other steroidogenic enzymes (17α hydroxylase [17-OH] and SCC [P450cSCC]) also contribute. A sensitive and convenient RIA is available to measure anti 21-OH antibody using recombinant ^{125}I-labelled 21-OH expressed in yeast. Discrepancy between ACA IFA and 21-OH RIA test results was reported among individuals with endocrine autoimmune diseases often associated with adrenal insufficiency. We evaluated concordance between ACA IFA and 21-OH RIA results in a large set of samples received for routine adrenal antibody testing. Methods: De-identified residual specimens (n=280) originally submitted to Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) for routine adrenal antibody testing by either IFA (n=140) or RIA (n = 140) were re-tested with both assays. Also included in the analysis were 1) results for an additional 264 specimens submitted for both testing with assays (ACA IFA and 21-OH Ab RIA), and 22 sera positive for mitochondrial antibodies. The ACA IFA was performed using monkey adrenal tissue (MarDx Diagnostics, Inc., Carlsbad, CA) and FITC-labeled goat antibodies to human IgG (Inova Diagnostics, Inc., San Diego, CA). Anti 21-OH testing was performed with a commercial RIA (Kronus Inc., Star, ID) employing ^{125} I-labeled 21-OH produced in yeast. Anti-mitochondrial antibodies (AMAs) were detected by IFA using rat kidney tissue (MarDx Diagnostics) and by Quanta Lite™ ELISA (Inova Diagnostics) employing recombinant antigen (MIT3). Results: The two assays yielded concordant results in 460 (83%) of the 554 samples, including 328 with negative and 132 with positive results, 94 samples were discordant. Samples with low positive results were the main contributors for IFA/RIA discordance: among 55 RIA+/IFA- samples, 49 showed RIA values close to the cut off and only 6 samples had values >10 U/mL. This discrepancy could be due to the fact that some of 21-OH epitopes recognized in RIA were "hidden" or not present in IFA substrate. Among 29 RIA-/IFA+ samples, most (18/29) had titers of 1:10 and only 4 had titers >1:40. The presence of other antibodies, either to steroid-producing cell antibodies or anti-mitochondrial antibodies, could cause these discrepancies. Presence of mitochondrial antibodies was identified in one sample. Conclusions: Low-positive samples are the major contributors to discrepancies between IFA and RIA results on adrenal antibody testing. The presence of anti-mitochondrial antibody may interfere with adrenal antibody testing by IFA.

A-219

Extreme Physical Stress Stimulates Bone Marrow-derived Circulating Stem/ Progenitor Cells that Mediate Tissue Repair: Possible Clinical Implications

I. Papassotiriou[1], E. Goussetis[2], K. Skenderi[3], M. Tsironi[4], G. Chrousos[5]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Stem Cell Transplant Unit, "Aghia Sophia" Children's Hospital, Athens, Greece, [3]Laboratory of Nutrition and Clinical Dietetics, "Harokopio" University, Kallithea, Greece, [4]Department of Internal Medicine, University of Peloponnesus, School of Nursing, Sparta, Sparta, Greece, [5]Department of Pediatrics, University of Athens Medical School, Athens, Greece*

Background: Autologous progenitor cells represent a promising option for regenerative cell-based therapies. Endothelial progenitor cells (EPCs) participate in vascular repair and angiogenesis, while circulating bone marrow-originated fibrocytes represent multipotent cells mediating tissue repair and remodeling after injury. Aging and cardiovascular risk factors, such as diabetes, however, affect circulating endothelial and bone marrow-derived progenitor cells, limiting their therapeutic potential. The "Spartathlon" ultradistance foot race (246Km continuous, prolonged, brisk exercise for up to 36h), is associated with profound physical strain, which renders it an ideal model of prolonged severe physical stress. The runners endure dramatic systemic and inflammatory changes, as their immune system functions intensively to cope with heart and skeletal muscle and other organ damage secondary to excessive physical strain. We hypothesized that this type of exercise might stimulate release of EPCs and other bone marrow-derived cells.

Athletes and Methods: We investigated the effect of physical stress on the number of circulating EPCs and fibrocytes, along with circulating molecules indicative of endothelial dysfunction and adipose tissue-derived proteins, in 20 "Spartathlon" athletes before, at the end and at 48 h post-race. The EPCs were obtained by culturing peripheral blood mononuclear cells (PBMC) under endothelial cell conditions (EndoCult) and were measured as colony-forming units (CFUs). Circulating fibrocytes were cultured from PBMCs in IMDM medium supplemented with IL-3 and M-CSF and identified as CD(45+)CD(14+)CD(34low)Collagen-I(+) fibroblastic cells. We also determined the plasma levels of endothelial dysfunction molecules E-, L- and P-selectins, soluble Intercellular Adhesion Molecule-1 (sICAM-1), soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1), and thrombomodulin (TM), along with adipose tissue-derived proteins leptin, adiponectin (ADPN), lipocalin-2 (NGAL), Retinol Binding Protein-4 (RBP-4), Plasminogen Activator Inhibitor-1 (PAI-1), Macrophage Migration Inhibitory Factor (MIF), IL-8 and Macrophage Chemoattractant Protein 1 (MCP-1) by means of immunoenzymatic techniques.

Results: Circulating EPCs increased by nearly ten-fold in peripheral blood at the end of the "Spartathlon" race (from 48±15 cells/ml to 464±36 cells/ml) and they remained increased (420±28 cells/ml) even at 48h post-race ($p>0.5$). Plasma levels of endothelial dysfunction molecules showed different patterns of responses: E-selectin, sICAM, sVCAM and thrombomodulin were increased significantly at the end of the race and returned to pre-race levels 48 h post-race, ($p>0.6$). Similarly, the adipose tissue-derived proteins NGAL, IL-8 and MCP-1 showed significant increases at the end of the race and returned to pre-race levels 48 h post-race, ($p<0.5$).

Conclusions: Our study demonstrates that acute inflammatory tissue damage induced by exhausting exercise increases EPCs but not fibrocytes. Given the ability of EPCs to promote angiogenesis and vascular regeneration and the association of fibrocytes with tissue fibrosis after persistent inflammation, we conclude that this kind of cell mobilization may serve as a physiologic repair mechanism in acute inflammatory tissue injury and a source of potential cell therapies in the near future. Furthermore, this study shows different patterns of adipose tissue-derived protein response to the systemic effort and inflammatory changes.

A-220

CDC Standardization Programs- Testosterone, Estradiol, and Vitamin D

J. C. Botelho, H. W. Vesper. *Centers for Disease Control and Prevention, Atlanta, GA*

Laboratory measurements are critical in patient care and public health decision making. However, the accuracy and reliability of these measurements prevent appropriate detection, treatment and prevention of diseases. The aim of CDC Standardization Programs is to standardize clinical measurements which ensure that accurate and comparable measurements are obtained regardless of the measurement procedure, location, and time. To achieve this goal, the CDC Standardization Programs are providing a comprehensive range of services and programs such as Reference Services, Standardization-Certification Programs, and Accuracy-based Quality Assurance Monitoring Services for testosterone (T), estradiol (E2), and vitamin D [25(OH)D].

As part of the Reference Laboratory Services the CDC has established higher order reference measurement procedures for T, E2, 25(OH)D2 and 25(OH)D3 in serum using LC-MS/MS. These measurement procedures are traceable to primary reference materials and to JCTLM certified reference measurement procedures. Using these reference methods, CDC assigns target values to sera used in its certification programs and by outside partners such as clinical and research laboratories, assay manufacturers, and proficiency testing providers. These materials are used for method comparisons, calibration, and trueness controls. CDC Standardization

CDC Standardization-Certification Programs are operating for T and E2 with the Hormone Standardization (HoSt) Program and total 25(OH)D with the Vitamin D Standardization-Certification Program (VDSCP). In both of these programs, quarterly blinded challenges are performed. Bias and imprecision assessments using established protocols and final assessment are made using criteria derived from biological variability. At present, 17 participants are enrolled in the HoSt-T Program (established in 2010) and 23 in VDSCP (established in 2013). Participants include clinical, academic, and pharmaceutical laboratories as well as manufacturers. Approximately 85% of participants have met the established criteria. Successful laboratories are published on the CDC website (http://www.cdc.gov/labstandards/hs.html). Over the past 4 years the CDC has provided 97 calibration verification serum sets to requestors and has had 85 enrollments in HoSt and VDSCP, which include many reenrollments. While participation has increased the success rate of participants has continued to improve as well. The testosterone HoSt Program has increased success rates by participants over the past 3 years from 79% in cycle 1 to 100% in cycle 3.

CDC Hormone Standardization Programs are endorsed and supported by key stakeholders such as the Partnership for Accuracy in Hormone Testing (PATH) and its affiliated organizations (i.e., AACC, The Endocrine Society, and American Urology Association). Furthermore, it collaborates with these organizations to further improve testing for other hormones such as thyroid hormones.

A-221

Quantifying Insulin-like Growth Factor-1: Inter-assay Variation Remains an Issue

H. Li[1], D. Bailey[2], L. Martin[2], T. Randell[2], K. Garen[2], G. Waite[2], A. S. Ptolemy[2]. *[1]Gamma-Dynacare Medical Laboratories, Brampton, ON, Canada, [2]Gamma-Dynacare Medical Laboratories, London, ON, Canada*

Background: As the main mediator of the somatotropic effects of growth hormone (GH), an accurate measurement of human insulin-like growth factor-1 (IGF-1) is required for the diagnosis and management of GH secretion disorders. However, the standardized measurement of IGF-1 continues to suffer from inter-assay variability, which may lead to inaccurate patient case decision making. In early 2013, the receipt of a vendor notification stating that the IGF-1 reagent lots deployed in our laboratory positively shifted patient median values prompted us to validate and ultimately deploy an alternate vendor's IGF-1 platform for patient testing. Recently, the primary vendor resumed IGF-1 reagent supply, triggering a secondary validation of these reformulated lots. These two studies specifically examined the inter-assay variability of IGF-1 measurements and the relative analytical performance of each test.

Methods: Linearity, intra- and inter-day precision, accuracy and sample carry-over were validated for IGF-1 measurements using the IDS-iSYS (Immunodiagnostic Systems) and the reformulated Immulite 2000 (Siemens Healthcare Diagnostics) assays, respectively. Patient correlation studies between the IDS-iSYS and the original and reformulated Immulite 2000 reagents were also respectively performed.

Results: The IDS-iSYS and reformulated Immulite 2000 assays had linear ranges of 10 to 1200 ng/mL ($R^2 = 0.998$, slope= 0.973) and 20 to 1700 ng/mL ($R^2= 0.999$, slope=1.02), respectively. At IGF-1 concentrations of 30.8, 249.1, 830.3 ng/mL and 45.0, 67.5 and 227.0 ng/mL the intra- and inter-day precision (%CV, N=20) of the IDS-iSYS and reformulated Immulite 2000 assays did not exceed 4.6% and 7.5%, respectively. The relative error (%RE) of the IDS-iSYS and Immulite 2000 methods respectively ranged from -8.4% to 1.5% and -3.5% to 7.5% for these precision studies. No significant carry-over was observed on either platform. Patient sample comparisons between the IDS-iSYS and the original Immulite 2000 formation showed significant bias (Deming regression: $y = 0.739x+35.87$, N=94, $R^2=0.988$). This method bias was exacerbated at IDS-iSYS derived IGF-1 concentrations >300 ng/mL (Deming regression: $y = 0.616x+114.88$, N=15, $R^2=0.981$, IDS-iSYS range= 256.3 to 770.1 ng/mL, Immulite 2000 range = 307.0 to 1128.0 ng/mL), relative to lower IGF-1 concentrations (Deming regression: $y = 0.854x+14.73$, N=79, $R^2=0.976$, IDS-iSYS range= 42.8 to 290.7 ng/mL, Immulite 2000 range = 31.5 to 351.0 ng/mL). Interestingly, this bias was less significant when patient results obtained with the reformulated Immulite 2000 reagents were correlated to those obtained with the IDS-iSYS (Deming regression: $y = 1.082x-9.9$, N=60, $R^2=0.989$, Immulite 2000 range = 25.0 to 352.0 ng/mL, IDS-iSYS range=31.0 to 352.0 ng/mL), although fewer samples with IGF-1 concentrations >300 ng/mL were included in this cohort.

Conclusion: Although the reformulated Immulite 2000 and IDS-iSYS IGF-1 assays offer acceptable analytical and clinical performance, a significant bias was noted with the original Immulite 2000 formulation. This difference was observed despite both Immulite formulations being traceable to the reference standard NIBSC 1st IRR 87/518. The IDS-iSYS assay is traceable to NIBSC 02/254. Laboratories should be aware that inter-assay IGF-1 variability must be carefully examined and its impact on the diagnosis and management of GH deficiency and acromegaly considered when testing platforms are changed.

A-222

Development of a Biochip Based Immunoassay for Quantification of Total Beta hCG in Serum

R. Duncan, A. McFall, S. Brockbank, A. Chacko, C. Richardson, P. Lowry, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormone family. It is heterodimeric and the alpha-subunit of hCG (92 amino acids) is identical to that of LH, FSH and TSH. The beta-subunit of hCG (145 amino acids) comprises the unique component of hCG and accounts

for the biological activity of this hormone. hCG interacts with the LH/hCG receptor and stimulates and maintains the corpus luteum after fertilization so it will not degenerate. The corpus luteum of pregnancy produces increasingly greater amounts of estrogen and progesterone for an additional ten weeks until the placenta takes over the secretion of these steroid hormones.This study reports the development of a biochip based immunoassay for the determination of total beta hCG in serum. This represents a new analytical tool for the detection of pregnancy. Methods: A sandwich chemiluminescent biochip based immunoassay applied to the Evidence Investigator analyser was employed. The capture antibody was immobilised and stabilized on the surface of the biochip and detector antibody was conjugated to HRP. Chemiluminescent signal was detected by digital imaging technology. The intensity of the signal is proportional to the analyte concentration in the sample. A correlation study was conducted using a commercially available immunoassay. Results: The assay was target specific showing <1% cross reactivity with FSH, LH and prolactin and <1% recovery of hCGα. The limit of detection was 0.914 mIU/ml for an assay range 0-2500 mIU/ml and the limit of blank was 0.388 mIU/ml. The intra-assay precision (n=23), expressed as %CV, was <7.5%. In the correlation study 80 serum samples were tested and the following linear regression equation was achieved versus another available *i*mmunoassay: y=1.5138x-185.07; r=0.984. Conclusion: This evaluation indicates applicability of the developed biochip based immunoassay for the detection of total beta hCG in serum. This represents a new analytical tool for the detection of pregnancy in test settings.

A-223

Performance Characteristics of Six Automated 25-Hydroxyvitamin D Assays: Mind Your 3's and 2's

S. P. Wyness[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah School of Medicine, Salt Lake City, UT*

Objective: Analyze the performance of 6 automated total 25-hydroxyvitamin D assays using 25(OH)D2/D3 and 25(OH)D3 only samples.

Methods: Access 2 and DxI 800 (Beckman Coulter*), ARCHITECT *i*2000$_{SR}$ (Abbott Diagnostics), ADVIA Centaur XP (Siemens), Liaison XL (DiaSorin) and Modular E170 (Roche Diagnostics) assays were evaluated for imprecision, method comparison and concordance. Imprecision used commercial control material tested in duplicate twice daily for 5 days. Method comparisons used residual serum samples with endogenous D2 and D3 (n=50) or D3 only (n=86). Comparisons with all 136 samples were intended to simulate real-world laboratory testing. Results were compared to an in-house LC-MS/MS method (traceable to NIST SRM 972) using Passing-Bablok regression and Bland-Altman bias plots. Acceptability criteria were coefficient of variation (CV) <10% and bias <15.8%.

Results: Imprecision was acceptable for all assays except E170 and Centaur (both CV 11%). Regression analysis of all samples in comparison to LC-MS/MS demonstrated under-recovery for ARCHITECT, DxI, E170 and Liaison assays (slopes 0.868, 0.983, 0.912, 0.834) while Access and Centaur over-recovered (slopes 1.013, 1.030). All correlation coefficients were below 0.95. Compared to D2/D3 samples, E170 and Centaur showed the greatest improvement in slope without D2 while Liaison was unaffected. Also, E170 under-recovered with D2/D3 and over-recovered in the absence of D2. Access, Centaur and DxI assays exhibited the opposite effect. Constant bias for all samples ranged from -3.3 (Centaur) to 1.7 ng/mL (ARCHITECT). Intercepts improved without D2 present for all assays except ARCHITECT and E170. Centaur constant bias improved the most in the absence of D2. Testing all samples, Centaur had the lowest overall bias (2%) and E170 (20%) and Liaison (22%) exceeded acceptable criteria. Testing D2/D3 samples, DxI and Access had the lowest bias (4%); ARCHITECT (26%), E170 (36%) and Liaison (29%) exceeded acceptable criteria with these samples. In the absence of D2 the Liaison still exceeded this limit (18%), the ARCHITECT had the lowest bias (1%) and E170, Centaur and Access were comparable to each other (8-9%). All assays over-recovered when analyzing vitamin D deficient samples (<20 ng/mL, n=31), with E170 (20%) and Liaison (19%) exceeding bias criteria. Concordance with LC-MS/MS at 20 ng/mL ranged from 77% (Centaur) to 89% (DxI). ARCHITECT, E170 and Liaison concordance improved without D2.

Overall, Access and DxI had slopes close to 1 and acceptable bias for all sample groups. Liaison had the lowest slopes and was not affected by D2. While ARCHITECT slope and intercept were not greatly affected by D2, bias and concordance improved without D2 present. E170 and Centaur assays were most affected by D2, based on improvements in slope, intercept or bias when D2 was absent.

Conclusions: It is important to consider the effects of D2 and D3 on individual assay performance. Assessing performance using total vitamin D may mask possible interferences in supplemented populations.

*Assays pending US FDA approval

A-224

Quantitation of Anti-Müllerian Hormone by the AnshLabs picoAMH ELISA Assay

J. Lu[1], C. Holt[2], M. Choi[2], K. Kalp[2], J. 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 School of Medicine, Salt Lake City, UT*

Background: Anti-Müllerian hormone (AMH) is responsible for regression of the female ductal system during embryonic development. It is produced by the male testes until puberty and the female granulosa cells until menopause. A highly sensitive AMH assay is ideal for investigation of infertility, menopause, ovarian reserve or monitoring granulosa cell tumors post-therapy. The picoAMH ELISA kit (Ansh Labs, Webster, TX, USA) is a new quantitative immunoassay that detects ultra-low concentrations of AMH in human serum. Here, we describe the analytical performance of the picoAMH assay.

Methods: Imprecision studies used manufacturer's controls (65 and 185 pg/mL) and patient pools (201 and 404 pg/mL) assayed in duplicate or triplicate once daily for 10 days. Dilution imprecision was tested using 1:10 and 1:100 dilutions of serum pools assayed in triplicate once daily for 5 days. Limit of blank (LOB) and limit of detection (LOD) were assessed using the blank and 6.3 pg/mL calibrators. Linearity was determined by serially diluting a high AMH serum sample in blank calibrator to create 5 samples tested in duplicate. Recovery was evaluated by adding the highest 2 calibrators to patient samples (84 and 202 pg/mL). Temperature stability was determined by storing 2 specimens (99 and 301 pg/mL) ambient for 24 hr, 4C for 7 days and -20C for 3 weeks. Effects of up to 3 freeze/thaw cycles were studied. Method comparison of 57 samples in the range of 80-181,000 pg/mL was performed using the Beckman AMH Gen II ELISA as the comparator method. Gender/age-specific reference intervals were established using fresh or biorepository serum specimens (6 mos-71 yrs, n=1,273).

Results: Imprecision and dilution imprecision studies showed total CVs ≤ 6.3 and ≤ 8.7%, respectively. LOB and LOD were 0.81 and 3.11 pg/mL, respectively. The assay was linear to 696 pg/mL. Recovery ranged 76-101%. AMH differed <18% at all storage temperatures and <5% after 3 freeze/thaw cycles. Deming regression of the method comparison yielded $y = 0.999 x - 0.226$, $R^2 = 0.99$. Eleven gender/age-specific reference intervals were established using non-parametric and robust statistics.

Conclusions: The AnshLabs picoAMH ELISA demonstrated performance close to the manufacturer claims and excellent correlation with the comparator method. This method lowered the LOD from the current 80 pg/mL to 3 pg/mL. We report gender/age-specific reference intervals for this assay that will be useful for clinical practice.

A-225

Insulin and leptin signaling in placenta from gestational diabetic subjects

A. Pérez-Pérez[1], P. Guadix[1], J. Maymó[2], J. L. Dueñas[1], C. Varone[2], C. González-Rodríguez[1], M. Fernández-Sánchez[3], V. SANCHEZ-MARGALET[1]. *[1]VIRGEN MACARENA UNIVERSITY HOSPITAL, SEVILLE, Spain, [2]University of Buenos Aires, Buenos Aires, Argentina, [3]IVI, SEVILLE, Spain*

Background: Insulin and leptin receptors are known to share signaling pathways, such as JAK2/STAT-3 (Janus kinase 2/signal transduction and activator of transcription 3), MAPK (Mitogen activated protein kinase) and PI3K (phosphoinositide 3-kinase). Both positive and negative cross-talk have been previously found in different cellular systems. Gestational diabetes (GDM) is a pathophysiological state with high circulating levels of both insulin and leptin. We have previously found that these three signaling pathways are activated in placenta from GDM patients to promote translation, involving the activation of leptin receptor. Now, we tested the hypothesis that both leptin and insulin receptors might contribute to this activation in a positive way that may become negative when the system is overactivated.

Methods: To answer this question we studied the activation of leptin and insulin receptors in placenta from GDM and normal pregnancies by Western blot. Besides, we performed in vitro studies with insulin and leptin stimulation of trophoblast explants to study PI3K and MAPK signal transduction pathways by Western blot using specific antibodies of phosphorylated proteins. Bands were scanned and data analyzed by Anova followed by Bonferroni´s post test.

Results: We have found that both leptin and insulin receptors are activated in placenta from GDM. In vitro stimulation of trophoblast explants with both leptin and insulin at submaximal doses (0.1 nM) potentiated the activation of PI3K and MAPK signaling, whereas preincubation with maximal concentrations of insulin (10 nM) and further

stimulation with leptin showed negative effect. Similarly, trophoblastic explants from GDM placenta, which presented high signaling levels, had a negative signaling effect when further incubated in vitro with leptin.

Conclusions: Insulin and leptin receptors have positive effects on signaling, contributing to high signaling levels in placenta from GDM, but insulin and leptin have negative effects upon overstimulation.

A-226

Ultrasensitive Luteinizing Hormone Assay on the MesoScale Discovery Platform

N. Vidal-Folch, S. K. Grebe, R. J. Singh, A. Algeciras-Schimnich. *Mayo Foundation, Rochester, MN*

Background: Luteinizing Hormone (LH) or Lutropin is a glycoprotein composed of α and β subunits secreted by the anterior pituitary gland after stimulation by gonadotropin-releasing hormone (GnRH). In children, LH is measured as an aid in the diagnosis of gonadal disorders such as central precocious puberty (CPP) and delayed puberty. Current automated immunoassays are sensitive to about 0.1 IU/L. These levels are appropriate for adults, but clinical studies suggest that basal LH reference ranges in pre-pubertal children are below the lower limit of detection of automated assays and a more sensitive assay is needed. **Objective:** Develop an ultrasensitive LH assay for pediatric patients using the electrochemiluminescence multi-array technology from MesoScale Discovery (MSD). **Methods:** The LH assay is a sequential two-site electrochemiluminescence laboratory developed test. A monoclonal biotinylated LH capture antibody is added to a streptavidin coated plate, incubated for 30 min and washed to remove unbound antibody. Sample is added and incubated overnight at 4ºC. After washing, a SULFO-TAG™ labeled detection antibody is added. After 2 h the plate is washed and counted on the MSD sector imager reader. The assay is calibrated against the WHO International Standard reference material from NIBSC (2nd IS 80/552). The performance characteristics of the assay were established over two different reagent lots and included determination of imprecision, limits of quantification and detection, linear measurement range and dilution linearity, spike recovery, interferences, sample stability and a method comparison with the Beckman Access LH assay. **Results:** Intra-assay and intra-assay imprecision on patient samples (0.03-25 IU/L) ranged from 1.3% to 3.1% and from 5.8% to 7.9%, respectively. The limit of detection was 0.004 IU/L and the limit of quantitation was 0.02 IU/L (15% CV). The linear measurement range was 0.02 to 28 IU/L. Average dilution linearity and spike recoveries were 92.9% (range 86-122%) and 101% (range 94-104%), respectively. The assay is not affected by hemolysis (up to 1000 mg/dL hemoglobin), lipemia (up to 1000mg/dL triglycerides) or bilirubin (up to 5mg/dL bilirubin). Repeat measurements showed <20% variability for serum and serum-separator tubes up to 14 days ambient or refrigerated and through 3 freeze/thaw cycles. Method correlation using Passing-Bablok regression fit against the Beckman Access LH assay was y=0.9119x-0.04042 and r=0.982 (N=200) in the concentration range of 0.3 - 28 IU/L. **Conclusion:** We have developed an ultrasensitive LH assay useful in the diagnosis of gonadal disorders in pediatric patients. The assay provides accurate results with a 10-fold improvement in functional sensitivity over existing automated assays.

A-227

Preanalytical validation of a serum normetanephrine, metanephrine and 3-methoxytyramine assay

O. M. Itkonen, U. Turpeinen, N. Tohmola, E. Hämäläinen. *Helsinki University Central Hospital, HUS Helsinki, Finland*

Background: Analysis of metanephrine (MN), normetanephrine (NMN) and 3-methoxytyramine (3MT) in plasma or serum has recently replaced the urinary assay in many laboratories for the diagnosis of pheochromocytoma. The aim of this study was to validate preanalytical factors of serum MN, NMN and 3MT assays.

Methods: We used samples from apparently healthy adult volunteers to study sample stability (n=25), sampling device (n=13), postprandial effect (n=7), intra-individual within-day variation and diurnal variation (n=7). Samples (200µL) with [2H3]-labeled internal standards were extracted with Oasis® WCX µElution plates (Waters), washed with water, methanol and 0.1% formic acid in acetonitrile and eluted with 2 x 50 µL of 2% formic acid in 95% acetonitrile-5% water. The eluent (25 µL) was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) employing an Agilent 1200 liquid chromatograph (Agilent Technologies), a 4000 QTRAP mass spectrometer (AB Sciex), and an Atlantis HILIC Silica 50x2.10 mm column (Waters). The LOQ of the assay was 0.025 nmol/L, the intra-assay CV was <7.2%, the inter-assay CV was <8.3%, and the linear range 0.025-5 nmol/L for MN, NMN and 3MT. Paired t-test was performed by Analyse-it for Microsoft® Excel 2003.

Results: Serum NMN and MN were stable (concentration changed <20%) for at least 7 days at room temperature and at +4°C, for 12 weeks at -20°C. NMN was stable during 1 and MN at least during 4 freeze-thaw cycles. No valid stability data of serum 3MT could be obtained because the concentrations were below the detection limit in the majority of our samples. NMN and 3MT concentrations were lower ($p \leq 0.032$) in samples drawn into Li-heparin plasma tubes (mean 0.41 and 0.03 nmol/L, respectively, Venosafe 60 USP U Lithium Heparin tube, Terumo) than in samples drawn into glass tubes (0.49 and 0.05 nmol/L, respectively), clotting catalyzator tubes (0.47 and 0.04 nmol/L, respectively) and SST™ II Advance gel tubes (0.47 and 0.04 nmol/L, respectively). All serum tubes were from Vacutainer. On contrary, MN was the highest in Li-heparin plasma (0.18 nmol/L), but the difference was significant only as compared to serum drawn into catalyzator tubes (0.17 nmol/L, $p=0.0165$). A regular breakfast meal had no effect on serum NMN, MN or 3MT concentrations ($p<0.075$ for all). There was no difference ($p>0.066$) in NMN and 3MT concentrations in samples drawn at 8 a.m. (0.48 and 0.03 nmol/L, respectively), noon (0.51 and 0.04 nmol/L, respectively) and 4 p.m. (0.45 and 0.04 nmol/L, respectively). However, MN concentration was 0.16 nmol/L at 8 a.m., 0.17 nmol/L at noon and 0.19 nmol/L at 4 p.m. ($p=0.0304$). The mean intra-individual within-day variation of NMN, MN and 3MT was 13% (range 7%-23%), 13% (range 3%-13%) and 22% (range 9%-36%), respectively.

Conclusions: To minimize assay variation due to preanalytical factors, we suggest that samples be transported to the laboratory at room temperature but stored frozen. Only 1 freeze-thaw cycle should be allowed before analysis, serum instead of Li-heparin plasma should be used, sampling should occur before noon and no fasting before sampling is required.

A-228

Functional Sensitivity of Five Automated Estradiol Immunoassays

W. E. Owen[1], J. A. Straseski[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: Estradiol (E2) is a steroid hormone produced primarily by the ovaries with small amounts produced in the testes and adrenal cortex. E2 measurement is used for assessing sexual development, fertility disorders, gynecomastia, estrogen-producing tumors and hyperplasia in the adrenal cortex. E2 is also used in monitoring fertility therapy for patients undergoing in vitro fertilization. Imprecision and method-to-method differences, especially at clinically important low concentrations, continue to be problematic for E2 immunoassays. We studied the functional sensitivity (FS) of five automated E2 immunoassays.

Methods: We evaluated the ARCHITECT i2000 (Abbott), DxI 800 (Beckman), ELECSYS E170 (Roche), and ADVIA Centaur and IMMULITE 2000 (Siemens) immunoassays. Five pools of different concentrations were each prepared by combining serum samples with comparable E2 concentrations as determined by LC-MS/MS. Pools were aliquotted and stored frozen (-70 ^{0}C) until testing. Imprecision was evaluated over 12 days using two lots of reagent and two calibrations. Five aliquots per pool (one per method) were thawed per day and assayed once per run, one run per day, two days per week, and three weeks per reagent lot (total=12 replicates). FS was determined by fitting a power function to the imprecision data using Excel.

Results: The FS's (ng/L) for ARCHITECT i2000, DxI 800, ELECSYS E170, ADVIA Centaur, and IMMULITE 2000 were determined to be 3, 39, 11, 30, and 22 respectively. All methods met manufacturer's claims except ADVIA Centaur (12.5 ng/L). Mean concentrations per pool are summarized in Table 1.

Conclusions: The ARCHITECT i2000 and ELECSYS E170 showed the best performance with FS's below 20 ng/L. However, it has been suggested that FS of 5 ng/L or lower are needed for clinical usefulness. Additionally, these immunoassays did not provide comparable mean E2 concentrations for the serum pools tested. Further harmonization of E2 immunoassays is required, particularly at lower concentrations.

Comparison of pool E2 cencentrations (ng/L) and FS (ng/L) by method

Pool		LC-MS /MS	ARCHITECT i2000	DxI 800	ELECSYS E170	ADVIA Centaur	IMMULITE 2000
1		15.4	25.8	19.1	15.5	29.5	25.5
2		36.0	38.0	39.3	33.6	41.0	41.6
3		101.0	86.1	93.1	92.3	89.8	88.8
4		202.0	160.3	215.6	207.2	183.3	210.3
5		651.0	524.8	738.8	782.3	631.1	634.9
FS		0.5	3	39	11	30	22

A-229

Development of a sensitive Dried Blood Spot Anti-Mullerian Hormone (AMH) ELISA

A. Kumar[1], B. Kalra[1], A. S. Patel[1], G. Savjani[1], L. Lechtenberg[2], C. R. Garcia[2]. *[1]Ansh Labs, Webster, TX, [2]University of Pennsylvania, Philadelphia, PA*

Background: The aim of this study was to develop a highly sensitive and simple dried blood spot human AMH ELISA to assess ovarian reserve.

Relevance: AMH has been reported to be strongly associated with age, antral follicle counts (AFC), FSH and has emerged as a clinically useful biomarker of ovarian reserve. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. Dried blood spot specimens stability makes it a practicable alternative to venous blood. It opens new possibilities in AMH testing, such as comparison of historical to current patient results; simplified blood sampling for patients in remote locations or for those who are homebound. Instead of traveling to a clinic to get blood drawn, a blood spot sample can be taken at a convenient site and mailed to a laboratory. This technology will be especially useful for monitoring ovarian function of physically challenged cancer patients undergoing chemotherapy.

Methods: A three-step, sandwich-type enzymatic microplate assay has been developed to measure AMH levels in two 7.9 mm dried blood spot disc in less than 6 hours. The assay measures human AMH and uses stabilized recombinant human AMH as calibrators (7-1000 pg/mL). This method uses a drop of whole blood collected on filter paper from a simple finger stick. The sample is eluted from the dried blood spot in an extraction solution and is added directly to the well. The assay measures the bio-essential AMH and does not exhibit interference by hematocrit in the extracted spot.

Results: Ansh Labs DBS AMH ELISA (AL-129), when compared to Ansh Labs US AMH ELISA (AL-105) using 56 matched serum and dried blood spot samples in the range of 62-18443 pg/mL yielded a correlation coefficient of 0.98 ($p < 0.0001$) and a slope of 0.96 with an intercept of -7.56 pg/mL. DBS AMH ELISA (AL-129) when compared to Ansh Labs picoAMH ELISA (AL-124) using 65 matched serum and dried blood spot samples in the range of 5-5240 pg/mL yielded a correlation coefficient of 0.99 ($p<0.0001$) and a slope of 1.02 with an intercept of -4.7 pg/mL. Serial dilution of seven extracted dried blood specimens (5000-11000 pg/mL) in the sample diluent showed an average recovery of 87-105%. Total imprecision, calculated on 3 controls over 40 runs, 2 replicates per run, was 5.84% at 22.58 pg/mL, 3.15% at 86.51 pg/mL and 4.34% at 373.18 pg/mL. The functional sensitivity of the assay calculated at 20% CV was 3.9 pg/mL.

Conclusion: A highly simplified, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age. The DBS results are comparable to serum based assays. The specimen stability, ease and low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

A-230

Nonalcholic Steato-hepatitis (NASH) in Type 2 Diabetes: Serum Body Fat-normalized Plasma Leptin Level is a Predictor of Serum Alanine Aminotransferase Level

H. S. Chaudhury[1], S. Akter[2], S. Murshed[2], M. K. Rahman[2], L. Ali[2]. *[1]International Medical College, Dhaka, Bangladesh, [2]Biomedical Research Group, BIRDEM, Dhaka, Bangladesh*

Background: Leptin is a multifunctional hormone which may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. Anti-steatotic function of leptin is well demonstrated in animal studies. Nonalcholic steato hepatitis (NASH) is common complication of metabolic syndrome and T2DM. In present study we investigated the role of leptin in the development of NASH in type 2 diabetes.

Methods: A total of 119 subjects were included. In Group I (Case), 59 newly diagnosed T2DM and in Group II (Comparison), 60 age (year, 36±4 vs. 35±4, M±SD) and Body Mass Index (BMI)-matched (kg/m2, 24.0±3.1 in Group I vs. 23.6±2.0 in Group II) healthy control subjects were included in this observational study. Plasma insulin (fasting and 30 min post-glucose) and leptin were estimated by Enzyme Immunoassay. Insulin secretory capacity (HOMA-B%) and insulin sensitivity (HOMA-S%) were calculated by Homeostasis Model Assessment using HOMA-CIGMA software. Fasting serum non-esterified fatty acid (NEFA) was measured by enzymatic-colorimetric method. Hepatocellular damage was assesed by serum alanine aminotransferase (ALT) which was measured by enzymatic colorimetric method. Serum ALT level > 30 u/l was considered elevated.

Results: The diabetic subjects showed highly significant β-cell dysfunction and also insulin resistance as evident from HOMA B% [20.2(4.2-89.6) in diabetic vs. 78.4(35.5-365.7) in control, $p<0.001$] and HOMA S% [84.6(39.1-226.4) vs. 118.8(22.0-3573.0), $p<0.004$]. Serum fat normalized leptin level was found significantly lower in diabetic subjects [ng/ml, 5.44 (0.65-34.7)] compared to controls [8.35 (1.36-55), p=0.012]. Diabetic subjects had higher prevalence of elevated serum ALT compared to control subjects (33% vs 62%, p <0/01). The fat-normalized serum leptin level inversely correlated with insulin secretory dysfunction. The serum ALT level was correlated with fat-normalized serum leptin level (r= -0.224, p=0.016), serum triacylglycerol (r= 0.372, $p<0.001$) and phase 1 insulin secretion (r= -0.213, p=0.024). The total NEFA level in the diabetic subjects was higher than control (mmol/l, 0.652±0.196 vs. 0.42±0.15, $p<0.001$).

Conclusion: The data suggest that a) Low plasma leptin in type 2 diabetes mellitus subjects is associated with insulin secretory dysfunction; and b) Elevated serum ALT in diabetic subjects is associated with lower level of fat-normalized leptin and decrease in phase 1 insulin secreation. c) The fat-normalized serum leptin is a predictor serum ALT level.

A-231

Predicted decrease of plasma 1,5-anhydroglucitol (AG) in presence of inhibitors of glucose reabsorption (SGLT2 inhibitors): potential utility of AG as a primary marker of drug effect

D. Fortuna, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Renal reabsorption of glucose under conditions of normoglycemia is essentially 100%. Drugs that inhibit renal glucose reabsorption, via inhibition of the main renal glucose transporter (sodium-glucose transporter-2, or SGLT2), have recently been approved for use in the U.S. for treatment of Type 2 diabetes (e.g., Invokana). Renal reabsorption of plasma 1,5-anhydroglucitol (AG, 1-deoxyglucose), an unregulated, non-metabolizable glucose analogue derived from diet, is normally >99%; normal plasma AG represents a balance between slow rates of input (5 mg/day) and excretion. In diabetes, plasma AG is often substantially decreased due to accelerated renal loss that occurs when glucose concentration is high enough to saturate reabsorption capacity (viz., under conditions of glucosuria). Correspondingly, plasma AG is likely to be directly affected by drugs that inhibit glucose reabsorption. Our objective was to examine this potential effect using an established mass balance model for AG and varying the AG reabsorption fraction according to the same degree of the effect on glucose reabsorption caused by the new reabsorption inhibitors.

Methods: We used a two-compartment AG mass balance model previously described (*Am J Physiol Endocrinol Metab* 1997;273:E821-E830). If displaced from steady-state, changes in body total AG (T) are given by $dT/dt = ki - T (GFR\ fe)/(1+K)/V$, where ki = AG input rate (5 mg/day), GFR = glomerular filtration rate (nominally 100 mL/min), K is the ratio between tissue and plasma compartments (K = 2.1), V is the plasma volume (nominally 3 L), and fe is the fractional excretion (0 to 1) of filtered AG. In normoglycemic steady-state, fe is <0.01 (= fe(ss)). According to literature, fractional excretion of glucose in presence of target blood concentrations of the SGLT2 inhibitor dapagliflozin is 35%-50%. We assumed an equivalent effect, fe = 0.35, for AG in the presence of inhibitor. Using normal, steady-state plasma AG = 21 ug/mL as an intial condition, we calculated the model-predicted time course of changes in plasma AG following a step increase in fe from fe(ss) to fe = 0.35.

Results: The model predicts an exponential decrease in plasma AG when fe is increased. For fe = 0.35, plasma [AG] transitions rapidly from a normal value (AG = 21 ug/mL) to a new steady-state (AG = 0.9 ug/mL) within approximately 24 hours, with t1/2 = 3.1 hours. According to the model, the same t1/2 would be operative for any starting plasma AG upon initiation of the same degree of inhibition of AG reabsorption. Low GFR will slow this effect (e.g., t1/2 = 6.8 hours for GFR = 45 mL/min) but will not affect the eventual net % change in plasma AG.

Conclusions: SGLT2 inhibitors are predicted to produce a rapid and substantial decrease in plasma AG. The effects of SGLT2 inhibitors would obviate the usual intent of AG measurement, which is to verify increasing plasma AG as a marker of improvement in glycemic control. Conversely, however, plasma AG measurement might potentially be useful in SGLT2 therapy precisely because it might (is predicted to) act as a direct marker for successful inhibition of reabsorption of hexoses.

A-232

Rapid and Cost-effective Determination of Plasma Renin Activity in Human EDTA Plasma by Two Dimensional Liquid Chromatography-Tandem Mass Spectrometry

T. Guo, B. Yue. *NMS Labs, Willow Grove, PA*

Background: Determination of plasma rennin activity (PRA) is a critical part in evaluation of primary aldosteronism, which accounts for at least 2% of hypertensive patients. PRA assay is used to quantitatively assess the capacity of renin to generate angiotensin I (Ang I) from angiotensinogen. PRA has traditionally been measured by radioimmunoassay (RIA) with fair sensitivity and consistency when optimal sample handling and incubation conditions were applied. However, RIAs lack specificity due to potential cross-reactivity between the Ang I antibody and other endogenous peptides, have a limited dynamic range, and require laborious sample handling. Several liquid chromatography tandem mass spectrometry (LC-MS/MS) based assays were reported to measure PRA using either off-line or on-line solid phase extraction (SPE). The off-line SPE based cleanup is labor-intensive and time consuming; while on-line SPE is not cost effective. We developed a rapid and cost-effective two dimensional LC-MS/MS assay to measure PRA in human plasma.

Methods: An API-5000 triple-quadrupole mass spectrometer (AB-Sciex) coupled with electrospray ionization (ESI) source and Shimadzu HPLC system was used to quantify Ang I in human plasma after incubation. Plasma samples with double-labeled degradation standard (DS) were incubated in a water bath at 37 ºC for three hours. Labeled internal standard (IS) were spiked prior to protein precipitation with acetonitrile. After centrifugation, the supernatants were transferred into injection vials. 70 µL extracted sample were injected onto a ZORBAX-C8 columns as the first dimension and a Synergi Polar-RP column with a water/acetonitrile/formic acid gradient as the second dimension. The ESI source was operated in positive ion mode with ionspray voltage at 4000 V and heater temperature at 400 ºC. Quantitation by multiple reaction monitoring (MRM) analysis was performed. Two ion pair transitions were monitored for the analyte and its IS/DS.

Results: The assay was validated as linear over the range from 0.15 to 150 ng/mL for Ang I. The lower limit of quantiation (LLOQ) was 0.15 ng/mL for Ang I. Within-run CVs were < 3.0% for all three levels of QC samples tested. Between-run CVs were 4.14% for low QC, 3.59% for mid QC, and 4.3% for high QC Samples, respectively. Recoveries ranged from 69% to 81% for Ang I. Mean carryover was <0.13% for Ang I in 3 runs. No interference was observed. Preliminary comparison with a validated LC-MS/MS method for PRA was assessed as follows: y = 1.06x + 0.03 (r=0.999, n=6).

Conclusion: LC-MS/MS method offers specificity superior to that of RIAs. This 2D LC-MS/MS method can rapidly measure PRA in human plasma within 3.5 minute. Compared to off-line SPE, the advantages of this method include simplicity, high throughput, and low cost. Thus, it can be routinely employed in a clinical environment.

A-233

Impact of angiotensin II receptor blockade on the renal cortical tissue renin-angiotensin system during the normoalbuminuric stage of diabetic mellitus in the rat.

N. Ishii[1], Y. Kurosaki[1], P. K. Carmines[2], Y. Yamada[3], T. Tsukushi[4], A. Imoto[1], T. Ichikawa[3], M. Yokoba[1], H. Suzuki[5], H. Ikenaga[6], Y. Aoki[7], T. Takenaka[8], T. Ichikawa[1], M. Katagiri[1]. *[1]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [2]University of Nebraska College of Medicine, Omaha, NE, [3]Kitasato University Graduate School of Medical Sciences, Sagamihara, Japan, [4]Kitasato University Hospita, Sagamihara, Japan, [5]Kitasato junior college of health and hygienic sciences, Minamiuonuma, Japan, [6]Ikenaga Clinic, Otawara, Japan, [7]Kanagawa Health Service Association, Yokohama, Japan, [8]International University of Health and Welfare, Sanno Hospital, Minato-ku, Japan*

Background: The circulating renin-angiotensin system (RAS) produces changes in plasma angiotensin II (AngII) levels as a mechanism for regulating blood pressure and maintaining fluid and electrolyte homeostasis. The renal tissue RAS can function independent of the circulating RAS. One key component of the renal tissue RAS is the (pro)renin receptor ((P)RR), which is able to bind either renin or prorenin. When bound to the (P)RR, prorenin catalyzes formation of angiotensin I from angiotensinogen (similar to the action of renin). Increased renal (P)RR expression has been reported to contribute to development of diabetic nephropathy (DN) through a pro-inflammatory mechanism (*Clin Exp Pharmacol Physiol*37:277-82,2010). Renal oxidative stress has also been implicated in DN, and RAS inhibition suppresses renal cortical oxidant production even during the early, normoalbuminuric stage of DM (*Clin Sci*124:543-52,2013).

Objective: The goal of this study was to evaluate the impact of systemic RAS inhibition (AngII receptor blocker;ARB), and the attendant suppression of renal oxidative stress, on the renal tissue RAS during the normoalbuminuric stage of DM.

Methods: Four groups of rats (n=5 per group) were examined: 1) STZ group: rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg,*i.p.*), 2) Sham group: rats receiving the STZ vehicle, 3) STZ+TLM group: STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group: TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. We quantified the following parameters in renal cortex: 3-nitrotyrosine (3-NT) production (an oxidative stress marker; by HPLC), AngII levels (by RIA), (P)RR expression, and expression of both angiotensin type-1 and type-2 receptors (AT_1R and AT_2R by western blotting).

Results: Similar to previous reports, blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups. Blood pressure did not differ among groups. GFR was increased in STZ group compared with Sham (P<0.05), and this was prevented by TLM-treatment (P<0.05 vs.STZ+TLM). Renal cortical 3-NT production was increased in STZ compared with Sham (P<0.05); however, TLM suppressed this phenomenon (P<0.05vs.STZ+TLM). Renal cortical AngII levels did not differ among groups. In contrast, STZ rats showed significant increases in renal cortical (P)RR (323±52% of Sham;P<0.05) and both of 42 and 58kDa AT_1R expression (286±6% and 228±5% of Sham, respectively;P<0.05). These changes were prevented by TLM treatment (P<0.05vs.STZ+TLM), although TLM did not alter either parameter in the Sham group. Renal cortex AT_2R expression was elevated in the STZ group (155±6% of Sham;P<0.05), and further increased by TLM-treatment (182±10% of Sham;P<0.05).

Conclusions: During the normoalbuminuric stage of DM in the rat, the renal cortex exhibits upregulation of major components of the intrarenal RAS (AT_1R, AT_2R and (P)RR) without a change in tissue AngII levels. The DM-induced changes in AT_1R and (P)RR expression are prevented by systemic AT_1R blockade, and may arise via oxidative stress. These observations indicate that the renoprotective effects of ARB may involve not only an antioxidant effect but also effects that rely on suppression of the intrarenal RAS.

A-234

Comparison of Immunoassays to Mass Spectrometry for Free and Total Testosterone in Men, Women, and Children

S. L. La'ulu[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: Circulating testosterone may be bound to albumin, sex hormone binding globulin (SHBG) or remain free. Measurement of these various forms of testosterone provides an overall assessment of androgen status and aids diagnosis of several conditions in men, women, and children. Study objectives were to compare 5 commercially available immunoassays to mass spectrometry for free and total testosterone in adults and children.

Methods: Residual serum samples from men (n=150), women (n=100), boys (n=25), and girls (n=25) were obtained after completion of clinical testing for total testosterone (TT) by liquid chromatography tandem mass spectrometry (LC-MS/MS). Free testosterone (FT) was determined in men using equilibrium dialysis (ED)/LC-MS/MS. All samples were further tested for TT and SHBG by the Abbott ARCHITECT *ci*8200, SIEMENS ADVIA Centaur and IMMULITE 2000, Beckman Coulter DxI, and Roche Modular E170. Albumin was measured using the Abbott ARCHITECT *ci*8200 and Roche c702. FT was calculated using the Vermeulen equation. For women, boys, and girls, calculated FT by immunoassays was compared to calculated FT using TT by LC-MS/MS.

Results: Comparisons using Deming regression for TT and FT in men and women are provided (Table). For boys and girls, slopes for TT ranged from 0.72 (IMMULITE) to 1.14 (ARCHITECT) and 0.84 (IMMULITE) to 1.76 (DxI), and slopes for FT ranged from 0.82 (IMMULITE) to 1.25 (ARCHITECT) and 1.06 (ARCHITECT) to 1.18 (DxI), respectively. Overall, more immunoassays under-recovered in men and women and over-recovered in boys and girls for both TT and FT. The average of absolute percent bias was highest in boys for both TT (92.4%) and FT (98.1%) compared to men (8.7% and 10.8%, respectively).

Conclusions: Consistent biases were not observed amongst methods and populations evaluated. Challenges with accurately measuring testosterone appear to remain in some immunoassays, but not all.

Total Testosterone					Free Testosterone				
Method	Slope	Intercept	R	% Bias	Method	Slope	Intercept	R	% Bias
Men (comparison to LC-MS/MS)					Men (comparison of calculated FT to ED/LC-MS/MS				
ARCHITECT	1.07	-0.87	0.985	8.6	ARCHITECT	0.98	0.66	0.954	8.2
Centaur	0.99	-41.0	0.906	-12.3	Centaur	1.00	-0.85	0.831	-12.5
DxI	0.76	34.3	0.970	-8.8	DxI	0.82	0.25	0.970	-14.1
E170	0.95	-7.83	0.985	-7.6	E170	0.86	0.45	0.959	-8.4
IMMULITE	0.81	25.3	0.922	-6.2	IMMULITE	0.82	0.42	0.906	-10.9
Women (comparison to LC-MS/MS)					Women (comparison to calculated FT using TT by LC-MS/MS)				
ARCHITECT	1.04	0.64	0.995	20.8	ARCHITECT	1.06	0.00	0.990	21.0
Centaur	0.89	7.15	0.943	22.0	Centaur	0.93	0.10	0.933	22.7
DxI	0.73	23.9	0.927	81.4	DxI	0.85	0.30	0.917	82.6
E170	0.93	-1.87	0.985	-9.9	E170	0.94	-0.02	0.990	-9.9
IMMULITE	0.71	10.2	0.970	-3.3	IMMULITE	0.76	0.12	0.970	34.3

Tuesday, July 29, 2014

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

Factors Affecting Test Results

A-235

Thrombin-Mediated Degradation of Parathyroid Hormone in Rapid Serum Tubes

S. L. La'ulu[1], J. A. Straseski[2], R. L. Schmidt[2], 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: Measurement of parathyroid hormone (PTH) is important for the clinical assessment of parathyroid disease and calcium homeostasis. Previous studies have demonstrated decreased stability of PTH in serum versus plasma specimens, although the precise mechanism for this difference has not been established. If thrombin activation during clot formation is responsible, the effect should be exacerbated in tubes containing additional thrombin. Exogenous thrombin is a constituent of Vacutainer Rapid Serum Tubes™ (RST; BD Diagnostics, Franklin Lakes, NJ), which include bovine thrombin to induce accelerated clot formation. As a known thrombin cleavage site exists in the 84 amino acid human PTH polypeptide, we hypothesized that *ex vivo* PTH cleavage in RST tubes might occur. Such a possibility has analytical and clinical implications, as intact PTH diagnostic tests are sandwich immunoassays that use paired capture/detection antibodies to the N- and C-terminal regions of PTH.

Methods: 1) *Screening Study*: Initial experiments were conducted to determine whether measurement of analytes with potential thrombin cleavage sites would be affected by RST tubes. Aliquots from previously collected serum specimens were incubated for 24 hrs in either an RST or a Serum Separator Tube™ (SST) before analysis on a cobas e602 immunoanalyzer (Roche Diagnostics, Indianapolis, IN). A subset of replicate experiments was performed on an ARCHITECT $i1000_{SR}$ (Abbott Diagnostics, Lake Forest, IL). 2) *Fresh Collections*: To verify findings in freshly collected specimens, three tubes of fresh blood were drawn from each of 10 healthy normal donors. Specifically, we collected one Plasma Separator Tube™ (PST), one SST, and one RST from each donor. After clotting and centrifugation, intact PTH assays on the cobas e602 were performed at multiple time points on aliquots held at ambient temperature or 4°C. Confirmatory experiments were conducted using exogenous thrombin and the direct thrombin inhibitor hirudin.

Results: In screening studies, PTH results were lower after specimen incubation in RSTs versus SSTs. These findings were confirmed in additional time-course experiments and on a separate immunoassay platform. In fresh collections, RST and SST specimens stored at room temperature also showed a decrease in PTH results (versus PST specimens) beginning at our earliest measurement time-point (approximately 1 hr post-collection). The magnitude of this decrease was more prominent in RSTs versus SSTs. A similar (but smaller and slower) decrease in PTH results in serum specimens was seen in aliquots stored at 4°C. Further studies confirmed that the decrease in measured PTH was thrombin-mediated, as it was blocked by hirudin.

Conclusion: The present study demonstrates that thrombin is responsible for the decrease in PTH results observed in RSTs. It is presumed that endogenous human thrombin, activated during clot formation, may be responsible for the smaller decreases observed in SSTs. As there is an incomplete understanding of which additional polypeptides may possess bovine thrombin cleavage sites, these studies provide a simplified screening strategy that laboratories can use when evaluating RSTs for assays at their own institutions.

A-236

The effect of different protease inhibitors in blood samples taken for parathormone, insulin, and prolactin analysis.

O. Baykan, A. Yaman, F. Gerin, O. Sirikci, G. Haklar. *Marmara University School of Medicine, Istanbul, Turkey*

Background: Proteolytic degradation by proteases can affect peptide hormone levels, which becomes especially important when there is a lag time between sampling and analysis. This should be taken into consideration when the samples are conveyed from peripheral to central laboratories. Five group of proteases (serine, cysteine, acid, metallo, and threonine) exist with various mechanisms of action. In our study we aimed to evaluate the protective role of different protease inhibitors on the degradation of parathormone (PTH), insulin, and prolactin in blood samples.

Methods: Blood samples (n=10) were collected from healthy volunteers into vacutainer tubes with gel seperator (Becton Dickinson, NJ, USA) with a) no additive, b), 1% protease inhibitory coctail (PIC) (Sigma) which inhibits serine, cysteine, and acid proteases, and aminopeptidases added immediately after blood sampling, c) PIC added after centrifugation (30 min after sampling) d) aprotinin which inhibits serine proteases (500 KIU/mL) (Sigma) added immediately after blood sampling and e) K2 EDTA (1.8 g/L). The samples were allowed to clot for 30 min and centrifuged at 1300xg for 10 min. Then, each batch of sample either stored at 4°C or at room temperature (RT) until analyzed at 6, 24, 48, and 72 hours and compared against baseline values. Insulin, PTH, and prolactin levels were measured with electrochemiluminescence immunoassay in modular E170 (Roche Diagnostics, Germany) analyzer. The desirable bias values were taken from the Westgard QC database.

Results: All parameters remained within desirable bias limits when stored at 4°C until 72 hours. PTH exceeded desirable bias limits when stored at RT longer than 24 hours. PIC addition before or after centrifugation inhibited protease associated PTH degradation. Since the PIC amount was less when added after centrifugation, a more economic application became possible. Insulin stored at RT decreased higher than desirable bias limits after storing longer than 6 hours and only EDTA preserved insulin at RT. Addition of PIC before centrifugation led to hemolysis which enhanced the insulin degradation through proteases. Prolactin remained to be stable under each condition.

Conclusion: These results shows that when the samples are conveyed between different locations, the preservation of peptide hormones should be kept in mind. Although this can be achieved with various protease inhibitors, storing the samples at 4 ºC from sampling until analysis, will work equally well.

A-238

Pre-analytical factors effecting Alzheimer's disease biomarker stability in CSF

D. R. Kozo, I. Baburina, J. B. Courtney, K. Williams, C. N. Baldasano, S. J. Salamone. *Saladax Biomedical, Inc., Bethlehem, PA*

Background: Evaluation of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD) is becoming increasingly important to improve the reliability of ante-mortem disease diagnosis to ensure proper patient management. Development of highly precise assays for $amyloid_{1-42}$ (Aβ42) and tau protein has allowed investigators to better characterize pre-analytical sample handling factors that may affect clinical interpretation. A prospective collection study was designed to model different CSF handling scenarios for storage conditions at the clinical site, followed by shipping to and then storage and handling at the testing site. **Objective:** Model CSF handling scenarios to identify conditions that would not significantly impact the determination of Aβ42 and tau protein. **Methods:** CSF was prospectively collected from 46 healthy individuals under an IRB approved protocol. A 10mL CSF aliquot was collected by lumbar puncture in the L3/L4 or L4/L5 interstitial space. One milliliter aliquots were stored and shipped from the collection site at: -80°C, -20°C, 4°C and 25°C. Using an immunometic chemiluminescent assay, 6 randomly selected patient samples were used to investigate storage, shipping and handling conditions, including thaw conditions, post thaw handling, and freeze/thaw cycles. Additional studies examined the influence of varying the storage tube manufacturer and the tube type and lot for a single manufacturer. **Results:** When stored at -20°C and 25°C, Aβ42 values were from 5 to 20% lower compared to the control stored at -80°C. For aliquots stored at ambient temperature for 48 hours, the Aβ42 values were 40% lower. There was little to no effect on tau concentrations across the range of storage temperatures. Aliquots initially stored at -80°C or ambient temperature showed the greatest decreases in measured Aβ42 levels (10 to 15%) following a second freeze/thaw cycle, whereas no loss of tau protein was observed following a freeze/thaw cycle. Thawing CSF samples at ambient temperature versus a water bath did not affect concentrations of either biomarker. Results were consistent between patients. Storing samples for 2h at ambient temperature post thaw resulted in less than 10% loss for either marker. The Eppendorf Lobind® tubes allowed transfer of CSF and storage at 4°C for up to six days without significant loss of either Aβ42 or tau protein biomarkers. No significant difference in either Aβ42 or tau protein concentrations was observed between different lots and sizes of these tubes. **Conclusion:** The measured level of Aβ42 was significantly affected by CSF storage, shipping and handling conditions, although there was no appreciable impact on tau. The utility of these markers in routine testing can be improved by using conditions at the collection and testing sites that minimize analyte losses. Immediate storage of freshly collected CSF at -80°C and shipment on dry ice gave the best Aβ42 recovery. These conditions will also preserve tau protein. Also, it was found that use of Lobind® tubes did not affect the measurement of either marker.

A-239

Using Factorial Design-of-Experiments (DOE) to Investigate Interactions among Pre-Analytical & Analytical Factors in the Laboratory

C. Hild, R. Paulsen, K. Whigham. *Aegis Sciences Corporation, Nashville, TN*

Loss of cannabinoids from solution during processing and analysis has been shown. Roth et al (1996) showed the impact of storage and kinetic conditions on THC-COOH loss due to analyte binding to surfaces. Stout, Horn, and Lesser (2000) show the contribution of storage temperature to THC-COOH loss in urine specimens. In addition to the factors commonly investigated, we investigate the interactive effects of storage, sample and processing factors on THC-COOH loss. The first behavior investigated is a consistent low bias of 10-12%; the second behavior studied is a less frequent, 30-60% loss of analyte encountered in some patient samples. Erratic losses of 30-60% was observed in less than 0.2% of specimens tested. Both pre-analytical as well as analytic factors, and the interactions amongst these factors, were theorized causes of these observed losses. In order to understand the causes of the bias as well as the outliers in measured concentrations, six handling, storage, and processing factors were investigated.

Methods The investigation of factor effects and interactive effects on the THC-COOH loss during storage, handling, and processing conditions is studied using a sequence of investigative studies. The impact of dilution method on THC-COOH loss was investigated across four analytical chemists. Each chemist prepared replicate dilutions by two different methods on a non-glucuronidated solution fortified to 100 ng/mL concentration. The first method prepared a dilution at a 2 mL exact volume; the second method removed 500 mL of excess solution after dilution preparation. Graphical analysis of variance (ANOVA) was used to investigate the impact of the dilution method on percent loss.

The outlier losses in THC-COOH observed during urinalysis by GCMS were studied using a blocked, fractional factorial design of experiment (DOE). These six factirs were: initial specimen state (thawed vs. never frozen), storage conditions (light vs. dark), vortexing, pipette transfer, glucuronidated vs. non-glucuronidated specimens, and temperature (42ºF vs. Room Temperature). 64 samples were tested across all levels of the factors. Impact on THC-COOH concentrations were evaluated through a least squares screening analysis that included both main effects and all pair-wise interactions.

Conclusions 1. The dilution method had a statistically significant effect on % loss. The dilution preparations that required removal of excess solution resulted in 40-50% loss of THC-COOH. This effect reproduced across all four chemists who performed dilutions.

2. The experiment showed three factors and two interaction effects as statistically significant. Non-glucuronidated specimen exhibited greater losses across the 64 samples with a maximal loss of 40%. Non-glucuronidated samples lost 20-30% more THC-COOH than did the gluceronidated samples. Transfers had the greatest effect on non-glucuronidated samples and had an additive effect with vortexing. Exact-volume pipetting reduced the impact of vortexing.

The control of kinetic and handling conditions is essential in order to reduce the loss of THC-COOH during normal processing of urine specimens. The relationships amongst the sample handling factors with state of conjugation provide insight into causes of THC-COOH loss in GC-MS analysis.

A-240

Developing a Cutoff for Urinalysis of Bloody Urine

S. Albahra, K. Born, R. Martinez, W. B. Furmaga. *UTHSCSA, San Antonio, TX*

Background: When bloody urine is submitted to the lab for testing, the most common procedure is to spin it down and test the clear supernatant. Clarity of supernatant is visually determined by technologist which caries a subjective bias. Additionally, plasma contents may significantly alter the results of testing, despite passing a visual inspection. In our project we were looking for objective criteria that define bloody urine which is acceptable for laboratory testing.

Methods: Both, hemolyzed whole blood (n=11) and fresh, non-hemolyzed blood where mixed with urine at known decreasing concentrations. After spinning down, the supernatants were tested by dipstick and by chemistry analyzer. The precipitants were submitted for microscopic examination.

Results: We found that blood has a significant impact on urinalysis even when it accounts for 0.8% of the urine volume. In this mixture, dipstick showed (+3) blood concentration, and the total protein as well as albumin level went up by 448% and 2240% respectively, when compared to the concentration in the urine. Significant differences were also noted in microscopic examination. Similarly, the mixture of hemolyzed whole blood and urine at 0.8% concentration showed an increase of 1762% in the total protein and 4560% in the albumin. The mixture of urine with blood at 0.08% concentration showed (+2) blood concentration by dipstick and an increased concentration of total protein by 160% and albumin by 700%. Microscopic evaluation was affected to a lower extent and the rest of the analytes tested within clinically acceptable ranges.

Conclusion: We have demonstrated that dipstick can be used as a method to evaluate acceptability of bloody urine for laboratory testing. A blood concentration of (+2), which correspond to 0.08% of blood volume within the urine, may consist of acceptable cut off for most of the analytes with exception of total protein, albumin and RBC/WBC on microscopic evaluation. If the blood concentration within the blood/urine mixture exceeds 0.08% the specimen is not acceptable for the majority of testing such as total protein, albumin, creatinine, specific gravity, and microscopic analysis.

A-241

The Effect of Hemolysis and Lipemia on 23 Analyte Values Measured on an Abbott c16000 Chemistry Analyzer

K. Doyle[1], P. Bach[2]. *[1]University of Utah, Salt Lake City, UT, [2]Intermountain Healthcare, Murray, UT*

Background: Medical laboratory test values may become erroneously elevated or decreased due to interfering substances or endogenous contamination such as hemolysis, icterus, or lipemia (HIL). Spectrophotometric-based assays are of particular concern as these can be affected by interferents that absorb incident light or inhibit transmitted light. The objective of this study was to evaluate the effects of hemolysis and lipemia on several analytes as determined on an Abbott c16000 chemistry analyzer with particular attention as to how interference could affect low, medium, or high baseline analyte values and how any observed changes could alter the clinical treatment of a patient.

Methods: Hemolysate was prepared from discarded whole blood specimens and Intralipid was obtained from the pharmacy. For each of 23 analytes to be measured, residual serum or plasma samples containing low, medium, and high concentrations of analyte were pooled to obtain a sufficient volume at a desired concentration. Hemolysate or Intralipid was added to these samples prior to analysis to obtain a final concentration of 65, 320, 1180 mg/dL hemoglobin (Hgb) or 50, 250, 1000 mg/dL triglyceride, respectively. Analyte concentrations and indices for hemolysis and lipemia were compared to unmodified specimens.

Results: Of the 23 analytes tested, 18 were partially or significantly altered by the presence of hemolysate or lipemia. Deviation from baseline values was caused either from increased analyte in hemolysate or Intralipid; or by interference of the spectral quantification of the analytes. Results of significant clinical interest are summarized below:

Interferent	Measured Analyte	Significance of Interference
Hemolysate	LDH	+ 1.2 U/L for every 1 mg/dL of Hgb
	AST	+ 5.7% of the Hgb value (U/L AST, mg/dL Hgb)
	Mg	+ 0.3-0.4 mg/dL per 100 mg/dL Hgb
	K	+ 0.3 mmol/L per 100 mg/dL of Hgb
	P	+ 0.2 mg/dL per 100 mg/dL of Hgb
	Total protein	+ 0.3 g/dL per 100 mg/dL Hgb
	Lactic acid	+ 1.0 mmol/L per 1000 mg/dL of Hgb
	Direct bilirubin	Both Hgb and analyte concentration dependent
	Triglycerides	+ 5 mg/dL per 100 mg/dL of Hgb
Intralipid	Mg	+ 0.1 mg/dL per 100 mg/dL of Intralipid
	Total Protein	+ 1 g/dL per 300 mg/dL of Intralipid
	Lactic acid	+ 1.1 mmol/L per 1000 mg/dL of Intralipid
	Direct bilirubin	Both lipid and analyte concentration dependent
	Triglycerides	Directly proportional

Conclusion: Our results provide a basis for predicting changes in analyte concentrations coordinate with hemolysis and lipemia indices on an Abbott c16000 chemistry analyzer, thereby enabling laboratory personnel to (1) assess the quality of the sample; (2) mitigate inaccurate test results; and (3) provide clinicians direction in interpreting results of specimens harboring interferents.

A-242

How to reduce TAT delays in a large Molecular Biology Laboratory

V. T. Alástico, L. C. Scarpelli, V. D. T. Niewiadonski, A. Alfieri, C. F. A. Pereira, N. Gaburo Jr.. *DASA, Sao Paulo, Brazil*

Background: Quality can be defined as the ability of a product or service to satisfy the needs and expectations of the customer. Turnaround time (TAT) is one of the signs

of laboratory service and is often used as indicator of laboratory performance. To improve the service's quality, the TAT should be constantly monitored and the outliers should have their causes investigated to avoid further delays.

Objective: The aim of this study was to measure and to reduce TAT delay in a large Molecular Biology laboratory.

Methods: Statistical study was performed during the period from January to December of 2013. In this period, the total number of tests reported and the number of tests release with delay were verified, based on reports obtained from our internal Laboratory Information System (LIS -Motion). TAT goals were determined according to the complexity of each test. For tests based on manual procedures; the goal was defined as 95% of the tests released monthly within the TAT established. For automated tests, the goal was defined as 98%. From July to December, the following actions were taken: meeting with the team to present the TAT data and mapping the process to identify the main causes of delays, such as pre-analytical (errors in patients input information, lack of documents needed for consistency analysis of the results) and analytical causes (routine days not well established, lack of trained labor, turnover). Additionally, some improvement alterations in the routine were done: training, define routines priority, changing platform from manual to automated. The data was monitored monthly using histograms for each test involved.

Results: Every month an average of 27.000 results are reported at DASA's Molecular Biology laboratory. From January to June the average percentage of delays was 0,89%, according to a report generated by internal management system (Motion). In the second semester, (July to December) the percentage of TAT delays was 0,49%. We observed that changing manual tests to automated platforms and team training were the most significant factors for reducing TAT delays.

Conclusion: In our experience, we were able to reduce the laboratory TAT delays in 45% by implementing strategies to improve the process and making people committed to the service's quality.

A-243

Bias, imprecision and uncertainty evaluation for some immunoassays

F. Yesildal[1], T. Ozgurtas[1], M. Serdar[2], I. Kurt[1]. *[1]Gulhane Military Medical Academy, Ankara, Turkey, [2]Acıbadem University, Faculty of Medicine, Ankara, Turkey*

Background: Bias (B), imprecision (I) and uncertainty (U) are important parameters in evaluating the analytical performance of a laboratory. Internal quality control (IQC) data can be used as an indicator of imprecision while external quality assessment (EQA) data can be used to detect biases. We can calculate the uncertainty of a measurement, using these data and other uncertainty resources. The purpose of this study was to evaluate the analytical performance of immunoassays in our core laboratory according to Fraser criteria.

Methods: B, I and U of 12 immunoassays were calculated according to EURACHEM/CITAC Guide, by using the IQC and EQA data of the period between January 2013 and December 2013. Cortisol, Estradiol, FSH, hCG, Insulin, LH, Prolactin, Parathormone, FreeT3, FreeT4, TSH and Testosterone assays were used for evaluation of analytical performance of immunoassay autoanalyzers - ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., USA). Desirable specifications for allowable total error (TE), I and B were used in evaluation according to Fraser criteria.

Results: Cortisol, estradiol, insülin, LH, prolactin, and TSH met the desirable specifications for B, I and TE. hCG, FreeT4 and testosterone performance were the worst of all and met none of the desirable specifications. Additionally, FSH and free T3 had imprecision problem while parathormone had inaccuracy problem (Table 1). **Conclusion:** Immunoassays are difficult tests to achieve standardization. There are many factors affecting the immunoassays and the amount of measurand is very small, so accuracy and precision are very important. When it is not possible to meet the optimum and desired specifications, minimum specifications may be considered. Uncertainty does not mean error or doubt about the measurement, but it is about the confidence of the result of measurements. Each laboratory should make this evaluation to determine whether there are systematic or random errors.

Table 1: Evaluation of each immunoassay according to Fraser criteria.

	Calculated Uncertainty	Comment (TE)	Calculated Bias	Comment (B)	Calculated Imprecision	Comment (I)
Cortisol	27,84	PASS	10,52	PASS	9,11	PASS
Estradiol	17,31	PASS	6,16	PASS	6,08	PASS
FSH	18,37	FAIL	7,37	PASS	5,48	FAIL
hCG	38,01	FAIL	16,20	FAIL	9,94	FAIL
Insulin	23,73	PASS	9,55	PASS	7,04	PASS
LH	16,78	PASS	6,78	PASS	4,95	PASS
Prolactin	14,83	PASS	5,70	PASS	4,74	PASS
PTH	28,95	PASS	12,64	FAIL	7,04	PASS
Free T3	12,72	FAIL	3,65	PASS	5,20	FAIL
Free T4	22,10	FAIL	8,13	FAIL	7,48	FAIL
TSH	16,19	PASS	5,36	PASS	6,07	PASS
Testosterone	32,29	FAIL	12,56	FAIL	10,14	FAIL

A-244

Sample recollection, an experience in a hospital where samples are collected by nurses, São Paulo, Brazil.

L. R. Almeida. *DASA, São Paulo, Brazil*

Background In recent years, there has been increasing interest in quality improvement and patient safety activities in healthcare. The clinical laboratory has a leader in the field of healthcare quality management with a focus on analytical quality born of its scientific background and was one of the first areas to use quantitative statistical control methods. However laboratories are now being asked to widen their focus to consider activities outside their immediate control. Accreditation agencies are increasingly requiring laboratories to go beyond analytical quality and take responsibility for the pre- and post-analytical (or extra-analytical) phases where most errors arise. Blood sample collections are performed by venipuncture for the implementation of this procedure it is required that the professional is qualified, have technical training and practical experience in nursing. During the analytical process it is identified that the sample cannot be analyzed due same pre-analytical interference, the most frequent causes are inadequate, insufficient material, hemolysis and clotted. Consistent final result does depend only by the analytical process, a well collected sample is also important. When the samples are collected by nurses it is expected a high number of recollection because nurses are more used to the patient care, laboratory practices is not part of the experience.

Methods: The laboratory as an outsource service follows the number of recollection monthly and to keep the total number of blood recollection according with standards recommendations of 2,0% is challenging once the procedure is hold by the nurse team. All recollection we analyzed considering only reasons related with the technics applied during the procedure. The causes of recollection are, inadequate material, insuficiente, hemolysis and clotted samples.

Results: In 2013 the laboratory of a private hospital received 414.902 solicitation of laboratorial tests, 0,7% of this total were recollected, 45% were from hemolysis, 38% clotted, 12% insuficiente material and 3% inadequate material. 91.428 patients were attended in this period.

Conclusion: Nurses that Work in the laboratory are more used to blood collection than the team that work with patient care, so the laboratory must follow the procedure done by the hospital and is responsible for improving these number of recollection by stablishing a training program , and also avaliate if the program is effective because the main reason of following these data is to reduce the impact to the patient.

A-245

Stability and clinical usefulness of thyroid hormones, TSH, GH, IGF1, BP3, SDHEA, and cortisol results in serum frozen at -20°C after long- term storage.

E. Kutasz, J. M. Lazzati, M. Maceiras, V. Zaidman, S. Rodriguez, M. Rubinstein, E. A. Chaler. *Hospital de Pediatría Garrahan, Buenos Aires, Argentina*

Background: Stored samples are commonly used. It would be necessary to verify if samples stored for a given time at -20°C in a serum bank maintain stability for subsequent clinical interpretation.

We believe that the definition of stability should be different for long-term or short-term storage conditions.

The concept of uncertainty (U) is useful for long-term storage conditions because it considers all sources of error in a result. Error may be associated with method precision (CVa%: analytical coefficient of variation) and with method accuracy (bias). Collaborative Peer Group data are used to calculate the bias of the results. Therefore

U is calculated according to the following formula: $U= 2\sqrt{(CVa)^2 + bias_{media}\%^2 + (CV_{group}/\sqrt{n_{lab}})^2}$. Where CV_{group} is group coefficient of variation and n_{lab} number of laboratories in the Peer group.

The reference change value (RCV: $Zx\sqrt{2} \times \sqrt{(CVa2 + CVi2)}$ Where Cvi is biological coefficient of variation) shows a significant change if two consecutive results are outside this range, which may be interpreted as a change in patient status.

Objective: To assess stability and/or clinical usefulness of the results of thyroid hormones, TSH, GH, IGF1, BP3, SDHEA, and cortisol in serum stored at -20°C for 8 months.

Materials: Statistically representative numbers of sera from pediatric patients were evaluated according the following process T0: measured between 1 and 2 hours post-extraction. T1: after 8-month storage at -20°C. .

Results were considered stable and/or clinically useful if T1 was within the 95%CI for U and RCV, respectively.

Results:

Analyte (n)	U % (95%CIT_1)	RCV % (95%CIT_1)
TSH (40)	11.81 (NS)	58.6 (CU)
T3 (40)	14.39 (S)	34.7 (CU)
T4 (40)	12.18 (NS)	28.3 (CU)
fT4 (40)	7.75 (NS)	34.0 (CU)
GH (27)	15.82 (NS)	20.0 (NCU)
IGF1 (27)	15.59 (NS)	20.8 (NCU)
BP3 (27)	There is not peer group	23.0 (CU)
CORTISOL (21)	16.42 (NS)	30.0 (CU)
SDHEA (27)	17.27 (NS)	28.6(CU)

Stable: S

Not Stable: NS

Clinically Useful: CU

Not Clinically Useful: NCU

Conclusions: Under these storage conditions only T3 remained stable and the results could be used for reference range and/or diagnosis-related group values. GH and IGF1 were not stable, and their results were not clinically useful. The remaining results were not stable but were clinically useful.

A-246

Factual or fictitious hypocalcemia?

M. Chen, B. C. Handy, E. A. Wagar, Q. Meng. *MD Anderson Cancer Center, Houston, TX*

Background: Calcium plays very important physiological functions. Measurement of serum calcium helps to identify many clinical disorders. Accurate results play a pivotal role in patient management. Unrecognized hypocalcemic emergencies can lead to significant morbidity or death. On the other hand, misdiagnosed hypocalcemia can result in inappropriate management and significant impact on patient care. Spurious hypocalcemia is not uncommon in clinical laboratory practice, and distinguishing false hypocalcemia from true hypocalcemia is essential. We report here two typical cases of hypocalcemia.

Methods: Total calcium and ionized calcium were measured from the original sample and redrawn blood samples to verify the results.

Results: Case 1 was a 19-year-old man with acute leukemia who underwent leukophoresis in which acid-citrate-dextrose formula A (ACD-A) was used as the anticoagulant. His initial blood chemistry results were as follows: serum calcium 7.9 mg/dL (normal range 8.4-10.2), ionized calcium 1 mmol/L (1.13-1.32), serum magnesium 2.0 mg/dL (1.8-2.9), serum phosphorus 8.3 mg/dL (2.8-4.6), blood urea nitrogen 25 mg/dL (8-20), and serum creatinine 1.26 mg/dL (0.70-1.30). The ionized calcium measured by point of care testing was 1.04 mmol/L (1.12-1.32). Repeat measurements from specimens collected next day showed consistently low total and ionized calcium: serum calcium 6.6 mg/dL (8.4-10.2) and ionized calcium 0.84 mmol/L (1.13-1.32) on chemistry analyzer. Case 2 was a 10-year-old boy with multiple endocrine neoplasia type 2 whose calcium level was initially unmeasurable. Calcium measurement was repeated several times from this sample and the results were the same. Magnesium and iron also were reported as undetectable from this sample, while potassium level was greater than 10.0 mmol/L. Specimen was recollected into a serum separator tube, and analysis of this sample revealed a calcium level of 9.4 mg/dL and potassium level of 4.5 mmol/L.

Conclusion: The low total and ionized calcium results in Case 1 are suggestive of factual hypocalcemia. The low calcium is presumed to be due to the chelation of calcium by citrate in the ACD-A during dialysis. Citrate-induced hypocalcemia is a major side effect of dialysis. Case 2 is clearly a fictitious hypocalcemia caused by EDTA contamination due to the wrong order of draw. Spurious hypocalcemia needs to be immediately recognized and appropriately interpreted in order to avoid misdiagnosis and unnecessary intervention.

A-247

Laboratory investigation of spurious hyperkalemia

M. Chen, B. C. Handy, E. A. Wagar, Q. Meng. *MD Anderson Cancer Center, Houston, TX*

Background: Pseudohyperkalemia is a laboratory artifact that is induced during the process of specimen collection, transportation, and preparation. The most common causes for spurious hyperkalemia include *in vitro* hemolysis, excessive tourniquet time or fist clenching during phlebotomy, contamination with potassium ethylenediaminetetra-acetic acid (K_2EDTA), and specimens collected from patients with hematological disorders such as leukocytosis and thrombocytosis. Factitious hyperkalemia occurs frequently in laboratory practice. Here we report two representative cases that presented with plasma potassium greater than 10.0 mmol/L, illustrating the investigation process for hyperkalemia.

Methods: Various types of peripheral blood specimens were collected. Repeat measurements of potassium were performed on different analyzers including Vitros Fusion 5.1, blood gas analyzer IL Premier 3000, and POCT. The results of these analyses were compared.

Results: Case 1 was an 84-year-old woman with chronic lymphocytic leukemia (leukocytes 181,300/μL). Her plasma potassium levels were persistently elevated (>10.0 mmol/L). Repeated measurements of potassium on Vitros Fusion 5.1 showed a potassium level >10.0 mmol/L in lithium-heparin plasma with or without gel but a potassium level of 3.6 mmol/L in serum. A sample collected into a lithium-heparin balanced syringe and analyzed by blood gas analyzer IL Premier 3000 showed a potassium level of 3.6 mmol/L, while a sample collected simultaneously in lithium-heparin showed a level of 11.1 mmol/L on Premier 3000. The patient's potassium level as determined by POCT was 2.6 mmol/L. Case 2 was a 10-year-old boy with multiple endocrine neoplasia type 2 who presented with a potassium level greater than 10.0 mmol/L. Surprisingly, the levels of calcium, magnesium, and iron in this sample were undetectable. In another specimen collected separately in a serum separator tube, the potassium was reported as 4.5 mmol/L and the calcium 9.4 mg/dL.

Conclusions: Severe hyperkalemia is a potentially life-threatening condition; immediate recognition and appropriate interpretation are critical. Case 1 is clearly a pseudohyperkalemia attributed to heparin-induced cell lysis in leukocytosis, whereas case 2 is confirmed due to contamination of K_2EDTA during specimen collection not following the order of draw. Venous blood collected in a lithium-heparin balanced syringe and analyzed by blood gas analyzer is recommended for potassium measurement in patients with leukocytosis. Standard operating procedures and order of draw should be followed for specimen collection. An investigation algorithm needs to be developed and the laboratory should follow the algorithm when pseudohyperkalemia is suspected.

A-248

Case Report: Multiple False Electrolyte Abnormalities In A Patient with Primary Biliary Cirrhosis Due To Extreme Hypercholesterolemia

M. S. Farooqi[1], L. D. Racsa[1], K. Jessen[2], S. Bosley[2], S. Nadkarni[2], I. A. Hashim[1]. *[1]University of Texas Southwestern Medical Center, and Parkland Health & Hospital System, Dallas, TX, [2]Parkland Health & Hospital System, Dallas, TX*

Background: Primary biliary cirrhosis (PBC) is an inflammatory, likely autoimmune, liver disease marked by the destruction of intrahepatic bile ducts. Common features of PBC include hyperbilirubinemia, pruritus, and elevation of plasma lipids, especially total cholesterol (TC). 96% of symptomatic patients are reported to have TC concentrations greater than 200 mg/dL, with an average of 377 mg/dL and a range of 77 mg/dL to 1035 mg/dL. We report a case of PBC where the finding of hyponatremia led to the discovery of a plasma TC level > 2000 mg/dL.

Methods: All measurements were made on patient plasma utilizing a Roche cobas® c501 instrument using manufacturer-supplied reagents and instructions unless otherwise specified.

Case Report: A 43 year-old female with PBC was admitted to the hospital after outpatient laboratory tests showed hyponatremia. Her complaints on admission included blurry vision, nausea, and significant pruritus. Physical exam was remarkable for scleral icterus; no xanthomas were noted. Relevant abnormal laboratory results

were: sodium, 121 mmol/L (reference range: 135-145); potassium, 3.0 mmol/L (3.6-5.0); chloride, 87 mmol/L (98-109); and total bilirubin, 10.0 mg/dL (0.2-1.3). She was started on intravenous (IV) fluids (0.9% sodium chloride). Subsequently, a lipid panel was ordered. Her previous lipid panel from 1.5 years ago showed a plasma TC concentration of 322 mg/dL (120-199). Her current lipid panel showed a TC concentration of 2156 mg/dL; triglycerides, 226 mg/dL (50-150); and HDL, 37 mg/dL (45-65). The LDL was unreportable. This was the highest total cholesterol value measured by our laboratory. An investigation took place to determine if this was an erroneous result. There was no evidence of contamination. The sample appearance was clear and not grossly viscous or lipemic. The hemolysis index of the sample was 3, icterus index, 11, and lipemic index, 73. No significant interference is expected below indices of 700, 14, and 2000, respectively, per the manufacturer. Furthermore, serial dilution of the specimen indicated no interferences. A second sample from the patient was obtained and showed a TC value of 2415 mg/dL; triglycerides, 299 mg/dL; and HDL, 42 mg/dL (LDL, unreportable). Treating these as accurate values, we investigated whether the patient's sodium concentration was falsely low due to hyperlipidemia. Direct ion-selective electrode measurement of the initial sample on a Radiometer ABL825 FLEX® analyzer showed a sodium concentration of 141 mmol/L (137-145); potassium, 4.4 mmol/L (3.6-5.5); and chloride, 105 mmol/L (101-111). Serum lipoprotein electrophoresis done at a reference laboratory confirmed the elevated TC level at 2295 mg/dL and found the major component to be lipoprotein X.

Conclusion: This case is important due to the degree of hypercholesterolemia, lack of lipemic sample appearance, and its link to multiple false electrolyte abnormalities. To our knowledge, there is only one other report of a PBC patient with a cholesterol level > 2000 mg/dL, and it, too, was associated with pseudohyponatremia significant enough to prompt clinical action. In this case, the clinical team stopped treatment with IV fluids upon learning of the patient's hyperlipidemia, and the patient was eventually discharged.

A-250

Biological Variation as a goal for comparability of thyroid stimulating and free-thyroxine hormones measurements in patients

J. Diaz-Garzon, V. Parrillas, P. Fernandez-Calle, R. Mora, M. Duque, J. Iturzaeta, R. Gomez-Rioja, A. Buno. *Hospital Universitario La Paz, Madrid, Spain*

Background: Laboratory testing of serum thyroid stimulating hormone (TSH) and free-thyroxine (FT4) are essential for the assessment of thyroid function. However, changes of methodologies could limit the application of clinical guidelines depending on the standardization status. The IFCC Working Group for Standardization of Thyroid Function Tests reports no significant differences in healthy individuals within TSH assays (when calibrators are traceable to WHO international standards). They used 10% as harmonization goal that is considered the current state-of-the-art of immunoassay comparability. The same occurs with FT4 quantification if methodologies are traceable to reference method.

Objective: To assess the comparability of patients' results between two TSH and FT4 immunoassays using Biological Variation (BV) criteria as comparability goal.

Material and methods: Following CLSI EP9-A2IR protocol, TSH and FT4 from 40 randomly selected patient serum samples were performed in two analysers, Elecsys (Roche) and Advia Centauro XP (Siemens) by duplicate. Both TSH methods were traceable to second and third WHO international standards (81/565 and 80/558) respectively. FT4 methods were traceable to different standards: Elecsys was traceable to the reference measurement procedure based on equilibrium dialysis and Centauro was traceable to internal standard using United States Pharmacopoeia material. Analytical imprecision of all methods were within desirable limits based on BV. BV desirable bias was set as analytical goal: TSH 7.82 % and FT4 3.34 %. Confidence interval at 95% (CI) of the predicted difference was compared to laboratory allowable bias at medical decision levels (MDL).

Results: Table shows the main results of the study.

TEST Units (Assayed range)	MDL	Difference	CI	Allowable BV desirable bias	Goal achieved
TSH µIU/mL	0.27	0.05	-0.10 to 0.19	0.02	Not
(0,37-4,71)	4.20	-0.71	-0.86 to -0.55	0.33	Not
FT4 ng/dL	0.90	0.1	0.06 to 0.13	0.03	Not
(0,87-1,63)	1.80	-0,18	-0.24 to -0.13	0.06	Not

Conclusions: Despite standardization efforts, applying biological variation criteria, comparability of TSH and FT4 results could not be guaranteed in patients. When a change of methodology occurs, laboratory should always perform studies in order to alert clinicians about the possible non interchangeability of patients' results with possible impact in their follow-up.

A-251

PATHFAST™ Presepsin (sCD14-ST) Sample Matrix Evaluation

E. Spanuth[1], M. Preusch[2], R. Thomae[3], E. Giannitsis[4]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]Department of Internal Medicine III, Intensive Care Unit, University Hospital Heidelberg, Heidelberg, Germany, [3]Mitsubishi Chemical Europe, Düsseldorf, Germany, [4]Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany*

Background Soluble CD14 is released from monocytes during activation by TLR4-specific inflammatory reaction against infectious agents yielding presepsin (sCD14-ST). Presepsin demonstrated powerful diagnostic and prognostic information in critical ill patients with infectious-inflammatory diseases. The objective of our study was to examine the suitability of different sample types for presepsin determination.#

Methods Whole blood samples were collected from 20 patients in serum, lithium heparin, K2 EDTA and Na3 citrate blood collection tubes (S-Monovette, Sarstedt, Germany). The patients were hospitalized with different degrees of infectious-inflammatory conditions at the intensive care unit (ICU).Serum and plasma samples were prepared from each whole blood sample by centrifugation (20 minutes at 2500 x g) and separation of plasma/serum within 2 hours after blood drawing. The native serum and plasma samples were measured using the PATHFAST™ Presepsin assay. For estimation of the inflammatory status C-reactive protein (CRP) values were determined in the heparin plasma samples using the cobas CRP assay (Roche Diagnostics).

Results No considerable differences of the presepsin concentrations measured in the different sample matrices were observed. The samples spanned a significant portion of the measurement range of the test. EDTA plasma samples ranged from 161 to 7400 pg/ml. The measurement of presepsin in the different sample matrices revealed CVs between 2.11 and 12.34 % showing a high concordance between the samples. The results are summarized in Tab. 1.

Tab. 1: Presepsin concentrations (ng/L) measured by using PATHFAST™ Presepsin in different sample matrices

	N	Mean	Median	Minimum	Maximum	IQR
Serum	20	1715	618	169	7251	273-2453
Heparin plasma	20	1749	635	182	7335	277-2610
Na-citrate plasma	20	1614	559	194	6997	265-2225
EDTA plasma.	20	1715	632	161	7400	223-2373

Conclusion The results demonstrated excellent comparability between the different sample matrices across the full measurement range suggesting that PATHFAST™ Presepsin may be useful for risk stratification of critical ill patients from inflammatory diseases in the emergency room and point-of-care setting.

A-252

Reference Intervals of Many Common Chemistry Tests are Affected by Advancing Age in Adults

A. K. Füzéry[1], Y. Qiu[2], M. La[2], G. S. Cembrowski[2]. *[1]Alberta Health Services, Edmonton, AB, Canada, [2]University of Alberta Hospital, Edmonton, AB, Canada*

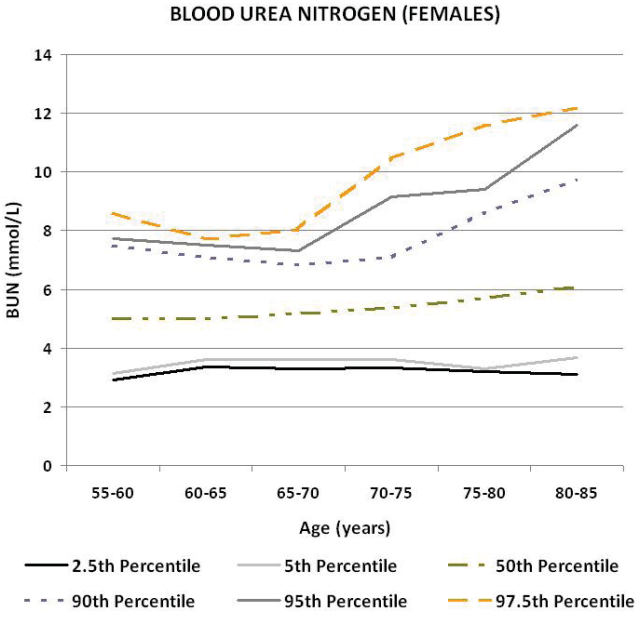
Objectives: More than 40 million Americans are over 65 years of age and their number will grow rapidly over the next 40 years. Current laboratory reference intervals have age-specific ranges for some tests but only for infants, children, and non-senior adults. Not enough is known about the effect of aging on many tests and geriatric reference intervals are not widely available. This study aims to determine the effect of advanced age in adults on chemistry reference intervals.

Methods: We compiled the results of common chemistry tests and pertinent demographic data of 55 to 85 year old US volunteers sampled in 4 cycles of the US National Health and Nutrition Examination Survey (2005-2006, 2007-2008, 2009-2010, 2011-2012). Reference interval diagrams were constructed with the 97.5, 95, 90, 50, 5, and 2.5th percentiles plotted against age intervals: 55-60 years (n=64 males, n=45 females), 60.1-65 years (53 M, 72 F), 65.1 to 70 years (50 M, 63 F), 70.1 to 75 years (50 M, 65 F), 75.1 to 80 years (51 M, 57 F), and 80.1 to 85 years (94 M, 83 F).

Only non-Hispanic whites with a waist circumference less than 105 cm (male) or 100 cm (female) were included in this study.

Results: The Figure shows a very interesting reference interval diagram. The increase in BUN with age is clearly apparent. We observed similar patterns for creatinine, potassium, osmolality, and total bilirubin. In contrast, we observed inverse relationships with age for albumin, ALT, and cholesterol. We found only minimal age-dependent relationships for ALP, AST, calcium, chloride, globulins, total CO2, iron, LD, sodium, phosphorus, and total protein.

Conclusions: Advancing age in the adult population affects many common chemistry tests. Our results suggest that it will be necessary to include age-appropriate reference intervals when reporting test results for geriatric patients.



A-253

Keeping Bacteria Under Control to Minimize Impact on Assays and Maximize Analyzer Uptime

M. Gauthey Baraou, S. Mabic. *Merck Millipore, Saint-Quentin-en-Yvelines, France*

Low bacteria count in pure water is particularly critical in clinical analyzers, because bacteria can generate numerous interferences in biochemistry and immunochemistry assays. The objective here is to describe several of those issues on assays and analyzer maintenance, and provide solutions to avoid bacterial contamination in water supplying the analyzer.

Typical impacts of bacterial contamination on assays include unstable calibrations, high absorbance of blanks, reference drifts, and errors on mean patient values. Those effects are observed, for instance, on the Arsenazo calcium assay, the potassium potentiometric assay, as well as immunoassays involving alkaline phosphatase (*e.g.* CTNI, fluorescence 6-MUP- and AMPPD-based assays). Those effects generated by the typical bacteria strains identified in clinical analyzers (*Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Caulobacter crescentus*) result from proteins and small organic acids released by bacteria.

Bacteria also have an effect on maintenance of the analyzer: an incomplete rinsing generates interferences with the 340 nm detection of assays using NAD/NADH chemistry and the frequency of sanitization can be significantly increased. Maintaining low bacteria level in the analyzer and the water purification system supplying the instrument can reduce downtime and minimize the risks of false results.

Some of the issues mentioned above and resulting from poor design can be avoided by selecting key purification technologies, such as ultrafiltration and germicidal 254 nm UV lamps. A reduction of two logs is observed on bacteria levels of water treated by UV mercury lamps (*ca* 100 cfu/mL to < 1 cfu/mL, using CLSI® recommended bacteria culture procedure). Blank variation in the CTNI assay was shown to decrease over a 30 day period, from a range of 18 to 52 mAU to a range of 20 to 26 mAU by adding an ultrafiltration step to the water treatment process. The ultrafiltration removes the alkaline phosphatase that is released by bacteria, and interferes with the biochemical cascade of the CTNI assay. The stability of the blank ensured a much higher reproducibility of the results and reduced the need for frequent calibrations of the assay.

Recommendations for the maintenance of the water purification systems are described to ensure a consistent supply of clean water to feed clinical analyzers, in-line with CLSI C3-A4 guideline.

A-254

Comparison of RBC hemolysis according to plasma and serum separator tubes among outpatient specimens

D. Ko, D. Won, T. Jeong, W. Lee, S. Chun, W. Min. *University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Republic of*

Background: In our laboratory, we use plasma separation tubes (PST) for chemistry analysis to obtain outpatient results rapidly. If lactate dehydrogenase (LD) measurement is required, serum separation tubes (SST) are used. PST can be used immediately after centrifugation with no need for clotting, and a greater volume of blood can be obtained. Although many studies have compared the results of blood chemistry analysis using PST and SST, there has been no evaluation of hemolysis using these types of tube. Herein, we compare the hemolytic index obtained using PST and SST, and apply this to quality control in the laboratory.

Methods: We analyzed the hemoglobin index of outpatients visiting the Asan Medical Center (Seoul, Korea) from January to December 2012. The hemolytic index, a quantitative serum index, was scored from 0 to 10 according to the concentration of hemoglobin (0-5.0 g/L) using the Toshiba-200FR automated instrument (Toshiba Medical Systems Co., Tokyo, Japan). Hemolytic index was classified by sample tube type (PST or SST), and significant hemolysis was defined as a hemolytic index of 2 or greater. In cases of significant hemolysis, electronic medical records were reviewed to identify the cause.

Results: Significant hemolysis was found in 0.66% (1,128 of 171,519) of the total specimens, 0.68% (1,051 of 154,886) of PST specimens, and 0.46% (77 of 16,633) of SST specimens. The mean hemolytic index in PST was 0.18 (SD: 0.43), which was significantly greater than that in SST (0.14, SD: 0.37) (P<0.001). The proportion of significant hemolysis was also higher in PST than in SST (P=0.001). The cause of significant hemolysis was identified in 48.1% (543 of 1,128) of the specimens; the causes of in vivo hemolysis were chemotherapy and prosthetic valve. The remaining hemolysis specimens (51.9%, 585 of 1,128) may have been due to complex sampling errors. Hemolysis of unknown cause was particularly common in pediatric samples.

Conclusion: The incidence of hemolysis was slightly higher using PST compared to SST, although both were <1%. We conclude that PSTs are thought to be more useful than SST in outpatient testing because they offer more rapid turnaround and can contain a greater sample volume in our laboratory.

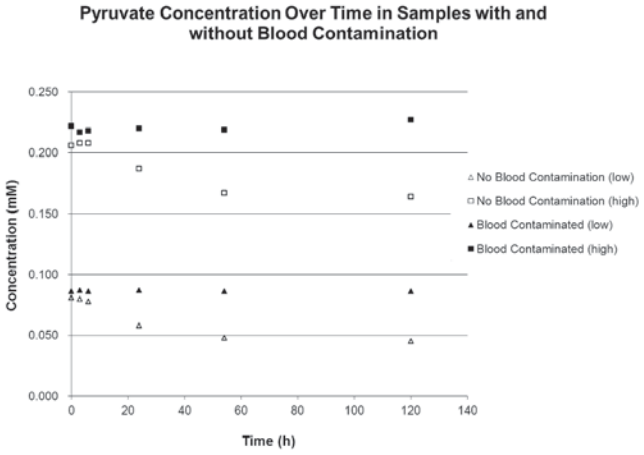
A-255

The effect of storage at room temperature on the measures of lactate and pyruvate in cerebrospinal fluid with and without blood contamination

L. Zhang[1], J. Gabler[2], D. Payto[3], M. Na[1], S. Wang[3]. *[1]Cleveland State University, Cleveland, OH, [2]ThermoFisher Scientific, San Jose, CA, [3]Cleveland Clinic, Cleveland, OH*

Background: Measurement of pyruvate and lactate, intermediates of carbohydrate metabolism, in cerebrospinal fluid (CSF) is important in evaluating disorders of the central nervous system. However, the stability data of these analytes in CSF is scarce in literature, especially for pyruvate. Objective: To assess the in-vitro stability of pyruvate and lactate at room temperature (RT) in CSF specimens with and without blood contamination. Method: Blood contaminated and non-contaminated CSF specimens were collected for this study. Separate pools were made for contaminated and non-contaminated samples. Unspiked pools were used for low concentration levels (0.09mM pyruvate and 1.9mM lactate) and high levels were spiked at 0.22mM pyruvate, 3.5mM lactate. Pools were stored at RT over 120h while duplicate samples were aliquoted at each of 6 time points (0h, 3h, 6h, 24h, 52h, 120h). Samples were deproteinized (12% trichloroacetic acid) and analyzed by enzymatic assays for both lactate and pyruvate. Results: Pyruvate concentrations were constant up to 6h in non-contaminated CSF samples at both concentrations (<5% decrease). The concentrations of pyruvate progressively decreased after 6h, reducing up to 45% after 120h and showing a greater instability at the low concentration level. In contaminated samples (hemolytic index=38), pyruvate concentrations showed no significant change through 120h (<3% decrease) at both high and low levels. At low concentration,

lactate concentration was constant through 48h in both contaminated and non-contaminated samples. High lactate concentration remained constant through 6h. Conclusion: Pyruvate and lactate at physiological concentrations in CSF specimens with and without blood contamination remain constant at RT up to 6h.



A-256

Performance Evaluation of Olympus AU2700 Plus by Six-Sigma Using Three Different Internal Quality Control Materials

C. Yılmaz Demirtas[1], M. Kocabıyık[1], F. Gucel[2], S. Elbeg[1], O. Gulbahar[1]. *[1]Gazi University Medical Faculty, Ankara, Turkey, [2]Etlik Zubeyde Hanım Gynecology Training and Research Hospital, Ankara, Turkey*

Background: The analytical performances of the instruments in the medical laboratories should be satisfactory. Six-sigma level is a measurement of quality in the evaluation and comparison of performance. We evaluated the sigma levels of the parameters in our comprehensive metabolic panel in a single instrument by using different internal quality control (QC) materials.

Methods: The chemistry instrument evaluated was Olympus AU2700 Plus (Beckman-Coulter, Brea, CA, USA) and the QC materials provided were Beckman Coulter (Beckman-Coulter, Brea, CA, USA), BioRad (Bio-Rad Laboratories, Hercules, CA, USA) and Randox (Randox Laboratories, North Ireland, UK). Our comprehensive panel included glucose, creatinine, uric acid, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol, triglyceride, calcium, potassium, magnesium, amylase, creatine kinase, gamma-glutamyl transferase (GGT), uric acid, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), sodium, chloride, albumin and total protein. We measured internal QC materials at two levels for 20 days consecutively. If a measurement was outside two standard deviation range then a rerun preformed. Analytical reproducibility assessed using the CLSI EP-5 protocol. CLIA criteria were the basis for the allowed total error values except for two parameters, which are GGT and direct bilirubin.

Results: Our data is expressed in the table with two cutoff values: six-sigma of more than six and less than three.

Conclusion: Health services aim zero error but being under the influence of many variables it is difficult to achieve this goal. Six-sigma methodology is useful in the planning of laboratory quality control process. The sigma level calculated for each test may provide insight about the quality. We found that the calculated sigma levels using different QC materials are similar in some parameters but different in others. We support the idea of using appropriate internal quality control material for each test and this procedure can guide in reducing the cost of poor quality.

Table 1: Calculated six-sigma values of three different QC materials.

QC material	Six-sigma >6	Six-sigma <3
Beckman Coulter level 1	Glucose, creatinine, uric acid, total bilirubin, ALT, ALP, total cholesterol, triglyceride, calcium, potassium, magnesium, amylase, creatine kinase, and GGT	Sodium, chloride, and total protein
Beckman Coulter level 2	Creatinine, uric acid, total bilirubin, AST, ALT, ALP, triglyceride, LDH, magnesium, amylase, and creatine kinase	Total protein, sodium, chloride, and GGT
BioRad level 1	Uric acid, total bilirubin, triglyceride, calcium, potassium, magnesium, and amylase	ALT, LDH, and sodium
BioRad level 2	Uric acid, ALP, triglyceride, potassium, chloride, magnesium, amylase, and creatine kinase	Urea, total bilirubin, AST, and sodium
Randox level 1	Uric acid, total bilirubin, total cholesterol, triglyceride, magnesium, amylase, and creatine kinase	Glucose, urea, AST, ALT, LDH, sodium, and chloride
Randox level 2	Albumin, uric acid, ALP, total cholesterol, triglyceride, magnesium, amylase, and creatine kinase	Glucose, LDH, and sodium

A-257

Bilirubin oxidase resolves bilirubin interference in a colorimetric acetaminophen assay

A. Abbadi, J. M. El-Khoury, E. Z. Reineks. *Cleveland Clinic, Cleveland, OH*

Background: Acetaminophen is a pain reliever found in over-the-counter and prescription medications. Acetaminophen toxicity is the most common cause of drug overdose and acute hepatic failure in the US. Measurement of acetaminophen levels in blood, once a suspicion of toxic ingestion has been established, is crucial for risk assessment, treatment and management. However, samples with high levels of bilirubin (icteric), which are very common in individuals with drug-induced hepatotoxicity, interfere with acetaminophen measurement in most commercial clinical assays and can lead to false positive results. Bilirubin oxidase (BOx) is an enzyme that catalyzes the oxidation of bilirubin to biliverdin. **Objective:** In this study, our objective was to explore the potential use of BOx as an additive to samples with high bilirubin concentrations to resolve acetaminophen interference. **Methods**: In experiment 1, pooled non-icteric heparinized plasma samples were spiked with acetaminophen and aliquoted. Aliquots were then spiked with acetaminophen-free samples containing different amounts of bilirubin. Acetaminophen and Icteric index (I-index) were measured at baseline and after a 30min incubation step with BOx (1 U/ml) at 37°C. In experiment 2, highly icteric patient samples (n=9, I-index>8) were analyzed before and after incubation with BOx (2 U/ml) at room temperature for 30min and 60min. All samples were analyzed using the Roche Integra 800, for which the manufacturer reports interference to acetaminophen with an I-index>4. Assay limit of detection is 15ug/mL. **Results**: See table. Experiment 1 demonstrated that BOx effectively removes bilirubin without affecting acetaminophen. Experiment 2 demonstrated that under normal lab conditions, BOx may be used to resolve false positive acetaminophen results due to elevated bilirubin. Data for 60min incubation not shown; all acetaminophen levels at 60min <15ug/mL. **Conclusion**: BOx can be used to resolve false positive acetaminophen results due to bilirubin interference. This suggests a potential role for BOx to resolve bilirubin interference in other clinical chemistry assays.

Experiment 1	Before BOx incubation		After BOx incubation (30 min at 37C)		Experiment 2	Before BOx incubation		After BOx incubation (30 min at RT)	
	I-index	Acetaminophen (ug/mL)	I-index	Acetaminophen (ug/mL)		I-index	Acetaminophen (ug/mL)	I-index	Acetaminophen (ug/mL)
Baseline	Non-icteric	22	Non-icteric	21	Patient 1	21.6	<15	8.8	<15
Sample 1	1.4	22	0.5	20	Patient 2	15.8	<15	7.4	<15
Sample 2	2.4	22	0.7	21	Patient 3	9.2	<15	6	<15
Sample 3	4	22	0.9	20	Patient 4	39.5	29	15.2	<15
Sample 4	7.7	23	1.4	19	Patient 5	40.2	24	12.7	<15
					Patient 6	8.9	<15	4.4	<15
					Patient 7	17.8	<15	5.5	<15
					Patient 8	16.4	<15	8.6	<15
					Patient 9	14.7	<15	6.1	<15

A-258

Case Report: Paternity case with three genetic incompatibilities

D. Moratori[1], J. P. G. Batista[1], M. Malaghini[2], L. C. Scarpelli[1], R. B. Moraes[1], O. Fernandes[1], N. Gaburo Jr.[1]. *[1]DASA, Sao Paulo, Brazil, [2]DASA, Paraná, Brazil*

Background: The occurrence of germ line mutations at microsatellite loci may cause problems in ascertaining non-fatherhood status in paternity testing. A mutation event of DNA markers caused by loss or gain of repetitive units is quite a common phenomenon in forensic practice and is becoming more frequent due the increased number of paternity tests performed worldwide.

Objective: To describe a case that revealed a potentially erroneous exclusion from paternity due to the presence of three genetic inconsistences between the alleged father and the child.

Methods: The case was performed during the routine of paternity test at DASA. The DNA from mother, child and alleged father were obtained from blood samples in FTA-treated cards. The punching process was automated with BSD600 Duet equipment (Life Technologies, Foster City, CA). A total of 47 loci were analyzed. The STRs (Short Tandem Repeats) markers amplification was performed using AmpFlSTR® Identifiler® Direct PCR Amplification Kit (Life Technologies). Additional STRs were performed using Powerplex 16 HS System (Promega Corporation, Madison, WI), NGM Select (Life Technologies) and others 26 "in house" loci. The detection was performed at 3130*xl* Analyzer (Applied Biosystems, Foster City, CA). Paternity index (PI) was calculated including the mutation rates according to American Association of Blood Banks (AABB) report.

Results: Out of 47 analyzed loci, three genetic inconsistencies between the alleged father and the child were detected: two mutations at STRs D2S1338 e D2S1776 and the presence of null allele at STR TPOX. The inconsistencies at STRs D2S1338 and TPOX were confirmed by 2 different commercial kits, while the STR D2S1776 was confirmed by co-amplification. The combined paternity index in the case was 19.897.397.674.899,85 which corresponded to final probability of paternity value higher than 99.9999%.

Conclusion: The overall number of cases performed in our paternity laboratory, since 2005, is more than 38.000. This is the first case that we have found three genetic inconsistences in a non-exclusion paternity investigation. This case emphasizes the requirement that an exclusion from paternity must be based on calculating the appropriate statistical estimations in every case.

A-259

Identification of key meta data to enable safe accurate and effective transferability of biological variation data.

W. A. Bartlett[1], F. Braga[2], A. Carobene[3], A. Coşkun[4], R. Prusa[5], P. Fernandez-Calle[6], T. Røraas[7], I. Leimoni[8], S. Sandberg[9]. *[1]Blood Sciences, Ninewells Hospital & Med School. Biological Variation Working Group, European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), Dundee, United Kingdom, [2]Luigi Sacco Univeristy Hospital, Biological Variation Working Group, EFLM., Milan, Italy, [3]Diagnostica e Ricerca, San Raffaele Spa, Biological Variation Working Group, EFLM., Milan, Italy, [4]Acibadem University, School of Medicine, Gülsuyu, Maltepe. Biological Variation Working Group, EFLM., Istanbul, Turkey, [5]Charles University and University Hospital Motol. Biological Variation Working Group, EFLM., Prague, Czech Republic, [6]Hospital Universitario La Paz. Biological Variation Working Group, EFLM., Madrid, Spain, [7]Norwegian Quality Improvement of Primary Care Laboratories (NOKLUS), Haraldsplass, Hospital., Bergen, Norway, [8]Euromedic S.A. Biological Variation Working Group, EFLM., Athens, Greece, [9]Haukeland University Hospital. Biological Variation Working Group, EFLM., Bergen, Norway*

Background: Biological variation data (BVD) are reference data with many applications in laboratory medicine. Appropriate transfer of BVD across populations and through time requires the user to have -knowledge of the characteristics of the population from which the data were derived -an understanding of how the data were derived and -an appreciation of the uncertainty that surrounds the reported estimates. As a consequence an estimate of within and between subject biological variations should be transmitted and adopted for use only if accompanied by a set of meta data that sufficiently characterises the BVD in those contexts. The Biological Variation Working Group (BVWG), set up by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), have undertaken work to identify a candidate minimum data set (MDS) to accompany published indices of within and between subject biological variations to enable this issue to be addressed.

Methods: The BVWG considered and discussed the content of published literature and web based databases to identify the key meta data to accompany BVD to enable safe accurate and effective transfer and application across populations and through time.

Results: Key meta data were identified under six main headings. Those are, with example subheadings: -

Target - *analyte and measurand, sample matrix, method characteristics.* Population characteristics - *demographics, state of well being, physical/physiological characteristics, medication* Study Characteristics - *study duration and design, power of study to detect BVD indices, model assumptions, and statistical approach.* Data Characteristics - indices of biological variability, confidence intervals, tests for model assumptions. Publication Details - *links to the original publication.* Data rating - *new concept to be developed to indicate the quality of the BV data against a set of key criteria.* **Conclusion:** Published reviews of the literature describing BVD for albuminuria, haemoglobin A1c, C-reactive protein and liver enzymes all indicate a high degree of heterogeneity in the approach to derivation and reporting of BVD. Published BVD are of varying quality, often poorly characterised and consquently applied inappropriately into clinical laboratory practice. This highlights the need for generation of recognised standards for these important data sets.

The working group believe that availability of a standardised minimum data set, as proposed above, will enable users to be more objective in the transfer of published BVD into their local and wider practice. This will prove challenging to deliver, and require mechanisms to facilitate the extraction of meta-data from publications for attachment to the BVD to enable onward transmission and transferability (e.g. incorporation into databases). This will require further development of the concept of a BVD data archetype incorporating internationally accepted coding systems (e.g. SNOMED, LOINC) and vocabularies.

The MDS has been identified as part of a larger programme of work being undertaken by the BVWG aimed at developing a critical appraisal checklist for papers containing BVD. This will enable the creation of the data rating concept included in the MDS.

A-260

Evaluation of Technopath Controls on the ARCHITECT Family of Instruments

D. Brault[1], A. Croce[2], L. Lennartz[3], M. Orth[4], J. Shih[5]. *[1]Hopital Tenon, Paris, France, [2]Ospedale Civile Sondrio, Sondrio, Italy, [3]Abbott Laboratories, Wiesbaden, Germany, [4]Vinzenz von Paul Klinikem, Marienhospital, Stuttgart, Germany, [5]Abbott Laboratories, Abbott Park, IL*

Introduction: Quality controls are important for laboratories to ensure that released results meet the required quality in regard to accuracy and precision. Consolidation of controls is an important trend in laboratories to simplify QC testing. Recently, multi-constituent controls (MCCs) have been introduced by Technopath Manufacturing Ltd (Ballina, Ireland) that cover a wide range of clinical chemistry and immunoassay analytes.

Objective: The goal of this study was to evaluate the performance of the Multichem S Plus, Multichem IA Plus and Multichem U controls on the ARCHITECT family of instruments. Precision and accuracy compared to the target value were evaluated.

Methods: Three European sites (Paris, France; Stuttgart, Germany; Sondrio, Italy) used the three controls for a minimum of thirty days in parallel with the lab's routine QC controls. Testing was performed on the ARCHITECT *c*8000, *c*16000, $i1000_{SR}$ and $i2000_{SR}$ instruments. Data presented here are from the following serum clinical chemistry analytes: (A)ALT, (A)AST, total bilirubin, chloride, total cholesterol, creatinine (enzymatic and picrate), glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassays: CA 19-9, CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, testosterone, estradiol and FSH; and on the following urine assays: chloride, creatinine (enzymatic and picrate), glucose, potassium, sodium and urea. The Multichem S Plus and IA Plus are serum based with three control levels; the Multichem U is prepared from human urine with two control

levels. All data were collected via AbbottLink for automated data retrieval. Means, standard deviations and ranges were calculated for all controls. Assay reagent lots and calibrator lots varied across the sites and within the sites.

Results: The results from twelve frequently performed clinical chemistry assays were analyzed. The %CV for the 12 assays with the Multichem S Plus control ranged from 0.42 to 4.71% at the individual sites. The %CV for the 6 assays with the Multichem U control ranged from 0.50 to 5.24% at the individual sites. For both controls, the majority of the CVs were less than 2%. The results from these eleven frequently performed immunoassays were analyzed. The %CV for the 11 assays with the Multichem IA Plus control ranged from 1.82 to 14.94% at the individual sites; however the majority of the CVs were less than 5%. Overall little variation was seen instrument to instrument, site to site or reagent lot to reagent lot.

Conclusions: The Technopath S Plus, IA Plus and U controls demonstrated similar performance to the routine internal laboratory quality controls. The use of these MCCs reduce the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise on quality.

A-261

Equivalency Testing of Serum and Plasma on the Siemens Dimension Vista® 1500 System at University Hospitals (UH) Case Medical Center

C. Schmotzer[1], T. Barker[1], D. Yakubek[2], M. B. Smith[2]. *[1]University Hospitals Case Medical Center, Cleveland, OH, [2]Siemens Healthcare Diagnostics, Newark, DE*

Background: UH Case Medical Center has 1032 beds and processes 6000 samples a day and 5.2 million tests per year. Consistent and rapid turnaround time (TAT) requires efficient processing and analysis of samples. Using plasma will eliminate clotting time and decrease pre-analytical processing aiming to shorten TAT.

Objective: To demonstrate the equivalency between serum (x) and plasma (y) on assays performed on the Dimension Vista System.

Methodology: Serum and lithium heparinized plasma samples were obtained from a single draw in individual patients in the hospitalized population and tested in parallel. Least squares regression analysis was performed on single determinations of serum (x) and corresponding plasma (y) samples. The criteria used for assessing equivalency were a slope of 0.90 to 1.10, an intercept that is clinically insignificant, a correlation coefficient greater than 0.95 and average percent bias (plasma-serum) less than 10% or the CLIA Total Allowable Error, whichever was greater.

Results: 46 assays (n=53-59 serum and corresponding plasma samples) evaluated met the above criteria. Table 1 lists data observed on representative assays of different method principles. The minimum and maximum slope, correlation coefficient and average percent bias observed for 46 assays are 0.92-1.05, 0.976-1.000 and -8.7 to 5.3%, respectively. Intercepts for these assays were clinically insignificant.

Table 1

Assay	Units	Slope	Intercept	Correlation Coefficient	n	Low	High	Average % Bias (plasma-serum)
1. ALB	g/dL	0.995	-0.037	0.977	56	2.1	3.9	-1.7
2. A1AT	mg/dL	0.947	6.875	0.983	57	116	376	-2.0
3. ALTI	U/L	0.977	-0.005	0.998	58	10	168	-2.3
4. ASL	U/mL	0.962	0.855	0.997	59	13	342	-2.9
5. AST	U/L	1.020	-0.780	0.998	58	7	232	-0.3
6. B2MIC	mg/L	0.992	0.014	1.000	58	1.21	33.19	-0.4
7. FERR	ng/mL	0.978	-1.096	0.999	58	21.2	3108	-2.4
8. FT4	ng/mL	0.991	0.013	0.993	58	0.67	1.97	0.1
9. GLU	mg/dL	1.053	-0.010	0.991	53	69	208	5.3
10. hsCRP	mg/L	1.009	0.360	0.997	55	0.16	164.2	1.6
11. IGG	mg/dL	0.991	-4.038	0.997	57	307	2605	-1.4
12. PBNP	pg/mL	0.986	18.027	1.000	56	25	14637	0.1
13. K	mmol/L	0.988	-0.072	0.976	56	3.2	4.6	-3.0

Other assays tested for serum vs. plasma equivalency: 1) ALP, 2) BUN, 3) CRP, 4) CL 5) CHOL, 6) CKMB, 7) C3,
8) C4, 9) CKI ,10) DBI, 11) ECREA, 12) FT3, 13) GGT, 14) HAPT, 15) HDLC, 16) IGA ,17) IGM,18) IRON, 19) LDI, 20) LDLC, 21) LIPL, 22) MYO, 23) PHOS, 24) PREALB, 25) RF, 26) NA, 27) TBI, 28) TIBC, 29) TP, 30) TRF, 31) TRIG, 32) TSH, 33) URCA

Conclusions The observed data on 46 assays support the equivalency of serum and plasma on the Dimension Vista System based on the predefined evaluation parameters to demonstrate serum and plasma equivalency.

A-262

25-Hydroxyvitamin D2: Prevalence and Impact on 25-Hydroxyvitamin D Measurement

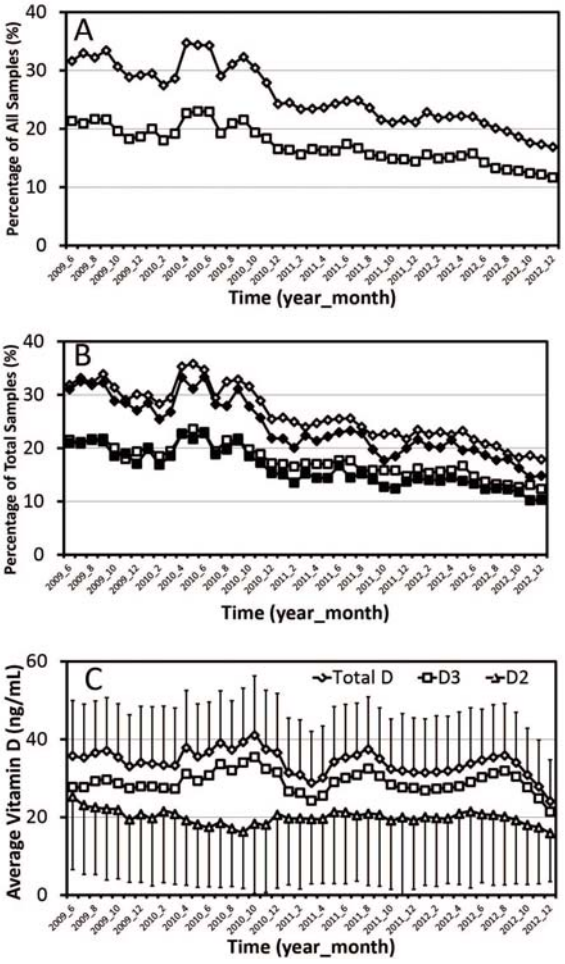
Y. Zhang, T. Kwong, M. Stolla. *Strong Memorial Hospital, ROCHESTER, NY*

Background: This study is to evaluate the distribution of 25OH-D2/D3 in a general patient population in western New York to provide insights into the current prevalence and the trend of vitamin D2 usage, and its impact on the accuracy of vitamin D measurement.

Methods: 25OH-D2 and 25OH-D3 results measured by in-house LC-MS/MS method at Strong Memorial Hospital at the University of Rochester Medical Center from June 2009 to December 2012 were retrospectively analyzed. The results were retrieved through the laboratory information system using specific parameters to include patients' age, gender, 25OH-D2, 25OH-D3, and total 25OH-D data. All the results during that period of time were included except research samples and the ones with missing data. The mean, standard deviation, overall distribution and monthly prevalence of 25OH-D2, 25OH-D3, and total 25OH-D were calculated using Statistica 10 Software. The significant levels were assessed using two tailed student T test in EXCEL. The limit of quantification (LoQ) for 25OH-D2 and 25OH-D3 were &lt 4ng/mL and &lt 6ng/mL, respectively. Total 25OH-D level of 60 ng/mL was considered potentially harmful.

Results: A total of 266,269 samples from were included for analysis. The percentage of samples with 25OH-D2 levels above assay LoQ decreased from 32% to 17% over the course of the study period. The percentage of samples with 25OH-D2 levels higher than those of 25OH-D3 decreased from 21% to 12% (see figure). Sixty-seven percent of the samples with 25OH-D2 levels above LoQ had serum concentrations of 25OH-D2 higher than those of 25OH-D3.

Conclusion: 25OH-D2, despite its gradual decrease in local prevalence, was still present in 17% of the patients tested in December 2012, many of whom had 25OH-D2 levels higher than those of 25OH-D3. To achieve accurate vitamin D measurement, clinical laboratories should assess the accuracy of their assays for both isomers, and if necessary, determine their local prevalence of 25OH-D2.



A-263

Stability Studies of Hemoglobin A1c (HbA1c) Based on Specimen Storage

K. A. VandenHeuvel, K. Morrison, P. Akl, K. E. Blick. *Un of OK Health Sci Ctr, Oklahoma City, OK*

Background: Hemoglobin A1c (HbA1c) is useful for the assessment of glycemic control in patients with known or suspected diabetes and, in some cases, for the diagnosis of diabetes. While freshly collected samples are preferred, in some instances it is necessary to test for HbA1c on stored, whole blood samples. Published studies suggest that both storage time and temperature can affect the HbA1c stability, therefore we investigated the effect of room temperature storage on HbA1c up to seven days, considering variables between specimens including levels of ambient glucose and labile HbA1c.

Methods: Twenty-five patients were included in the study; samples collected from each patient included whole blood (EDTA) and plasma (heparin). Patients were selected to reflect a wide range of HbA1c and glucose values: HbA1c; 4.8-14.7 % and glucose; 95-558 mg/dL. The whole blood samples were stored at room temperature (20°C) then analyzed for HbA1c by HPLC (Variant II Turbo) on days Zero, 4, and 7; whole blood glucose levels were measured on the Abbott i-Stat while baseline plasma glucose levels were also measured on the UniCel DxC800 (Beckman Coulter).

The HbA1c values obtained on days Zero, 4, and 7 were compared by two statistical methods: 1) the means for days 4 and 7 were compared to day Zero levels using the two-tailed paired Student's t-test, and 2) linear regression analysis to assess the degree of change in regression line slope over the incubation period.

Results: The HbA1c values obtained on Day Zero ranged from 4.8 to 14.7% (mean=9.1 +/- 3.6%) representing a wide spectrum of normal and abnormal values; values obtained on Days 4 and 7 ranged from 5.4 to 15.7% (mean=9.1+/- 3.7%) and 5.3 to 15.9% (mean=9.0+/- 3.9%) respectively. The Student's t-test indicates that the Day 4 and Day 7 means are not statistically different from that observed on Day Zero (p=0.86, Day 4; p=0.57,Day 7). Baseline ambient glucose ranged from 95 to 558 mg/dL (mean=217+/-144); baseline labile HbA1c ranged from 1.1 to 6.2% (mean=2.5+/-1.6%). Linear regression analysis of the Days 4 and 7 HbA1c values versus those on Day Zero indicates only a 3 percent proportional difference on Day 4 with an observed slope of 1.03. The latter change could result in an increase of up to 0.18% at the 6.1% cutoff. However, on Day 7, a proportional difference of 8 percent was observed (slope=1.08) suggesting that older stored samples show a consistent positive proportional bias amounting to as much as 0.5% at our current reference range cutoff of 6.1. The Day 4 and 7 regression lines show a good fit (Day 4,Syx=+/-0.37,r^2=0.991; Day 7, Syx=+/-0.74,r^2= 0.967).

Conclusion: We conclude that HbA1c can be performed on whole blood samples stored at room temperature for up to 4 days, irrespective of ambient glucose levels, with no statistically significant change in levels. However increases of up to 3% from baseline should be expected. Test results for HbA1c on whole blood samples stored for 7 days show an unacceptably high degree of proportional error of 8 percent.

A-264

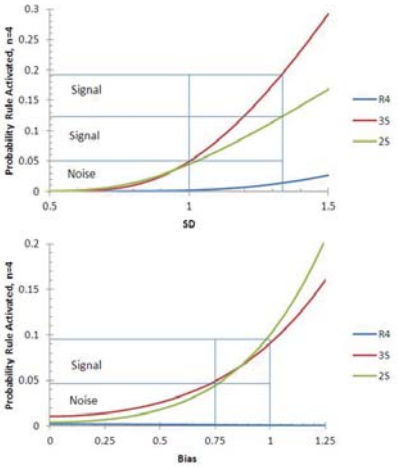
The message content of quality control rules

M. H. Kroll, C. Garber, C. Bi, S. Suffin. *Quest Diagnostics, Madison, NJ*

Background: We assessed the ability of three quality control (QC) rules (R_{4S} [R4], 1_{3S} [3S] and 2_{2S} [2S]) to provide signal compared with noise and discriminate between imprecision (random error) and bias. QC rules should identify unacceptable runs and discriminate specifically between bias (B) and random error (standard deviation, SD). Comparing the probability of signal with that of noise quantifies the message content. Appropriate corrective action depends on signal, not noise, and requires discrimination between errors types.

Methods: We calculated the number (*N*) of expected combinations of quality control messages for each rule based on the number of control materials. We calculated baseline probabilities (*P*) of rule activation using the cumulative distribution function (CDF), varying bias (B) from 0 to 1.25 and SD from 0.5 to 1.5, assuming the following allowable errors: **$Error_{Total}$=3, $Error_{Bias}$=0.75; and $Error_{SD}$=1.** ***P(R4)=2{CDF(B,SD,-2)}{1-CDF(B,SD,2)}; P(3S)={CDF(B,SD,-3}+{1-CDF(B,SD,3)}; P(2S)={CDF(B,SD,-2)}^{2}+{1-CDF(B,SD,2)}^{2}. P(Noise)=P(RuleActivation|B=0.75,SD=1); P(Total)=P(rule-activation|B>0.75,SD>1); P(Signal)=P(Total)-P(Noise) P(CombinationActivation)=N*BaselineProbability=N_j*P_j(Signal); j=[R4,3S,2S],i=[SD,Bias].*** **Results:**The number of message combinations (*N*) is *n/2* for R4, *n* for 3S, and *3(n/2-1)+1* for 2S for *n-controls*; for 4-controls: N=2 for R4, N=4 for 3S, and N=4 for 2S. The figure shows the *CombinationActivation* probabilities, for n=4 controls, as a function of bias or SD. Given bias=1 or SD=1.33, *CombinationActivation* probabilities are as follows: P_{R4}(Noise)=0.0012, P_{R4}($Signal_{Random}$)=0.012 (<3% messages), P_{R4}($Signal_{Bias}$)=0.000; P_{3S}(Noise)=0.049 (21% of 3S messages), P_{3S}($Signal_{Random}$)=0.142 (77% of 3S Signals), P_{3S}($Signal_{Bias}$)=0.042; P_{2S}(Noise)=0.045 (25% of 2S messages), P_{2S}($Signal_{Random}$)=0.077 (58% of 2S Signals), P_{2S}($Signal_{Bias}$)=0.056.

Conclusion: The 3S and 2S rules impart significant *noise* (22% of messages). R4 specifically identifies random error but represents < 3% of messages. 3S and 2S discriminate poorly between random and bias errors, with only 77% of messages representing random errors for 3S and 58% for 2S. The quality control rules demonstrate inadequacies in ascertaining run acceptability and discriminating between imprecision and bias.



A-265

Comparison of Uncertainty Values Between Core and Emergency Laboratories for Routine Biochemical Parameters

S. Tapan[1], M. Uyanik[1], E. Sertoglu[2], I. Kurt[1]. *[1]Gülhane School of Medicine, Department of Clinical Biochemistry, Ankara, Turkey, [2]Ankara Mevki Military Hospital, Anittepe Dispensary, Department of Biochemistry, Ankara, Turkey*

Background/Aim: Most regulatory authorities that use International Organization for Standardization (ISO) Standards to assess laboratory competence require an estimate of the uncertainty of measurement of assay test results. According to ISO 15189, the uncertainty of measurement is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand. ISO 15189 requires that "The laboratory shall determine the uncertainty of results, where relevant and possible". In this study, we aimed to calculate the uncertainty of measurement of our core and emergency laboratory, in terms of routine biochemistry tests.

Materials and Methods: Study was conducted at the Laboratory of Department of Medical Biochemistry in Gulhane School of Medicine, Ankara, Turkey. Internal and External Quality Control (IQC and EQC, respectively) results of two Olympus AU2700 (device 1 and 2 in core laboratory) and one Olympus AU 640 (device 3 in emergency laboratory) autoanalyzers were assessed in the scope of this study. 24 parameters were evaluated between a period of January 2013-December 2013. The calculations were derived from the EURACHEM/CITAC and AACB guides. Then we assessed the analytical performances and uncertainty of measurements according to CLIA, Rilibak, Fraiser and Six Sigma.

Results: Considering the evaluation criteria; the calculated uncertainty according to AACB of three devices met the expectations for in all parameters. However, Cl^- results of device 1 and 3 were determined above CLIA while K^+ results of device 1 and Na^+ results of device 3 were above Rilibak' criteria when calculated according to EURACHEM. Moreover, calculated total error (TE) rates of Na^+, K^+, Cl^-, calcium and total protein were above Fraser' TE rates. Cl^- and urea in all devices; total protein, albumin, Na^+, K^+ and calcium in device 3; Na^+, creatinine and ALP in device 2 were under 3 sigma, according to six sigma.

Conclusion: This study shows that the Core Biochemistry Laboratory of Gulhane School of Medicine Hospital results met the expectations within the appropriate limits of total error defined by CLIA, Rilibak, Fraser in most of the parameters. However, in emergency laboratory, most results were found to be close to the upper limit or above the criteria. As expected, it's quite difficult for emergency laboratories, that work for 24 hours a day and 7 days a week, to meet all criteria due to shift and personnel changes. Establishing uncertainty of measurement guidelines for clinical laboratories can accelerate the pace of understanding both for clinicians and clinical laboratory practitioners.

A-266

Variables impacting the analytic false positive rate for Cardiac Troponin T on the Roche cobas e411

A. M. Wockenfus, C. D. Koch, K. J. Hartung, B. S. Karon. *Mayo Clinic, Rochester, MN*

Background: We have observed rare occurrences of analytic false positive results for cardiac Troponin T (cTnT) using plasma separator tubes (PST) and the STAT Troponin T assay on the Roche cobas e411 immunoassay analyzer. Internal and external (assay manufacturer) discussions identified several potential sources of error: cellular debris, intermittent carryover, electrical supply fluctuation, and inadequate sample centrifugation. PST has historically been used for cTnT testing because it allows for faster processing than serum tubes. Previous studies have demonstrated PST specimens contain more cellular debris. This increases the likelihood of micro-clot formation in the analyzer sample path; which could negatively impact the assay accuracy. The goal of our study was to identify and minimize variables associated with analytic false positive cTnT results while maintaining acceptable turnaround time.

Methods: Our Emergency Department (ED) patients receive a panel consisting of cTnT measurement at 0, 3, and 6 hours after presentation. To capture analytic false positive results, all samples with 0 hour cTnT ≥ 0.04 ng/mL, or 3 or 6 hour samples demonstrating a change of ≥ 0.03 ng/mL from 0 hour value, were repeated. All samples were repeated in this manner between 2007 and 2012. An analytic false positive was defined as a sample that upon repeat analysis demonstrated a change in results of +/- 0.04 ng/mL for cTnT values ≤ 0.10 ng/mL or +/- 20% for values >0.10 ng/mL. Initial attempts to reduce analytic false positives included adjusting centrifugation times from 3 to 5 minutes; and manufacturer-performed instrument and measuring cell performance verification when false positives were noted. To address relatively higher concentrations of cellular debris in PST samples, a rapid clot serum tube (RST) was implemented mid-2012. RST tubes fully clot within 5 minutes, drastically reducing the clot time (up to 30 minutes) of traditional serum tubes. Finally, the electrical power source for the analyzer was stabilized and a redesigned measuring cell was installed, both at the recommendation of the manufacturer.

Results: Neither increasing centrifugation time, performance verification after false positives, nor installation of the redesigned measuring cell significantly impacted the rate of false positives observed (1 in 2200). A slight decrease in analytic false positives was observed when switching from PST to RST (1 in 2200 vs. 1 in 2375, respectively). The most significant decrease in false positive rate was observed when the electrical power was stabilized while using RST. Under these conditions false positive rate decreased to approximately 1 in 5000. In order to determine if this decrease would also be observed with PST; RST vs. PST was re-evaluated with electrical stabilization. RST continued to show a decreased false positive rate.

Conclusion: The combination of electrical power supply stabilization and implementation of RST decreased the rate of analytic false positive cTnT results on the cobas e411 to approximately 1 in 5000. This facilitated rapid analysis of cTnT samples while eliminating the need to repeat positive results for patients presenting to the ED.

A-267

Effects of 49 Different Rare Hb Variants on HbA1c Measurement in Seven Methods

R. L. Schmidt[1], S. L. La'ulu[2], S. E. Hanson[3], C. L. Rohlfing[3], R. R. Little[3]. *[1]University of Utah School of Medicine and ARUP Laboratories, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]University of Missouri School of Medicine, Columbia, MO*

Background: Hemoglobin A1c (HbA1c) is recommended for routine monitoring of long-term glycemic control in patients with diabetes and for use in diabetes diagnosis. Previous studies have shown interference from the four most common heterozygous Hb variants (HbAS, HbAE, HbAC, and HbAD) with some HbA1c assay methods. Here we examine analytical interference from 49 different less common variants with 7 different HbA1c methods using various method principles (ion-exchange HPLC, boronate affinity HPLC, immunoassay, and enzymatic).

Methods: Hb variants were screened using the Bio-Rad Variant II beta thal short program, confirmed by alkaline and acid electrophoresis, and identified by sequence analysis. The Trinity ultra2 boronate affinity HPLC method and Roche Tina-quant immunoassay were used as primary and secondary comparative methods, respectively, since these methods are least likely to show interference from Hb variants. Other methods included were the Tosoh G7 and G8, Bio-Rad D-10 and Variant II Turbo, and Diazyme Enzymatic. Identified variant samples (n=88) and a group of non-variant (AA) samples (n=92) were analyzed by each method; there were multiple samples for some variants. Samples with HbF >10% (based on G8 %HbF) were excluded. To eliminate any inherent calibration bias, results for each method were adjusted using regression verses the ultra2 with AA samples. When results from the two comparative methods matched within 10%, all other methods' calibration-adjusted results were compared and judged to be acceptable if within 10% of the ultra2 result. For five variants (one sample each), results from the two comparative methods were discordant and this could not be explained by an amino acid substitution that would affect binding of the Tina-quant antibody; these results were excluded from further evaluation.

Results: Almost all variant samples were recognized as such by the ion-exchange HPLC methods by the presence of abnormal peaks or results outside the reportable range. For most variants (80%), interference (>10% difference from ultra2 or no result reported) was seen with one or more of the ion-exchange methods. Following manufacturer instructions for interpretation of chromatograms usually, but not always, prevented reporting of inaccurate results. For 14 variants studied, the Diazyme results were inaccurate; this method does not detect the presence of variants and all results are reported.

Conclusions: In order to insure that accurate HbA1c results are obtained for all patients, it is important to know if a patient has a hemoglobin variant and how that variant can affect their HbA1c results. Laboratories must be cautious about reporting results when the presence of a variant is suspected. Manufacturers may need to clarify and/or tighten their criteria for accepting results and update their software to flag potential variants in an effort to reduce the likelihood of incorrect HbA1c results being reported.

A-268

Evaluation of HbA1c measurement in Trinidad and Tobago

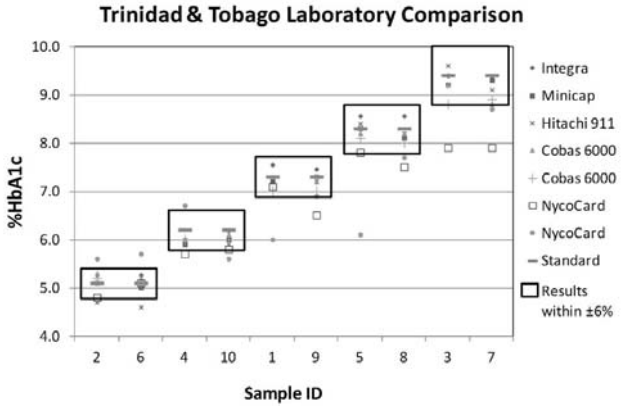
R. R. Little[1], C. L. Rohlfing[1], D. E. Goldstein[1], P. Ladenson[2], M. Rastogi[3]. *[1]University of Missouri School of Medicine, Columbia, MO, [2]Johns Hopkins University School of Medicine, Baltimore, MD, [3]Sangre Grande Hospital, Sangre Grande, Trinidad and Tobago*

Background: HbA1c is recommended for routine monitoring of long-term glycemic control in patients with diabetes and also for diabetes diagnosis. The prevalence of diabetes in Trinidad and Tobago is over 12%. Although HbA1c testing is offered by public and private sector laboratories, no HbA1c proficiency testing (PT) has previously been performed. Therefore, Johns Hopkins Medicine International and the Diabetes Diagnostic Laboratory (DDL) at the University of Missouri organized a pilot HbA1c Proficiency Testing Study for 7 key laboratories as part of the Trinidad and Tobago Health Sciences Initiative.

Methods: Sets of 10 samples containing blinded duplicates were created from five whole blood pools with HbA1c levels from 5.1 to 9.3% HbA1c and shipped to participating laboratories. To assess within-day imprecision, the pooled estimate of the SDs between the duplicates (Sp) was calculated; 0.229 was the acceptable limit based on the current NGSP HbA1c standardization program monitoring criterion. To assess accuracy, each laboratory's results were compared to an NGSP Secondary Reference Laboratory (SRL9 using Tosoh G8).

Results: One laboratory reported results as IFCC %HbA1c; these were aligned to NGSP using the NGSP/IFCC master equation [NGSP%=0.915(IFCC%)+2.15]. Methods used by the laboratories included Roche Tina quant on Cobas Integra, Cobas 6000 and Hitachi 911, Sebia Minicap, and Axis-Shield NycoCard. All laboratories except the two using the NycoCard showed acceptable imprecision. All results from three laboratories (one each using Sebia Minicap, Roche Cobas 6000, and Roche Cobas Integra) were within 6% of the NGSP SRL assigned values; most results from the two laboratories using the NycoCard were outside 6%.

Conclusion: Because inconsistent HbA1c results can negatively impact patient care, it was recommended that all laboratories report NGSP % HbA1c and those using the NycoCard use a more precise method. Laboratories are instituting changes based on these findings and a future study will re-assess performance.



A-269

Identifying G6PD Deficiency: Defining the Lower Reference Limit and Evaluating Pre-analytical Sources of Error

B. Suh-Lailam, D. Grenache. *University of Utah, Salt Lake City, UT*

Background: Glucose-6-phosphate dehydrogenase (G6PD) protects red blood cells (RBCs) from oxidative damage by regenerating NADPH. G6PD deficiency is the most common X-linked enzymopathy that results in hemolytic anemia triggered by oxidative stress. Diagnosis of G6PD deficiency is facilitated by determining its activity in RBCs and interpreting the result against the lower limit of a reference interval (LLRI). Despite the use of similar methods, the LLRI for G6PD varies considerably between laboratories (range, 4.6-8.8; N=4). The objectives of this study was to 1) validate the LLRI of the G6PD reference interval used by our laboratory (7.0-20.5 U/gHb) and 2) to identify pre-analytic sources of error that may influence the accuracy of G6PD test results.

Methods: A G6PD reference interval was determined empirically and by the Hoffman method. For the empirical approach, whole blood was collected from 120 reference subjects (59 males and 61 females; 19-75 years) and G6PD determined by kinetic spectrophotometry using commercially available reagents (Trinity Biotech USA) at 37°C. For the Hoffman method, 20,736 G6PD test results were extracted from the laboratory information system and linear regression performed over the linear portion of the cumulative frequency distribution. The LLRI and upper limit of the reference interval (ULRI) were calculated as LLRI=2.5(slope)+intercept and ULRI=97.5(slope)+intercept. The effects of pre-analytical sources of error were investigated using samples submitted to the laboratory for G6PD testing and included the method of vortex mixing, incubation time for RBC lysis, and analytical dwell time. The study was approved the University of Utah Institutional Review Board.

Results: G6PD activities in 120 reference subjects ranged from 9.0-14.7 U/gHb (median=11.6). The non-parametric reference interval was 9.9-14.1 U/gHb. G6PD activities in the 20,736 clinical subjects ranged from 0.3-86.2 U/gHb (median=13.2). 6.1% of the samples were less than the current LLRI of 7.0 U/gHb. The Hoffman technique yielded a reference interval of 9.9-16.6 U/gHb. Applying this LLRI to the clinical subjects identified 8.9% as deficient. The method of vortex-mixing was evaluated in 40 samples and did not significantly affect G6PD activity. Compared to the reference technique (single-tube with single-tube vortex mixer) which produced a mean G6PD activity of 13.0 U/gHb, the use of a tube rack with multi-tube vortex mixer or a tube rack with single-tube vortex mixer produced mean activities of 12.9 and 13.2 U/gHb, respectively ($p>0.24$). The incubation time for RBC lysis after vortex-mixing did affect G6PD activity. Compared to the reference time of 5 minutes, a 3-minute incubation had no effect (mean difference 0.2 U/gHb, $p=0.05$) but 15- and 30-minute incubation times produced significantly lower results (mean difference -1.0 and -2.1 U/gHb, respectively; $p<0.0001$). Analytical dwell time (40 minutes) had no significant effect on G6PD activity as determined from 70 aliquots of a whole blood sample that produced a CV of 1.5% at a mean of 15.6 U/gHb ($r=0.12$, $p=0.32$). Conclusions: The G6PD LLRI should be 9.9 U/gHb as determined empirically and by the Hoffman method. The incubation time for RBC lysis is a variable that should be controlled when performing G6PD testing.

A-270

Diurnal Variation of analytes: an underestimated pre-analytical factor in clinical chemistry

M. Abou El Hassan, B. Hoffman. *University of Toronto, Toronto, ON, Canada*

Objectives: Diurnal variation is a well known cause of biological variation. We set out to identify analytes affected by diurnal variation, the amplitude of the within day difference and whether collection instructions specified the impact of collection time on test interpretation.

Design and Methods: We identified analytes reported to undergo diurnal variation through a review of Tietz's Textbook of Clinical Chemistry and through a search of Pub Med to capture recent citations. We checked if the time of collection, and its potential impact on test interpretation, was specified in the posted collection procedures issued by two large commercial labs in Ontario.

Results: Our search identified 21 analytes affected by diurnal variation. As expected, cortisol, the archetypal diurnally changing analyte, was the most cited. Hormones represented the major class of identified analytes, but fasting plasma glucose (FPG), MR-proANP, and hepcidin were other noteworthy inclusions. Diurnal amplitude ranged from a low of 1.1 for FPG (still sufficiently large to degrade test performance in ruling diabetes in or out using the cutpoint of 7.0 mM) to a maximum of 65-fold for melatonin. Of the 14 analytes listed in posted collection instructions, the importance of the time of collection was specified for only 5 (36%).

Conclusions: The confounding effect of diurnal variation on test interpretation is underappreciated given that cautions regarding when analytes should be collected were issued in collection instructions only 36% of the time.

A-271

Can platelet aggregation testing by Multiplate be influenced by one minute tourniquet application?

G. Lima-Oliveira[1], G. Lippi[2], G. L. Salvagno[3], S. Gaino[3], G. Poli[3], M. Gelati[3], G. Picheth[1], G. C. Guidi[3]. *[1]Federal University of Parana, Curitiba, Brazil, [2]University of Parma, Parma, Italy, [3]University of Verona, Verona, Italy*

Background: The study of the role of platelets in the pathogenesis of ischemic vascular diseases and the monitoring of antiplatelet drugs require reliable platelet-function tests. Presently several techniques are in use to measure platelet aggregation.

Assessment of platelet function by multiple electrode aggregometry (Multiplate® Roche, Germany) is common in laboratory practices. Indeed, sample collection and processing are essential steps for quality assurance, so that the fulfillment of standardized preanalytical conditions are key factors in maintaining patient safety. The collection of diagnostic blood specimens for Multiplate is traditionally performed by medical staff using a tourniquet for evidencing veins. The Clinical and Laboratory Standards Institute H03-A6 document, recommends the use of the tourniquet for localizing suitable veins and the tourniquet time can not in any case be extended over 60 sec. This study was aimed to assess the impact of 60 sec tourniquet application on platelet function evaluated by multiple electrode aggregometry (Multiplate).

Methods: Ten volunteers, after 12-hours fasting, were maintained seated during 15 minutes to eliminate possible interferences from both lipemia and blood distribution due to different posture. After this time frame, 6mL of blood were collected by venipuncture with a 20G straight needle directly into two 3.0mL Hirudin Blood Tube for Multiplate® analysis (proprietary vacuum tube 06670105001, Roche Diagnostics GmbH, Penzberg, Germany) from two different procedures: Procedure I (no stasis) - a radial vein was localized on right forearm by a subcutaneous tissue transilluminator device without tourniquet, to prevent interference from venous stasis. Procedure II (stasis) - a radial vein was localized on the left forearm by tourniquet application during 60 sec prior to venipuncture. To eliminate any potential interference due to either the contact phase or the tissue factor all 1st tubes from each volunteer for both procedures were discarded. All diagnostic blood specimens were mixed gently and carefully by five times inversion, as recommended by the manufacturer. All samples were processed for assessment of platelet function (ADP-test®, ASPI-test®, COL-test®, RISTO-test® and TRAP-test®) by multiple electrode aggregometry (<15 min after collection) on the same Multiplate (Roche Diagnostics GmbH, Penzberg, Germany). In the Multiplate Test Cell, activated platelets adhere to and aggregate on the sensor wires. This leads to an increased resistance between the sensor wires, which in continuously recorded and expressed via the area under the curve in arbitrary units. The significance of differences between samples was assessed by paired Student's t-test after checking for normality by the D'Agostino-Pearson omnibus test. The level of statistical significance was set at $p<0.05$.

Results: All platelet functions tested showed lower values from samples collected with tourniquet than without tourniquet. Significant differences ($p<0.05$) were observed for RISTO-test (-16%), ADP-test (-10%) and TRAP-test (-8%) determination in samples collected after 60 sec tourniquet application.

Conclusion: The significant variations after 60 sec tourniquet application suggest that platelets could undergo a sort of pre-activation by venous stasis, thus decreasing the sensitivity to subsequent activation by agonists. Furthermore, this effect can compromise clinical results interpretation and jeopardize patient safety. In conclusion tourniquet application should be avoided during phlebotomy for Multiplate analyses.

A-272

Impacts of sample volume and stopper on the stability of ethanol in lithium heparin plasma

S. N. Narla, D. Brass, S. Dean, B. Horne, Y. Zhu. *Medical University of South Carolina, Charleston, SC*

Background: It is recommended that ethanol samples should be analyzed immediately upon opening the tube, but there is no evidence about how long the ethanol is stable in real specimen tubes without stoppers. The objective of this study is to evaluate the stability of ethanol with different sample volumes in unstoppered and stoppered tubes at room temperature (RT).

Methods: Heparin plasma samples with ethanol ≥ 10 mg/dL were selected for this study and stored at -20°C until analysis. Ethanol was analyzed on UniCel DxC800 Systems by an enzymatic assay. To determine the impact of stopper on ethanol at RT, 30 plasma samples with a volume of 100 µL stored in 5 mL plain tubes at RT with and without stoppers and ethanol was measured after 1, 2 and 3 hours of baseline measurement. To determine the influence of sample volume on ethanol stability, 30 heparin separator tubes each with a total volume (including cells, gel, and plasma) of 3, 4 and 5 mL with plasma volume of 0.5, 1 and 2 mL respectively were stored at RT without stoppers and ethanol was measured after 1, 2 and 3 hours of baseline measurement. The allowable total error (ATE) for ethanol was 25%.

Results: The average ethanol concentrations in samples with different volumes with and without stoppers and recoveries were shown in table1.

Conclusions: Ethanol concentrations are within ATE for samples stored for at least 3 hr without stopper at RT with a minimum of 0.5 mL plasma in original heparin plasma separator tubes and for samples stored in plain tubes with stoppers at RT for at least 3 hr with a minimum volume of 100 µL, whereas ethanol in this low volume of sample in plain tubes is unstable for more than 2 hr at RT without a stopper.

Table1: Stability of ethanol with various volumes at room temperature

		Base Line	1h	2h	3h
100 µL of P* (NS)	Mean ± SD (mg/dL)	143 ± 95	126 ± 84	107 ± 72	91 ± 60
	R (%)	100	88	76	66
100 µL of P* (ST)	Mean ± SD (mg/dL)	143 ± 95	144 ± 95	144 ± 95	144 ± 96
	R (%)	100	101	101	101
3 mL TV (0.5 mL P*) (NS)	Mean ± SD (mg/dL)	142 ± 94	141 ± 94	137 ± 92	131 ± 87
	R (%)	100	99	96	92
4 mL TV (1 mL P*) (NS)	Mean ± SD (mg/dL)	153 ± 100	150 ± 98	147 ± 95	143 ± 94
	R (%)	100	98	95	94
5 mL TV (2 mL P*) (NS)	Mean ± SD (mg/dL)	142 ± 73	141 ± 72	138 ± 71	136 ± 71
	R (%)	100	99	97	96

TV: Total Volume, P*: Plasma, R: Recovery, ST: Stopper, NS: No-Stopper

A-273

Evaluation of a method to detect prozone/hook effect/antigen excess phenomenon for Free Light Chain quantification using a simple pooling protocol

V. De Guire[1], F. Courjal[2], R. Leblanc[1], M. Malvaso[1], M. Piché[1], H. Harbec[1], A. Roméro Ospina[1], J. Amyot[1], N. Tétreault[1]. *[1]Maisonneuve-Rosemont Hospital, Montreal, QC, Canada, [2]The Binding Site Group, Birmingham, United Kingdom*

Background: Antigen excess is an important issue that can lead to underestimation of patient's results and misdiagnosis. With patient samples which can range from less than 1mg/L to over 100 000mg/L, serum free light chain (FLC) measurement is especially prone to this interference. Some institutions choose not to implement on site testing for FLC, due to the lack of an instrument platform which can automatically detect antigen excess and in which concerns over workflow interruptions and personnel costs inhibit implementation of an antigetn excess protocol.

Methods: We propose a methodology based on sample pooling for a fast and cost effective method to detect of antigen excess phenomenon for serum free light chain quantification. Our strategy was evaluated on the Immage (Beckman) and on the BNII (Siemens) nephelometers using the Freelite® assay (The Binding Site).

Results: First, we evaluated the sensitivity of our strategy using a pool of 15 mixed samples spiked with different concentrations of kappa or lambda free light chain. Secondly, patients with antigen excess ranging from 1192 to 8500 mg/L of kappa free light chain were efficiently detected using our strategy. Finally, a preliminary evaluation of the size of the pools was conducted using pools ranging from 15 to 42 different samples. Based on the precision of the method, our preliminary data suggest that the number of samples in a pool can reach up to 43 different patients to detect an antigen excess of 1192 mg/L.

Conclusion: We described and validated a strategy based on sample pooling for detection of antigen excess on free light chain measurement. This approach could be suitable for any laboratory measuring serum free light chain that does not currently have an instrument platform for detection of antigen excess.

A-274

Performance Evaluation of Rapid Test Strips for the Identification of EDTA-Containing Specimens

K. L. Scholes[1], S. L. La'ulu[2], A. T. Ence[1], H. L. Logan[1], R. L. Parker[1], F. G. Strathmann[3], J. R. Genzen[3]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]University of Utah, Department of Pathology, Salt Lake City, UT*

Background: Most laboratory assays have specimen acceptability requirements. Proper identification of ethylenediaminetetraacetic acid (EDTA) containing specimens is frequently necessary when investigating mislabeled specimens, suspected primary tube mixtures, and aliquots. The purpose of this study was to evaluate the performance of commercially available EDTA test strips using biological specimens.

Methods: Studies included human sera and plasma specimens, bovine calf serum, and phosphate-buffered saline. Specimens were applied to test strips (Quantofix® EDTA; Macherey-Nagel) using a pipet ("drop mode"). These test strips are embedded with bismuth nitrate, xylenol-orange, and citric acid to detect chelating agents such as EDTA. Test strips were blotted to remove excess fluid; visual evaluation was made at 15 seconds. Reactions were scored on a scale from red (no EDTA), orange (low EDTA), to yellow (EDTA). A NODE+Chroma color detector (Variable, Inc) on a custom 3D-printed test strip channel was also used to capture quantitative RGB values

on a Bluetooth-linked iPhone 5, thus permitting additional statistical analysis and graphical display. Potentially reactive chelating agents tested included alpha lipoic acid (ALA), deferoxamine (DEF), 2,3-dimercapto-1-propanesulfonic acid (DMPS), dimercaptosuccinic acid (DMSA), K_2EDTA, Na_2EDTA, ethylene glycol tetraacetic acid (EGTA), and penicillamine (PEN). Results were compared to K^+, Ca^{2+}, Mg^{2+}, and serum indices (Roche cobas 8000), as well as a sodium tetraphenylborate method of K^+ detection. Finally, supernatant bismuth concentrations were evaluated using inductively coupled plasma mass spectrometry (ICP-MS).

Results: Test strips detected EDTA in specimens taken from the following primary tube types: royal blue (Na_2EDTA), tan (K_2EDTA), lavender (K_2EDTA), and pink top (K_2EDTA). Test strips did not falsely detect EDTA in serum tubes including gold (serum separator), red (serum), and orange top (thrombin / serum separator), although three discordant red top serum results - due to pour off and/or aliquot errors - were identified using EDTA test strips in preliminary experiments. Test strips did not falsely detect EDTA in most other plasma specimens, including light green (lithium heparin / plasma separator), green (sodium or lithium heparin), and light blue top (sodium citrate), although one type of gray top tube (potassium oxalate with sodium fluoride) produced an orange "low EDTA" reaction. EDTA test strip reactivity was observed with a variety of chelating agents (order of reactivity: DMSA≈EGTA>K_2EDTA=Na_2EDTA>DMPS>PEN; ALA and DEF were non-reactive at 10 mM), although in general reactivity was only observed at concentrations higher than might be expected during chelation therapy. No pH effect was observed between pH 2-12. Lipemia did not interfere with test strip performance. Marked hemolysis caused an orange appearance to otherwise yellow reactions from EDTA-containing specimens, while visible icterus caused a slightly brown appearance to normally red reactions from non-EDTA specimens. ICP-MS demonstrated that bismuth levels were higher in specimens which had test strips dipped into the solution, arguing that a "dip mode" method may interfere with certain clinical assays.

Conclusions: EDTA test strips reliably detected the presence of EDTA in clinical specimens. Indeterminate or "low EDTA" orange results may require further investigation. "Dip mode" can produce analytical complications for assays which measure (or are interfered by) test strip constituent reagents.

A-275

Lack of tube mixing can be validated as regards ISO-15189 standard - preliminary validation

G. Lima-Oliveira[1], G. L. Salvagno[2], G. Lippi[3], G. Brocco[2], M. Pantani[2], W. Volanski[1], F. G. Rego[1], G. Picheth[1], G. C. Guidi[2]. *[1]Federal University of Parana, Curitiba, Brazil, [2]University of Verona, Verona, Italy, [3]University of Parma, Parma, Italy*

Background: Gentle and careful mixing by inverting 5 times is standardized as good laboratory practice by manufacturers and endorsed by Clinical Laboratory Standard Institute. Correct mixing after blood collection is claimed to be important. Nowadays laboratory quality managers have evidenced that phlebotomists do not mix vacuum tubes as recommended by manufacturers. As regards ISO-15189 international standard, all necessary improvements and potential sources of nonconformities, either technical or concerning the quality management system, shall be identified and all laboratory process shall be validated. The aim of this study was to evaluate whether it is really necessary to mix both K2EDTA- and lithium heparin-vacuum tubes immediately after blood collection.

Methods: Blood collection: Samples from 100 volunteers were drawn in three 3.0mL vacuum tubes containing 5.9mg K2EDTA and in three 3.5mL vacuum tubes with 52.5USP U of lithium heparin and gel separator. To eliminate any potential interference due to either contact phase or tissue factor, ~2mL of blood were preliminarily collected in a discard tube without additive. Blood collection was accurately standardized, including the use of needles and vacuum tubes of the same lot.

Processing: All vacuum tubes (one from each kind of additive) were processed using 3 different methods. Method 1: Gold Standard (M1): All specimens were mixed gently and carefully by inverting 5 times as recommended; Method 2: Rest time (M2): All specimens remained 5 min in upright position, followed by gentle careful mixing by inverting 5 times; Method 3: No mix (M3): All specimens were left in upright position without mixing afterwards. The influence of the primary tube mixing procedure was evaluated for routine hematology- and clinical chemistry-testing by paired t-test.

Laboratory testing: All samples were processed for routine hematological testing immediately after collection (<15min) on the same Sysmex® XE-2100D, Automated Hematology Analyzer (Sysmex Corporation®, Kobe, Japan). Routine clinical chemistry was performed on the same Cobas® 6000 c501 module (Roche Diagnostics GmbH, Penzberg, Germany). The parameters tested included erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, RBC distribution width, reticulocytes, white blood cells count and differential, including neutrophils, lymphocytes, monocytes, eosinophils and basophils, platelet count and mean platelet volume, glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, total protein, albumin, C-reactive protein, urea, creatinine, uric acid, alkaline phosphatase, amylase, pancreatic amylase, aspartate aminotransferase, alanine aminotransferase, g-glutamyl transferase, lactate dehydrogenase, creatine kinase, total bilirubin, direct bilirubin, phosphorus, calcium, magnesium, iron, sodium, potassium, chloride, lipase and hemolysis index.

Results: No fibrin filaments or microclots were observed in any samples. Significant differences ($P<0.01$), were found only for: a) erythrocytes (0.5%) and haematocrit (1.1%) when M1 was compared with M2; b) alanine aminotransferase (-3.0) when M1 was compared with M3; c) erythrocytes (-0.9%) and haematocrit (-0.8%) when M2 was compared with M3.

Conclusion: This preliminary evaluation has shown that K2EDTA- and lithium heparin- tube mixing after collection with evacuated system appears unnecessary. Moreover, this outcome indicates that not mixing vacuum tubes should not viewed as a non conformity for quality system and in conclusion the lack of tube mixing can be validated as regards ISO-15189 standard.

A-276

Comparability of urine total protein assays in patients with monoclonal proteinuria

C. D. Giesen, K. S. Lockington, T. S. Larson, J. C. Lieske. *Mayo Clinic, Rochester, MN*

Background: Urine contains various proteins including albumin, Tamm Horsfall protein, and low molecular weight proteins. Several different reagents and instrument platforms are used for urinary total protein measurement, with no established gold standard. All methods appear to measure albumin consistently, yet detection of non-albumin proteins appears more variable. This study compared the performance of five different reagent kits using urine from patients with and without kidney disease and also with known monoclonal M-spikes.

Methods: Urine samples submitted for random urinalysis (RUA) and monoclonal protein electrophoresis testing were analyzed using four pyrogallol red urine protein assays (Pointe Scientific Microprotein Reagent Set, Canton MI; Quantimetrix QuanTtest Red, Redondo Beach CA; Wako Diagnostics Autokit Micro TP, Richmond VA; and Siemens Healthcare Diagnostics Total Protein_2 (Urine), Tarrytown NY), and one benzethonium chloride assay (Roche Diagnostics Total Protein Gen. 2, Indianapolis IN) on the Roche cobas 6000 c501. Pyrogallol red assays were all performed using identical instrument settings, while the benzethonium chloride assay was performed per manufacturer's instruction. The Wako pyrogallol red assay served as the reference assay for the analysis. Samples were electrophoresed and stained on a SPIFE 3000 system (Helena Laboratories, Beaumont TX). M-spike quantitation was performed using a Helena Laboratories Quick Scan 2000. The Quick Scan 2000 scans and calculates the relative percentage of each electrophoretically separated protein fraction based on its staining intensity. The original urinary concentration of the M protein band is obtained by multiplying its relative percentage on the gel by the urinary total protein concentration.

Results: Among RUA samples with varying levels of proteinuria (1- 2632 mg/dL; mean=130 mg/dL; median=52 mg/dL), Passing and Bablok regression analysis revealed good correlation between all methods and the reference (Wako) assay (Pointe: y=1.15x+1.90, n=100; Quantimetrix: y=0.97x+0.39, n=325; Siemens: y=0.99x+5.04, n=25; Roche: y=1.01x+4.31, n=100). In the urine samples with a known M-spike but total protein content >25% albumin, linear correlations with the Wako assay were still reasonably good (Pointe: y=1.0273x+7.8232 R^2=0.9985, n=18; Quantimetrix: y=0.9549x-3.6582 R^2=0.9974, n=22; Siemens: y=1.0114+4.1414 R^2=1, n=4; Roche: y=0.9496x+11.007 R^2=0.9946, n=18). Urine samples with M-spike concentrations <100 mg/24 hours also compared reasonably well, regardless of the albumin percentage (Pointe: y=1.0731x+3.0911 R^2=0.9564, n=44; Quantimetrix: y=0.9101x+0.4658 R^2=0.9781, n=54; Siemens: y=0.9059x+6.181 R^2=0.9843, n=10; Roche: y=1.0416x+3.0244 R^2=0.884, n=44). However, among samples with M Spikes ≥100 mg/24 hours and albumin ≤25%, comparisons between assays were quite variable (Pointe: y=1.1156x+10.509 R^2=0.8207, n=52; Quantimetrix: y=0.2386x+18.907 R^2=0.6697, n=66; Siemens: y=0.9536x+15.617 R^2=0.9735, n=14; Roche: y=1.2409x+15.676 R^2=0.7977, n=52).

Conclusions: All urine protein assays in this study compared well for testing random urinalysis samples obtained from a population of patients with and without kidney disease in whom albumin is usually a major protein species. However, these total protein assays performed quite variably when testing urine samples that contained large quantities of monoclonal protein, particularly those with <25% albumin and

a calculated M-spike greater than 100 mg/24 hours. In particular, urine total protein assays can vastly underestimate protein excretion in certain patients with large M-spikes.

A-277

Elevated Cerebrospinal Fluid Total Protein caused by Povidone-Iodine (Betadine) Interference

V. Gounden, Z. Zhao. *National Institutes of Health (Clinical Center), Bethesda, MD*

Background: The measurement of cerebrospinal fluid (CSF) total protein (TP) is useful in the diagnosis of meningitis and the detection of other inflammatory diseases. The laboratory was contacted by a clinician concerned with a clinically discrepant elevated CSF TP. The specimen received for chemistry testing was the first tube of the CSF collection. The CSF TP was 417 mg/dL (reference interval 15- 45 mg/dL) and CSF glucose was 87.2 mg/dL (reference interval 40-70 mg/dL). CSF cell counts were: red blood cells 6/mm3 and white cell count 1/ mm3. Another specimen from the patient (tube 2) taken at the same time and submitted to hematology was analyzed for CSF TP. The CSF TP result of tube 2 was 20 mg/dL and was confirmed on repeat analysis.

Objectives: To investigate falsely high CSF TP results, suspected to be caused by preparation of collection site by povidone-iodine (Betadine) solution.

Design and Methods: We performed interference studies to determine the effect of Betadine, iodine only and povidone only on the CSF TP concentration. A CSF diluent was prepared by pooling clear CSF specimens to which reagent grade water was added to give a final ratio of 90%:10% v/v CSF to water. An initial sample of the CSF diluent and either the povidone-iodine or iodine only or povidone only solution was prepared to give a final ratio of 90%:10%v/v CSF to solution. This initial sample was then serially diluted with the prepared CSF pool to give different final concentrations of povidone-iodine/iodine/ povidone. CSF TP was then measured for these prepared samples using the laboratory routine chemistry analyzer, the Siemens Dimension Vista (Siemens Healthcare Diagnostics, Tarrytown, USA). This assay involves the reaction of protein in the sample with the pyrogallol red (PGR) sodium molybdate complex to form a bluish-purple colored complex, which absorbs at 600 nm. Further CSF TP measurements were made on the Siemens Dimension Xpand using the PGR method. In addition to this CSF samples were analysed for TP at a reference laboratory using the modified biuret method. To investigate if the positive interference was a result of the iodine in the Betadine solution and its direct absorbance at the same wavelength as the PGR- protein complex, spectrophotometric absorbance studies were also performed.

Results: The experimental data of the interference confirmed a positive interference for the PGR assay when Betadine containing povidone or povidone alone were added to a CSF sample. The Betadine solution did not interfere the modified biuret method for TP. The false positive TP interference was clinically significant (> 10% change) even at Betadine concentrations (as low as 0.0025%) where the CSF sample appeared clear on visual inspection. Spectrophotometric studies of the Betadine solution and patient's sample showed no absorbance at 600nm.

Conclusions: Low levels of povidone-iodine contamination of CSF specimens can lead to clinically significant positive interference for TP results. Alternate iodine solutions not containing povidone should be used for preparation of sites for CSF collection.

A-278

Extending the Time Restriction on Transit Time for Lactate Measurement

L. Racsa[1], C. Eggert[2], M. Mohamed[2], P. Kutscher[2], I. Hashim[1]. *[1]University of Texas Southwestern Medical Center, Dallas, TX, [2]Parkland Health & Hospital System, Dallas, TX*

Background: Plasma Lactate is useful for assessing tissue perfusion in critically ill patients. When using sodium fluoride/potassium oxalate (FlOx) as a preservative, test reagent manufacturers require that blood specimens be processed within 15 minutes from collection. This transit time limit, however, cannot always be met, particularly when the laboratory is distant from patient care, as the case in our institution. 3% of lactate samples arrive late into the laboratory and are thus rejected. In an attempt to reduce the number of rejected lactate specimens, we examined the effect on lactate values following extended transit time to 30 minutes.

Methods: Lactate samples were collected in triplicate from 50 patients (with prior physician orders for lactate) and from 50 normal volunteers. One sample set was kept at room temperature (RT) and processed at 15 minutes from collection according to manufacturer's instruction. Of the remaining two sample sets, one was kept at RT and the other on ice (4°C) for 30 minutes before lactate analysis. Lactate was measured using Roche Cobas® LACT2. Accuracy was determined with lactate values obtained at 30 minutes (both RT and 4°C) compared to the standard 15 minutes. Intra-assay precision was obtained using 10 aliquots from the same samples spanning the analytical measuring range. Inter-assay precision studies were performed on 20 patients.

Results: Lactate values ranged from 0.6 to 25.4, and from 0.7 to 2.1 mmol/L in patients and volunteers, respectively. There was good correlation between RT lactate values measured at 15 and 30 minutes (r= 0.9993, bias 0.04). There was good correlation (r=0.9992, bias -0.06), between RT lactate values measured at 15 minutes and those at 30 minutes (4°C). There was good correlation (r=0.9993, bias -0.01) between RT and 4°C lactate values measured at 30 minutes. Intra-assay imprecision for RT samples processed at 15 minutes ranged from 1.6%, 1.0%, 1.1%, and 0.9% at lactate levels of 1.3, 5.5, 9.0 and 12.4 mmol/L respectively. For RT samples processed at 30 minutes, imprecision ranged from 0%, 1.2%, 1.0%, and 0.8% compared with 4°C samples, 0%, 1.3%, 0.9%, and 1.1%, at lactate levels of 1.3, 5.5, 9.0 and 12.4 mmol/L respectively. Inter-assay imprecision for RT samples processed at 15 minutes ranged from 0.0-6.7%; RT samples process at 30-minutes ranged from 0.0-13.1% compared with 0.0-10.2% for samples kept at 4°C. All precision studies met our acceptance criteria of <14%.

Conclusion: The performance of the assay for samples kept for 30 minutes either at RT or 4°C was not significantly different from those kept at RT and processed at processed at 15 minutes. Our institution changed the lactate processing protocol to 30 minutes, leading to a reduction of lactate order rejections from 3% to less than 1%.

A-279

Measurement of Serum Total Calcium Using the 5-nitro-5'-methyl-BAPTA Method in the Presence of Four Gadolinium-based Contrast Agents

C. A. Wittwer, L. J. Ouverson, D. R. Block, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Some gadolinium-based contrast agents administered to patients undergoing MRI procedures are known to interfere with the widely-used o-cresolphthalein complexone (o-CPC) dye method used to measure serum total calcium. Patient samples with serum calcium <7.0 mg/dL using this method were routinely re-tested in the clinical laboratory with an Arsenazo III method to avoid reporting falsely decreased calcium results due to gadolinium interference. Roche Diagnostics recently introduced a calcium reagent formulation which utilizes a different ion-selective indicator, 5-nitro-5'-methyl-BAPTA (NM-BAPTA).

Objectives: (1) Verify the manufacturer's claim that gadolinium-containing MRI contrast media at therapeutic concentrations does not interfere with serum total calcium measurement utilizing the NM-BAPTA method. (2) Evaluate laboratory efficiencies gained by implementing a serum calcium method that is not affected by gadolinium-based contrast agents.

Methods: Gadolinium-based contrast agents commonly used at Mayo Clinic were added to five residual patient serum pools with varying concentrations of calcium (6.5-10.3 mg/dL). Gadodiamide, gadobenate dimeglumine, gadoxetate disodium and gadofoveset trisodium were each added to serum pools yielding final concentrations of 0.1, 0.25, 0.5, 1.0, and 2.0 mmol/L contrast agent. Total serum calcium was measured utilizing the o-CPC and NM-BAPTA methods on a Roche Cobas c501 and c701 (Roche Diagnostics) and the Vitros 350 Arsenazo III method (Ortho Clinical Diagnostics). Results obtained were compared to control serum pools with de-ionized water added to account for dilution. Absolute differences between total calcium concentrations in samples with and without contrast agent were calculated. The number of patient samples with calcium <7.0 mg/dL, cost of maintaining a secondary method and turn-around time were assessed over a one year time period.

Results: Addition of gadodiamide at final concentrations of 0.1, 0.25, 0.5, 1.0 and 2.0 mmol/L lowered serum calcium results by an average (mg/dL) of 0.3 (range 0.1-0.5), 0.5 (range 0.2-0.8), 0.8 (range 0.4-1.3), 1.4 (range 0.9-1.9) and 2.4 (range 1.7-3.0) mg/dL, respectively, using the o-CPC method. There were no differences between calcium results in control and gadodiamide-containing samples using the Arsenazo III or NM-BAPTA method (average absolute difference 0.0 mg/dL, range 0.0-0.3 mg/dL). In samples containing gadobenate dimeglumine, gadoxetate disodium and gadofoveset trisodium, serum calcium results differed by <0.2 mg/dL between contrast-added and control samples (average absolute difference 0.0 mg/dL, range 0.0-0.2 mg/dL) at all concentrations of contrast agent using the o-CPC, NM-BAPTA, and Arsenazo III methods. During 2013, 732 patient samples were re-tested using the Arsenazo III method costing >$1,200 in additional reagent, calibrators and quality

control. Turn-around time for reporting of calcium results was 25-35 minutes longer for specimens requiring re-testing.

Conclusions: The Roche Diagnostics NM-BAPTA method for measuring serum total calcium does not show clinically significant interference in the presence of four gadolinium-based contrast agents. By implementing a calcium method that is free from gadolinium interference, the laboratory improved quality and reduced the risk of reporting falsely decreased serum calcium results due to gadolinium interference. Efficienies gained included eliminating a secondary Arsenazo III calcium method, reducing patient re-testing, and eliminating the reporting delays and costs associated with re-testing.

Tuesday, July 29, 2014

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

Hematology/Coagulation

A-280

Clinical Utility of Hematological Parameters to Predict Sepsis Prior to Clinical Presentation in Medical Intensive Care Unit Patients

S. W. Njoroge, M. Thompson, J. M. Colon-Franco, S. Litt, D. A. Anderson, T. W. Rice, A. P. Wheeler, A. Woodworth. *Vanderbilt University School of Medicine, Nashville, TN*

Introduction: Sepsis is characterized by pathogen invasion into the bloodstream and the host's response to this invasion. Early detection of sepsis allows for rapid initiation of therapy, decreases morbidity and mortality, and reduces healthcare expenses.

Objective: To investigate the diagnostic utility of hematological parameters to predict the onset and severity of sepsis in Medical Intensive Care Unit (MICU) patients prior to clinical presentation of systemic inflammatory response syndrome (SIRS).

Methods: This retrospective cohort study employed 125 MICU patients with SIRS. These patients were identified by software that scans electronic medical records and alerts investigators when a MICU patient meets SIRS criteria. Several hematological parameters were quantitated on the Sysmex XE 5000 analyzer from specimens collected 24 or 48h prior to the patient meeting SIRS criteria. Procalcitonin was quantified in residual plasma using the Vidas B.R.A.H.M.S® PCT assay (bioMerieux, Inc). The diagnoses of non-infectious SIRS, sepsis and sepsis severity were blindly adjudicated by 2 MICU physicians as: SIRS (n=70) and Sepsis (n=55; severity: sepsis n=7, severe sepsis n=22, and septic shock n=26). Receiver operator characteristic (ROC) curves were generated to evaluate the diagnostic utility of these hematological parameters to predict sepsis.

Results: Areas under the ROC curves (AUC) for each parameter to predict sepsis or severe sepsis/septic shock are listed in table 1. Four hematological parameters WBC, ANC, % neutrophils, and IGC, were significantly different between septic and non-septic patients and between patients with early/no sepsis vs. severe sepsis or shock. Additionally, WBC, ANC, and % neutrophils showed significantly improved diagnostic strength to predict sepsis when compared to a sepsis biomarker, procalcitonin.

Conclusions: Certain hematological parameters measured before onset of overt symptoms of systemic inflammation accurately predicted patients who developed sepsis, severe sepsis and shock. The diagnostic utility of these markers may be improved by combining them into logistic regression models.

Table 1: Diagnostic Utility of Selected Hematological Parameters Measured 24 - 48 Hours Prior to Onset of SIRS to Predict Sepsis and Sepsis Severity

	Sepsis vs. No Sepsis			Early/No Sepsis vs. Severe Sepsis and Shock		
Predictor	**AUC**	**95% CI**	**P value**	**AUC**	**95% CI**	**P value**
Procalcitonin (PCT)	0.67	[0.57-0.76]	0.001	0.69	[0.60-0.79]	<0.001
White Blood Cell Count (WBC)	0.7	[0.61-0.79]	<0.001	0.68	[0.58-0.77]	<0.001
Red Blood Cell Count (RBC)	0.52	[0.42-0.62]	0.74	0.53	[0.43-0.64]	0.54
Immature Granulocyte Percentage (IG-%)	0.60	[0.50-0.70]	0.07	0.61	[0.51-0.71]	0.04
Immature Granulocyte Count (IGC)	0.66	[0.57-0.76]	0.001	0.66	[0.57-0.76]	0.002
Neutrophils (Neut %)	0.72	[0.63-0.81]	<0.001	0.74	[0.65-0.83]	<0.001
Absolute Neutrophil Count (ANC)	0.71	[0.62-0.80]	<0.001	0.71	[0.62-0.80]	<0.001
Platelet Count	0.52	[0.41-0.62]	0.74	0.52	[0.42-0.63]	0.64

A-281

Comparison of coagulation factors assays in two automated platforms

S. TUFIK, M. C. FERES, M. C. DE MARTINO, M. P. MIRANDA, S. C. F. DA SILVA. *AFIP, SAO PAULO, Brazil*

Background: The determination of coagulation factors II, V, VII and X in the plasma is indicated to diagnosis congenital or acquired deficiency factor, to distinguish dysproteinemias, to aid in therapeutic monitoring of concentrated prothrombin and oral anticoagulants and to verify the function of protein synthesis in the liver. This study aims to evaluate the analytical performance of factors II, V, VII and X measured in Siemens BCS XP System in comparison with Stago STA Compact System performance.

Methods: In a normal population, 18 samples (n = 18) were collected with citrate as anticoagulant. Within 4 hours after collection, samples were analyzed by coagulometric method simultaneously on Siemens BCS XP System, using optical detection and Siemens deficiency factor plasmas and on STA-Compact using mechanical detection. In order to establish correlation between methodologies, we evaluate coefficient of correlation, slope and intercept.

Results: Statistical data is summarized in the table below.

Analytical performance comparison for deficient plasmas						
Assay	**n**	**Regression eq.**	**r**	**Mean**	**SD**	**Selection range**
Factor II	18	y=0.3996x + 66.885	0.64	94	18	67% - 133%
Factor V	18	y=0.6791x + 13.734	0.94	109	23	72% - 153%
Factor VII	18	y=1.2714x – 35.975	0.91	120	24	78% - 217%
Factor X	18	y=0.7043x + 20.576	0.90	123	21	87% - 155%

Conclusion: We observed good correlation between platforms for the V, VII and X factors. For Siemens BCS XP, matrix of factor II is of human origin, providing better performance and low correlation with Stago platform on which the matrix is a mixture of human serum and bovine plasma.

A-282

Plasma cell myeloma with rare presentation

L. B. Zuppani[1], E. H. J. T. Dauar[1], S. S. Rodrigues[1], O. Fernandes[1], C. L. Oliveira[1], O. F. D. Dias Neto[2], J. Tabacof[2], D. P. Jardini[3], F. B. F. Carvalhares[3]. *[1]DASA, São Paulo, Brazil, [2]Clínica Oncológica Paulista, São Paulo, Brazil, [3]Hospital Paulistano, São Paulo, Brazil*

Background: Plasma cell myeloma is a multifocal plasma cell neoplasm associated with an monoclonal immunoglobulin called M-protein in serum and/or urine. The disease spans a clinical spectrum from assyntomatic to aggressive forms and due to deposition of abnormal immunoglobulin chains in tissues. The diagnosis is based on a combination of pathological, radiological and clinical features. Over 90% of cases of mieloma occur in patients 50 years old or older. The median age at diagnosis is 70 years. Only 2% of cases begin before the age 40.

Objective: The objective of this case is to bring awarness to atypical presentations of plasma cell myeloma that may hinder its diagnosis. In the present case the difficulty in diagnosis is due to the atypical age at onset, absence of monoclonal immunoglobulin in the protein electrophoresis, hypocelularity of bone marrow without typical elements of the pathology and presence of histiocytes and plasma cells with cytoplasmatic inclusions.

Clinical case: CLMS, 38 year old woman, addmited to Hospital Paulistano due to metrorrhagia and pelvic pain. CBC: hemoglobin 5.6 g/dl, platelets: 86,000/ mm^3. Radiographic study: lytic lesions in the hip. Blood marrow count : hipocelularity of all myeloid cells; 1.6% of plasma cells with abnormal morphology; presence of cells with crystallized cytoplasmic imunoglobulin with a lamellar pattern. Protein electrophoresis with absence of M-protein. Blood Immunofixation with presence of monoclonal kappa without correspondence with heavy chain IgA, IgG or IgM. Urinary Immunofixation: presence of monoclonal kappa without correspondence with heavy chain IgA, IgG or IgM. Bone marrow biopsy with imunohistochemistry report: CD68 clone PG-M1 positive in nomerous histiocytes with crystallized cytoplasmic imunoglobulin. Kappa positive in most plasma cells with large cytoplasm. CD 138 positive in about 40-50% of cells. FDG-PET: hypermethabolism in the lithic lesions of the iliac crest. Increased FDG uptake in the bone that corresponds to bone marrow activity.

Conclusion: The enlarged lamelar cytoplasm of plasma cells and histiocytes, probably correspond to the cytoplasmic accumulation of immunoglobulin. The identification of these cells as plasmocites, associated with the imunohistochemistry, FDG-PET and blood and urinary immunofixation allowed the diagnosis of Plasma Cell Myeloma of Kappa chain.

A-283

Validation of Thrombin-Antithrombin III Complex by Enzyme-Linked Immunosorbent Assay in Humans, Non-Human Primates, and Canine Citrated Plasma to Support Pre-clinical and Clinical Coagulation Studies

C. Phanthalangsy[1], J. Stejskal[2], R. Giovanelli[1], J. Wisniewski[1], S. Ramaiah[2]. *[1]Pfizer, Groton, CT, [2]Pfizer, Andover/Cambridge, MA*

Background: The conversion of prothrombin into active thrombin is a significant event within the coagulation cascade. Thrombin is primarily inhibited by antithrombin (ATIII) in which results in a stable inactive proteinase/ inhibitor complex. The concentration of thrombin-antithrombin III complex (TAT) can be measured, and represents a sensitive clinical biomarker for the diagnosis of thrombotic disease in the coagulation cascade.

Methods: Siemens Enzygnost® TAT micro immunoassay (Catalog #:OWMG15) was validated in human, non-human primate (NHP), and canine citrated plasma. The concentration of TAT was measured in-vitro quantitatively through a sandwich enzyme immunoassay and used two different antibodies directed against thrombin and ATIII, respectively. The first incubation step consists of TAT binding to peroxidase-conjugated antibodies that are attached to the surface of the microtitration plate against thrombin. The second incubation step consists of a reaction in which the enzyme-conjugate antibodies are bound to the free ATIII determinants. Any unbound constituents and excess enzyme-conjugated antibodies are removed by a series of washes after each incubation. The enzymatic reaction between hydrogen peroxide and chromogen is stopped by the addition of diluted sulphuric acid. This results in a color intensity change which is proportion to the concentration of TAT. TAT concentrations are measured photometrically through the SPECTRAmax 384plus reader within the kit standard concentration range of 2 to 60 µg/L. For higher TAT concentrations, the sample was diluted using Sekisui TAT deficient material (Catalog #: 203) for humans and normal TAT levels (<4 µg/L) for NHP and canine.

Results: Intra-assay was established through 5-10 replicates and the %CV was ≤4.6 for human, ≤10.8 for NHP, and ≤8.3 in canine. Inter-assay precision was established through a minimum of 3 separate assay runs and the %CV was ≤1.50 for human, ≤15.5 for NHP, and ≤11.4 in canine. The limit of blank was established by analyzing 10 replicates and determined to be 0.135 µg/L for human and 1.3 µg/L for NHP. The lower limit of quantitation was determined as 2.36 µg/L with a %CV of 11.28 in human and 2.10 µg/L with a %CV of 15.7 in NHP. The upper limit of quantitation was determined as 60.15 µg/L and %CV of 4.58 in human and 60.0 µg/L and %CV of 13.4 in NHP. Dilutional linearity was determined using samples near the upper limit of the assay calibration curve, and spiked recovery was determined using samples spiked with several concentrations of kit calibrators. All results were within 20% of the expected value. Sample freeze/ thaw stability was performed using samples with concentrations near the lower and middle limits of the assay calibration curve and consist of 4 freeze/thaw cycles. All results were within 20% of the expected value. Sample stability was performed for human and canine samples frozen at -80 °C. Human samples were stable up to and including 3 months, and canine samples were stable up to and including 2 weeks.

Conclusion: All outlined criteria for the validation of TAT in human, NHP, and canine were met and used to support pre-clinical and clinical coagulation studies.

A-284

Inevitable is fasting time for coagulation laboratory tests - Preliminary evaluation

G. Lima-Oliveira[1], G. L. Salvagno[2], G. Lippi[3], E. Danese[2], G. Poli[2], M. Gelati[2], M. Montagnana[2], C. Recchi[2], M. Saggiorno[2], G. Picheth[1], G. C. Guidi[2]. *[1]Federal University of Parana, Curitiba, Brazil, [2]University of Verona, Verona, Italy, [3]University of Parma, Parma, Italy*

Background: Errors in the preanalytical phase generate further work or additional investigation that may cause unnecessary procedures for patients. This study was aimed to evaluate the inevitability of fasting time for coagulation laboratory tests.

Methods: The first blood sample was collected from 10 healthy volunteers at fast (12h). Immediately after blood collection, the volunteers consumed a standardized meal (Table 1).

Table I. Nutritional composition of standardized meal

Nutritional composition	Slice of cheese	Yorgut	Slice of bread	Chocolat snack	Fruit juice	Total
Number (overall weight)	1 (25 g)	1 (125 g)	2 (46.8 g)	1 (20.7 g)	1 (200 g)	417.6
Kcal	64	134	126	105	134	563
KJ	266	562	532	438	572	2370
Protein (g)	4.4	4.1	4.2	1.1	0.8	14.6
Carbohydrate (g)	0.8	19.4	22	12.7	32	86.9
Sugar (g)	0.8	N/A	3	10	N/A	13.8
Total lipids (g)	4.6	4.4	2.4	5.5	0	16.9
Saturated lipids (g)	3.1	N/A	0.8	3.7	0	7.6
Fibre (g)	0	N/A	0.9	0.2	2	3.1
Sodium(g)	0.3	N/A	0.206	0.02	0	0,606
Calcium(g)	0.133	0.131	N/A	N/A	N/A	0.264

The following blood samples were collected 1, 2 and 4 hours after the end of the meal. Each phase of sample collection was standardized, including use of needles and vacuum tubes of the same type and lot. Coagulation tests included the following: activated partial thromboplastin time (aPTT sec), prothrombin time (PT sec), fibrinogen (mg/dL), antithrombin III (AT %), protein C (PC %) and protein S (PS %). The significance of differences between samples was assessed by paired Student's t-test after checking for normality by the D'Agostino-Pearson omnibus test. The level of statistical significance was set at $p < 0.05$.

Results: One hour after food intake, variations were observed for PT (-2.3%, P=0.45) and AT (1.9%,P=0.04). Two hours after meal differences were observed for aPTT (-4.0%, P=0.03) and PT (-3.8%,P=0.57). Statistically significant increases could be observed four hours after the meal only for AT (3.0%,P=0.02). The results of fibrinogen, PC and PS were not influenced by the meal at any time points.

Conclusion: Significant variations of aPTT, PT and AT coagulation laboratory tests after a standardized meal were observed. In conclusion these preliminary outcomes had shown that the fasting time should be carefully considered when performing these tests, in order to prevent spurious results and reduce laboratory errors especially in the therapeutic monitoring.

A-285

Mean platelet volume in patients with pre-eclampsia

W. Lee, S. Cho, E. You, Y. Jeon, H. Lee, T. Park. *Kyung Hee Medical school, Seoul, Korea, Republic of*

Background Larger platelets have greater haemostatic efficiency than smaller ones by producing larger amounts of vasoactive and prothrombotic components. Mean platelet volume (MPV) is a useful marker indicating alteration of platelet activity which shows association with various inflammatory diseases. Pre-celampsia (PE) is a disease characterized by endothelial damage, elevated intravascular platelet activation while increased MPV in the second of third trimester of pregnancy has been reported. The aim of this study is to evaluate possibility of MPV as a marker of recovery from PE or eclampsia after delivery.

Methods Twenty-one pre-eclamptic and one eclamptic women who gave birth at Kyung Hee Medical Center during January 2011 to June 2012 were include in the study. The results of white blood cells (WBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and MPV were obtained using an automated hematologic analyzer, Advia 2120 (Siemens Diagnostics, Tarrytown, NY, USA). Medical records were analyzed retrospectively. Postpartum laboratory data of 16 patients with existing serial results of the day of delivery, a day after and two days later were analyzed retrospectively.

Results Nineteen patients had preterm delivery and three had term delivery. Major comorbidities of diabetes mellitus (DM) and myoma were presented in eight patients. Both MPV and platelet count were shown to have decreasing tendency over the observed period, although statistical significance was not shown (P=0.152 and P=0.327, respectively). A ratio of MPV/PLT was slightly increased without statistical significance (p=0.222). Longitudinal serial data of six postpartum days in 5 pre-eclampsia women showed definitely decreasing tendency.

Conclusions MPV has shown decreasing tendency during postpartum periods following PE or eclampsia. These results demonstrate reduced platelet activity and decreased maternal intravascular systemic inflammation after resolution of pregnancy. This study provides evidence that MPV is able to reflect the recovering state from PE or eclampsia in postpartum periods. Most of the previous

studies were focused on increased MPV at pregnant period of PE. Meanwhile, we carefully suggest MPV could be supportive surrogate marker as recovery from inflammatory state after delivery in PE based on these results of our study. Large scale prospective studies are required to affirm these findings and elucidate the underlying mechanism to induce changes of MPV in PE or eclampsia.

A-286

Capillary electrophoresis identification and laboratory evaluation of a complex protein finding in a patient serum

L. B. Porto, P. R. C. Souza, L. L. Leite, Z. R. M. Cardoso, C. F. A. Pereira. *DASA, RIO DE JANEIRO, Brazil*

Background: A 74-years old female was evaluated by her primary care physician, who ordered basic routine tests. All results were within the reference intervals, except the serum electrophoresis, which revealed two unusual intense bands expressed in the gamma region.

The aim of our study was to better characterize the finding according to the guidelines in order to provide more information to the physician.

Methods: Laboratory analysis was conducted by performing a high resolution capillary electrophoresis using the Capillarys® (Sebia) for protein identification. In order to define abnormal protein type, immunofixation of Immunoglobulins and κ and λ light chain, antisera was performed using agarose gel and reagents with the Hydrasys® Electrophoresis System (Sebia). Subsequently, the direct measurement to define the level of immunoglobulins and light chains was performed by nephelometry using BN II® (Siemens Healthcare Diagnostics).

Results: Immunofixation with Immunoglobulins and κ and λ light chain antisera showed the presence of two monoclonal bands against IgG λ and an IgA κ compatible with a biclonal gammophaty. The direct nephelometric measurement revealed a normal IgG level of 1010 mg/dl, a high IgA level of 849 mg/dl, a normal IgM level of 60.5 mg/dl, a high level of κ light chain level of 416.0 mg/dl and λ light chain level of 228.0 mg/dl. The κ/λ ratio of 1.82 was within the reference interval.

Conclusion: In this particular case, the initial diagnosis was apparently a polyclonal distribution, and although not initially requested, the use of complementary tests like quantification and immunological identification, by immunoglobulins profile, were important to help the physician to streamline the diagnosis, monitor and stratify the risk of this biclonal pathology.

A-287

Mean platelet volume (MPV) in patients with chest pain

G. Lobreglio, F. Sicuro, M. Renis. *Azienda ASL Lecce Presidio Ospedaliero Vito Fazzi, Lecce, Italy*

BACKGROUND AND OBJECTIVES. Variation in platelets size is indicative of change in platelet function; mean platelet volume (MPV) is directly related to platelet activation in vivo, which plays a central role in the pathogenesis of many vascular diseases. Recent studies have shown conflicting results about the relationship between increasing MPV and cardiovascular disease. This work was aimed at investigating if there is a difference in mean platelet volume between patients with acute myocardial infarction (AMI) and subjects with other cardiac diseases or non-cardiac chest pain, owing to platelets activation in acute coronary syndromes.

METHODS. The study included 5927 consecutive patients with acute non traumatic chest pain presenting to the emergency department of a medium-sized hospital over a period of 30 months, from January 2011 to June 2013. The median age of study participants was 61 years (range, 47 to 93); 3051 (51,5%) were male, 2876 (48,5) were female. EDTA anticoagulated whole blood and serum samples were obtained on admission from all patients. According to the Universal Definition of Myocardial Infarction, AMI was diagnosed in patients showing a rise and/or fall of cardiac troponin I (cTnI) above the diagnostic threshold for MI (0,30 ng/mL), typical changes of electrocardiogram, imaging evidence of new loss of viable myocardium or presence of an intracoronary thrombus. MPV was measured in the course of complete blood count with Sysmex XE-2100 automated hematology analyzer using the Hydro Dynamic Focusing and Direct Current Detection. cTnI was measured by Architect *i* System with chemiluminescent microparticle immunoassay (CMIA) from Abbott Laboratories.

RESULTS AND CONCLUSIONS. AMI was diagnosed in 652 (11%) patients with chest pain (364 men, 288 female). Mean MPV measured at hospital admission was 11.2 ± 0,7 femtoliter (fL) in patients with AMI and 10.4 ± 0,5 fL in all other subjects, with a mean difference of 0.8 fL (95% CI 0.6 – 1.1; P < 0.05). These results show that MPV is higher in patients with acute myocardial infarction compared to those with other cardiac diseases or non-cardiac chest pain, suggesting a substantial role of platelets activation in the formation of the thrombus that occludes the culprit coronary arteries. Therefore, MPV is a potentially useful biomarker of platelet activity in the setting of coronary atherothrombotic events.

A-288

Targeted Metabolomic Profiles Are Strongly Correlated With Metabolic Alterations In Patients With Sickle Cell/Beta Thalassemia Disease

I. Papassotiriou[1], F. Panetsos[2], T. Livadara[2], A. Tzivaras[3], E. Voskaridou[4]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Analytical Chemistry Laboratory, Bioiatriki, Athens, Greece, [3]Blood Transfusion Center, "Aghia Sophia" Children's, Athens, Greece, [4]Thalassemia Center, "Laikon" General Hospital, Athens, Greece*

Background: The complex pathophysiology of Sickle Cell Disease (SCD) makes unlikely that a single therapeutic agent will prevent or reverse all SCD complications. Metabolomic analysis might help in the characterization of the endogenous and exogenous effects of potential new treatments. Metabolites are small molecules that are chemically transformed during metabolism and provide a functional readout of cellular state. Metabolites serve as direct signatures of biochemical activity and are therefore easier to correlate with phenotype. The metabolome is typically defined as the collection of small molecules produced by cells and offers a window for interrogating how mechanistic biochemistry relates to cellular phenotype. There are very few reports providing comprehensive measurements of metabolites present in blood and almost no reports on metabolites changes associated with SCD. In this context we aimed to detect and to quantify targeted metabolites' abnormalities in patients with Sickle Cell/beta thalassemia disease (HbS/βThal) and their implication in pathways that might be of interest to prevent vaso-occlusion and/or to monitor the effects of new therapies on SCD.

Patients and Methods: Thirty adult patients with HbS/βThal were enrolled in the study, while 20 apparently healthy individuals matched for age served as controls. Targeted metabolome analyses based on aminoacids and carnitines were performed after extraction from dry blood spots (DBSs) on filter paper using High Performance Liquid Chromatography followed by tandem Mass Spectrometry (LC/MS/MS), with derivatization (AB SCIEX 5500 triple quadrupole and QTRAP® LC/MS/MS Systems, Framingham, MA, USA) with reagents provided by Chromsystems Instruments & Chemicals, Germany. The injection volume was 10 µL and the analysis lasted ca. 2 min.

Results: The main results of the study showed that: a) fifty metabolites were separated in patients and controls samples, b) mapping the results of analyses, patients with HbS/βThal compared to controls had 17 metabolites with significantly lower concentration, 10 metabolites with comparable concentration and 23 metabolites with significantly higher concentration, c) L-arginine and L-ornithine concentrations were significantly lower in patients HbS/βThal compared to controls, 9.3±2.6 vs 14.7±3.7 µmoles/L, ($p<0.01$), and 116.0±15.0 vs 211.2±19.5 µmoles/L, ($p<0.06$) and d) carnitine, acetylcarnitine and propionylcarnitine correlated significantly positive with reticulocyte production index ($p<0.001$).

Conclusions: Our findings showed significant alterations in whole blood metabolome of patients with HbS/βThal. Also we demonstrated the very important metabolic abnormality of Nitric Oxide biosynthesis pathway due to the low concentration of the aminoacids serving of substrates in this cycle and disturbances in carnitine and acylcarnitines homeostasis. These abnormalities in the metabolome reflected the hemolysis, inflammatory process and pulmonary hypertension observed in these patients.

A-289

Validation of Fluorescence in Situ Hybridization assay for detection of rearrangements involving the Mixed Lineage Leukemia gene

F. K. Marques, W. G. Teixeira, P. C. Ângelo, E. C. C. Mateo, A. C. S. Ferreira. *Instituto Hermes Pardini, Vepasiano, Brazil*

Background: The Fluorescence *In Situ* Hybridization (FISH) testing has become an important tool of clinical practice in many laboratories dealing with neoplastic deseases. The FISH is more sensitive than conventional chromosomal analysis because can detect a specific alteration in both dividing and nondividing cells and small population of abnormal cells. Although the performance of the most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior implementation of assay for clinical use. Clinical laboratories must independently adopt protocols for verify the performance of the assay. Rearrangements involving the Mixed-Lineage Leukemia gene (MLL) represent 10% of abnormalities detected in acute leukemias, which in many cases represent poor prognosis. Thus the rapid identification of these rearrangements is needed to guide prognosis and treatment.

Objetive: To validate FISH assay for detection of rearrangements involving the MLL gene following recommendations from the American College of Medical Genetics (ACMG).

Methods: We use the MLL dual-color breakapart probe manufactured by Cytocell®. In the familiarization phase the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. 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 reference range (normal cutoff) were estimate the false positive rate from 20 uncultured bone marrow samples that would be unlikely to harbor a MLL rearrangement. Two analysts score 500 interphase cells (250 per analyst). All MLL probe signal patterns were recorded. The normal cutoff for each signal pattern was calculated using the BETAINV and CRITBINOM function available in Microsoft Excel.

Results: The MLL breakapart probe presents two portions of the gene, differentially labeled: the proximal 5 'labeled with green fluorophore, the distal 3' labeled with red fluorophore. A typical result of using this probe should show 2 fusions of green and red signals (2F). The separation of the fusion signals indicates the presence of MLL rearrangement. The clinical validation of FISH showed rearrangement involving MLL gene, in agreement with conventional karyotyping. The probe demonstrated 100% specificity and analytical sensitivity. Among the 20 bone marrow samples analyzed, six had one or two false positive cells, which have been designated by the abbreviation 1F1R1G (one fusion, one red, one green). MLL atypical probe signal patterns were also found: 1F, 3F, 1F1G and 2F1G. The cutoffs obtained with BETAINV function was validated for counting 200 cells. The signal patterns and respective normal cutoff are 1F1R1G (2.3%), 1F(4.4%), 3F(1.4%), 1F1G(3.0%) and 2F1G(1,4%). The BETAINV function does not allows a cutoff of zero and show minimal change with increasing cell score. It can minimize the problem of false positive signals that may be due to probe random loss or gain of signals, diffuse probe signals in cells with less condensed chromatin, probe size and design, sample type and others.

Conclusion: The FISH for MLL gene rearrangements with Cytocell ® probe was considered approved.

A-291

Thrombocytopenia In Children with Malaria

C. E. Lekpor[1], W. Ababio[2], R. Ansah[3], S. Amankwah[4], F. A. Botchway[5]. *[1]Department of Medical Laboratory, Kwame Nkrumah University Of Science and Technology, Kumasi, Ghana, [2]Hematology Department, Korle Bu Teaching Hospital, Accra, Ghana, [3]University of Ghana, Accra, Ghana, [4]Chemical Pathology Department, University of Ghana Medical School, Accra, Ghana, [5]Department of Child Health, Korle Bu Teaching Hospital, Accra, Ghana*

Background: The objective of this study was to access the occurrence and severity of thrombocytopenia in children with malaria at the Child Health department of Korle Bu Teaching Hospital between January and June 2013.

Methods: It was a retrospective study, done at the Child Health Department of Korle Bu Teaching Hospital. Data regarding positive cases of malaria <

12 years attending the Out Patient Department and admitted at the Emergency Room between January 2012 and June 2012 were obtained. Patients were further assessed for thrombocytopenia and its severity. Data were analyzed by Chi square test using SPSS version 13.0.

Results: A total of 131cases were included in the study with a mean age of presentation of 8 years. Plasmodium falciparum was identified in 119 (90.8%) patients while Plasmodium malariae in 6 (4.5%) patients and Plasmodium ovale in 3 (2.3%) with 3 (2.3%) cases or mixed infections of Plasmodium falciparum and Plasmodium ovale.

Thrombocytopenia was observed in 93(71%) cases, of which 40(31%) cases had mild, 56(43%) cases moderate and 34(26%) cases had severe thrombocytopenia. Thrombocytopenia was equally found in falciparum, malariae and ovale infections with no significant difference in severity between falciparum, malariae and ovale species.

Conclusion: Thrombocytopenia is frequently seen in malaria and it is not dependant on type of malaria. In any acute febrile illness, thrombocytopenia should alert one to the possibility of malaria.

A-292

Hemophilia C with a Cys482Trp Mutation in the F11 Gene

J. Yoo[1], B. Kim[2], J. Choi[2]. *[1]NHIS Ilsan Hospital, Goyang, Korea, Republic of, [2]Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: Coagulation factor XI (FXI) is a member of the "contact pathway" and is either activated intrinsically by coagulation factor XII (FXII) or by thrombin, which is produced by an extrinsic pathway and plays an important role in hemostasis. Factor XI deficiency, also known as hemophilia C, is a predominantly autosomal recessive genetic bleeding disorder. The F11 gene encodes the FXI protein, and mutations in the F11 gene have been found in patients with FXI deficiency. We report the first case of a heterozygous mutation (Cys482Trp) in the F11 gene, resulting in a mild FXI deficiency in a Korean patient.

Methods: A 14-year-old male patient with intermittent epistaxis was admitted to the hospital because of increased epistaxis frequency. The patient did not have any abnormal medical history that would have indicated bleeding tendency, apart from being treated for allergic rhinitis. The blood test results were as follows: leukocyte count, 5.6×10^9/L; hemoglobin level, 159 g/L; and platelet count, 215×10^9/L. The blood coagulation test showed normal prothrombin time but prolonged activated partial thromboplastin time (aPTT; 45.9 seconds, reference range, 20.9-35.0 seconds). The level of von Willebrand factor was within the reference range. The activity levels of factors II through XII were within the reference ranges, whereas FXI showed slightly decreased activity (26%; reference range, 60-140%). The patient's genomic DNA was extracted from the collected whole blood sample by using the Easy-DNA Kit (Invitrogen Corporation, Carlsbad, CA, USA). Exons 7, 8, 11, and 13 of the F11 gene were amplified by polymerase chain reaction (PCR). Direct sequencing of the amplified regions was performed by using the ABI Prism 3500dx automated genetic analyzer (Applied Biosystems, Foster City, CA, USA), the same primers that were used to amplify the 4 exons of the F11 gene by PCR, and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). To determine whether the patient had a DNA mutation, Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) was used to compare the patient's DNA sequences to the reference DNA sequence (GenBank accession number, NM_000128.3). The guidelines of the Human Genome Variation Society (HGVS) were used to identify the sequence variations.

Results: Direct sequencing of the proband demonstrated a heterozygous mutation, c.1500C>G (p.Cys482Trp) of exon 13 in the F11 gene.

Conclusion: The Cys482Trp missense mutation identified in our patient had been previously reported in England, but has never been reported in Korea. Owing to the small number of FXI deficiency cases with associated mutations that have been reported in Korea to date, further studies are warranted to contribute to the development of a database that would help clarify the distribution of mutations present in the Korean population. Such a database would also facilitate studies aimed to clarify the possibility of a founder effect for F11 gene mutations. Furthermore, these efforts would help improve the molecular and genetic diagnostic strategies used for Korean patients.

A-293

The utility of flow cytometry in the diagnosic of hemolytic anemias

B. Alvarez, A. González, D. Velasco, N. Del Amo, M. Medrano, L. Conejo, A. Vlagea, J. Alonso, J. Villarrubia, R. Guillén, F. Cava Valenciano. *Laboratorio Central BRSalud, Madrid, Spain*

Background: Hereditary spherocytosis (HS) is the most common inherited anaemia in Northern Europe and North America. The red cells in HS show abnormal morphology attributable to a deficiency or dysfunction of one of the red cell cytoskeleton protein (spectrin, ankyrin, band 3 and/or protein 4-2). Recently is used the flow cytometric test that measures the fluorescence intensity of red cells labelled with the dye eosin-5-maleimide (EMA), which reacts covalently on the first extracellular loop of band 3 protein. By this technique a decreased expression of Band 3 in the red membrane surface results in a lower mean fluorescence intensity of EMA. The aim of our study is to differentiate between HS and immune and nonmembrane-associated haemolytic anaemias, such as autoimmune hemolytic anemia (IHA) and Thalassemia (TL).

Method: The EMA-binding test was performed as described by King et al. with minor modifications in 150 controls, 44 HE, 17 IHA and 15 Thalassemia patients, using a BD FACSCanto II flow cytometer.

Results: Significant difference in EMA mean fluorescence intensity (MFI) results was obtained between HE and all of the groups studied (control group ($p<0.001$); IHA (P=

p<0.001), and TL patiens ((p<0.001)). There were differences between IHA and TL patients (p<0.022) while we don't find significant differences between TL patiens vs control group (p=0.533). See figure 1.

Conclusion: Measuring the fluorescence intensity of EMA labeled red cells by flow cytometry could be a powerful tool in the study of hemolytic anemias being a method available in most haematology laboratories. In our case has been shown to be effective for the discrimination of hereditary spherocytosis and IHA versus normal controls and other hemolytic anemias. We found a specific pattern of EMA expression in IHA probably due an increased level of reticulocytes in these patients.

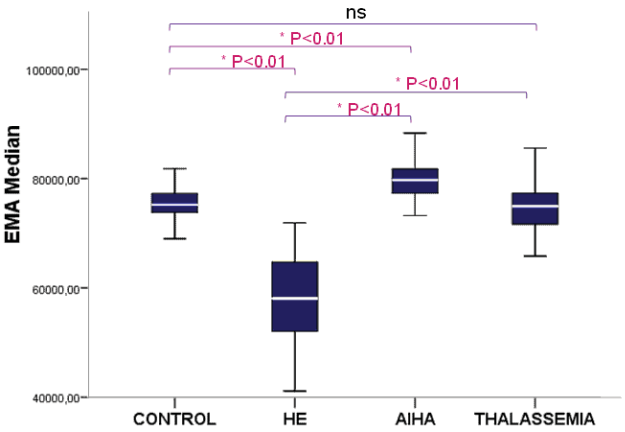


Figure 1: Differential MFI of EMA in the different groups of patients. Data show the mean and standard deviation of EMA. p: statistical significant level (Mann-Whitney U non parametric test for independent samples). MFI: Mean fluorescence intensity. EMA: eosin-5-maleimide. NS: non significant

A-294

Development of An Immunoturbidimetric Assay for the Determination of Haptoglobin Incorporating a New Ready to Use Reagent

S. Baxter, S. McElhatton, M. Rodriguez, J. Campbell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: The primary function of the plasma protein haptoglobin is to bind to free haemoglobin thereby preventing haemoglobin-driven oxidative tissue damage, the renal excretion of iron and the subsequent kidney damage following intravascular hemolysis. The plasma levels of this protein are reduced during episodes of hemolysis and the measurements are used in the diagnosis of haemolytic anaemia. Haptoglobin is also a positive acute-phase protein with immunomodulatory properties, the levels of this protein are elevated in inflammatory, infectious processes and in malignancies. This study reports the development of an assay for the determination of haptoglobin in serum samples, which incorporates a new ready to use reagent leading to a simplified procedure and a reduction of handling errors prior to analysis. The assay is applicable to a variety of automated analysers. This is of value for application in clinical laboratories.

Methods: The assay is immunoturbidimetric, the sample containing haptoglobin reacts with anti (human) haptoglobin antibody; insoluble complexes are formed allowing quantitative measurement at 340 nm. The amount of complex formed is proportional to the concentration of haptoglobin in the sample. The reagent is liquid and ready to use. The assay is applicable to a variety of automated systems. Accelerated stability of the reagent was assessed: three lots of reagent in the final packaging were nonstressed (stored at 2^0C to 8^0C) or heat-stressed (at 30^0C, 37^0C and 40^0C for 2, 4, 8, 13, and 26 weeks) and run in parallel. Within-run precision was assessed by testing serum samples at defined medical decision levels, 2 replicates of each sample were assayed 5 times for 1 day. Correlation studies were conducted using a commercially available assay system.

Results: Initial evaluation assigned 56 weeks real time stability for the reagent. The assay showed a sensitivity of 0.19 g/L and was linear up to 3.66 g/L. Prozone was not observed to a concentration of 4.65 g/L. The within-run precision for levels 0.85 g/L and 2.85 g/L, expressed as %CV, was 1.0% and 1.2% respectively. In the correlation study 95 serum patient samples were tested and the following linear regression equation was achieved: y = 1.03x - 0.04; r = 0.999.

Conclusion: The results indicate applicability of this immunoturbidimetric assay to the determination of haptoglobin in serum samples. The inclusion of a new ready to use reagent leads to a simplified procedure and a reduction of handling errors prior to analysis. This is of value for application in clinical laboratories.

A-295

Free Protein S correlation between two systems

S. TUFIK, M. C. FERES, M. C. DE MARTINO, M. P. MIRANDA, S. C. F. DA SILVA. *AFIP, SAO PAULO, Brazil*

Background: Protein S is a vitamin K plasma protein synthesized by the liver. During blood coagulation, protein S forms a complex with APC which bonds to phospholipids surface and speeds up the proteolytic inactivation catalyzed by APC of the factors Va and VIIIa. The free portion of protein S corresponds to 40% of total protein S. A total or acquired protein S deficiency is associated with a venous thromboembolism risk. There are three types of hereditary deficiencies: type I involves a reduction in total and free protein S levels; type II, a rare form, presents fall in protein S activity and normal levels of total and free protein S; and finally, type III presents normal levels for total protein S and low levels for the free form. Acquired protein S deficiency may be caused by liver dysfunction, nephrotic syndromes and vitamin K deficiency, pregnancy, treatment with L-asparaginase, estrogen therapy, virosis and disseminated intravascular coagulation. This study aims to evaluate the free protein S analytical performance on Siemens BCS XP System compared to obtained by Elite Pró IL system to decide on the introduction of these tests in routine.

Methods: Fresh and frozen blood samples were dosed (n=32), collected with citrate as an anticoagulant. The sample range selection situates between 40% and 122%. Plasma was analyzed within two hours after unfreeze simultaneously in Siemens BCS XP, using Siemens INNOVANCE Free PS-Ag, and IL Protein S, applying the turbidimetric method through optical detection for both systems. Correlation coefficient, slope and intercept were evaluated to examine the correlation degree between the methodologies of this study.

Results: Comparison resulted in a regression equation y = 0.908x + 17.35 and a correlation coefficient r = 0.83. The simple concordance index is 84%, concordance chance 0.63%, kappa index of 0.57, classified as moderate concordance. From five discordant samples, four are very close of a normality value and don't cause, therefore, clinical impact.

Conclusion: We conclude the Siemens INNOVANCE Free PS-Ag analytical performance on Siemens BCS XP is comparable to Elite Pró IL. Thus, these tests were approved for laboratory routine implementation.

A-296

A Dual Monoclonal Antibody Chemiluminescent ELISA for the Detection of Hepcidin-25

J. Chapo, A. Jones, K. R. Pitts. *Corgenix, Inc., Broomfield, CO*

Background: Iron is essential for all metazoans. Hepcidin is the peptide hormone that helps maintain plasma iron homeostasis and is an important clinical biomarker. It is first synthesized as an 84-amino acid precursor which is then proteolytically processed into a 60-amino acid pro-peptide and subsequently into the mature and bioactive 25-amino acid peptide. Hepcidin-25 regulates iron bioavailability by binding to the iron exporter ferroportin, causing its internalization and degradation from the surface of duodenal enterocytes, placental trophoblasts, macrophages and hepatocytes. Dysregulation of plasma iron homeostasis contributes to the anemia associated with chronic diseases such as infection, rheumatoid arthritis, and various cancers. As hepcidin-25 is a key regulator of iron homeostasis, a reliable method to detect hepcidin-25 levels in a blood sample would greatly improve our understanding of how to impact the anemia of chronic disease. Here we report the development of a robust immunoassay to measure hepcidin-25 in a blood sample.

Methods: A monoclonal antibody pair (capture and detection) was characterized and optimized for assay development. The capture antibody was coated onto opaque white 96 well plates while the detection antibody was conjugated to horseradish peroxidase (HRP) using standard immunochemistry methods. Antibody coat concentrations and conjugate detection concentrations were systematically titrated to achieve optimal sensitivity and dynamic range. Numerous iterations of coating and blocking buffers were assayed to further enhance assay manufacturability and consistency. Finally, in-process testing of linearity and precision was conducted to ensure robust performance to the end-user.

Results: The assay is specific for hepcidin-25, and does not cross-react with either hepcidin-22 or hepcidin-20. Linearity testing was performed over multiple kit lots and demonstrated a linear range of 60-6000pg/mL, spanning both normal and disease state levels of hepcidin-25 previously reported. Precision of the assay ranges from 5.0-14.0%CV, with sample recoveries falling between ±20%. The assay was used to test a set of normal and non-hematologic cancer samples. We found that, while a range of hepcidin-25 levels was observed in non-hematologic cancer samples, the assay was able to differentiate between normal and disease state sample populations.

Conclusions: Unlike other hepcidin assays, ours utilizes paired monoclonal antibodies that facilitate the direct detection of hepcidin-25 in a sandwich ELISA configuration. The assay also takes advantage of chemiluminescent detection to improve the sensitivity and stability of signal. Taken together, the data support the utility of this monoclonal-based chemiluminescence sandwich ELISA for the specific detection of hepcidin-25.

A-297

Evaluation of the Newly-Developed Serum Ferritin Measurement System, Point Reader™ and Point Strip™ Ferritin

R. Kubota[1], K. Kanamori[2], Y. Kawai[3], N. Sakai[1], K. Shiba[2]. *[1]Saitama Prefectural University, Saitama, Japan, [2]Bunkyo Gakuin University, Tokyo, Japan, [3]USHIO INC., Tokyo, Japan*

Background: Serum ferritin is widely measured to evaluate the iron levels in the body. Point Reader™ (USHIO, Tokyo, Japan) and Point Strip™ Ferritin (ASKA, Kanagawa, Japan), a newly-developed serum ferritin measurement system, is based on the principle of immunochromatography. This system measures serum ferritin concentrations in five minutes and may allow detection of the early iron-deficiency. Therefore, the objective of this study was to evaluate the performance of this new system. **Methods:** Unidentified residual serum specimens with known ferritin concentrations were used. Serum ferritin concentrations were measured using Point Reader and Point Strip Ferritin according to the manufacturer's instructions. The absorbance of phosphate buffered saline was measured to ascertain the sensitivity. The reproducibility of the test was determined by taking 8 replicate measurements of the 3 standards that had low, medium, or high levels of ferritin (12.0, 39.7, and 78.8 µg/L, respectively). For the correlation test, serum ferritin was measured using N-assay LA Ferritin (NITTOBO, Tokyo, Japan) on TBA-40FR Accute (TOSHIBA, Tokyo, Japan). **Results:** The minimum detection limit of the serum ferritin using Point Reader and Point Strip Ferritin was determined to be 10.0 µg/L. In addition, the measurement range of this system was 10.0-100 µg/L as determined from linearity testing. This system has a 3.8% reproducibility in a low control, 6.3% in a medium control, and 6.1% in a high control. We examined the correlation between serum ferritin concentrations obtained using this system (y) and those obtained using TBA-40FR Accute (x) (n = 107). The linear regression equation was $y = 1.07x + 0.84$, and the correlation coefficient (r) was 0.956. **Conclusions:** The newly-developed Point Reader and Point Strip Ferritin measurement system is simple and provides fast measurement of serum ferritin concentrations. This system can be used to assess the iron deficiency during pregnancy as well as in infants and blood donors.

Point Reader™ Point Strip™ Ferritin

A-298

Mathematical Model-based Estimation of Red Blood Cell Clearance Identifies Low Iron States in Patients with Normal Complete Blood Counts

H. Patel, J. Higgins. *Massachusetts General Hospital: Center for Systems Biology, Boston, MA*

Introduction The healthy human hematologic system is held in a state of dynamic equilibrium by the carefully regulated processes of (1) cell production, (2) cell maturation in the peripheral circulation, and (3) cell clearance. The resulting steady state is routinely quantified by measurements such as hematocrit (HCT), hemoglobin (HGB), or red blood cell count (RBC). Diseases and other conditions perturbing one of these processes may trigger compensation in the others. For instance, decreasing iron availability will eventually lead to iron deficiency and anemia, but prior to the development of anemia, a decrease in RBC production may be compensated by a delay in RBC clearance. This compensatory delay in clearance will maintain the steady state -- and will also confound our ability to discover the problem because the HCT, HGB, and RBC will remain normal. We hypothesize that an estimate of the rate of the underlying RBC clearance process would serve as a useful screening biomarker of decreasing iron availability, helping to identify seemingly healthy patients whose iron levels are close to abnormal or are already abnormally low. These patients should have iron levels checked and may need to be followed more closely.

Methods We used a mathematical model of RBC population dynamics to infer RBC maturation and clearance rates for patients from routine CBC and reticulocyte counts performed on an Abbott CELL-DYN Sapphire automated hematology analyzer. We first established a normal range for the estimated RBC clearance threshold, defined the probability of clearance as a function of an RBC's volume and hemoglobin content. We identified patients whose CBCs were entirely normal according to existing CBC indices but whose RBC clearance thresholds fell below the 5th percentile of the normal range. We then measured ferritin levels for these patients at the time of their entirely normal CBC.

Results We found that among patients with an entirely normal CBC and a low modeled RBC clearance threshold, 5% had abnormally low ferritin levels, and 19% had ferritin levels that were within 5% of the lower limit of the normal range. Low and low-normal ferritin was 4x more prevalent in this low clearance group than in a control group whose RBC clearance threshold was normal. We also found a statistically significant relationship between the estimated RBC clearance threshold and the ferritin level, with lower clearance threshold associated with a lower ferritin level.

Conclusions Iron deficiency anemia is an early sign of a number of important diseases, such as colon cancer, gastric cancer, celiac disease, and more. Our results suggest that RBC clearance threshold is often reduced, possibly as compensation to maintain desired steady states. This compensation confounds our ability to detect the illness by measuring HCT or HGB. But by using a mathematical model of *in vivo* RBC population dynamics, we can estimate a patient's clearance threshold and identify patients likely to have low ferritin levels. These patients would benefit from direct iron level assessment and further revaluation to identify the underlying cause of the decreased iron availability.

A-300

Distribution of hemoglobinopathies and thalessemias in a Northern Alberta population

T. Higgins, k. Rodriguez Capote. *DynaLIFEDx,, Edmonton, AB, Canada*

Background DynaLIFEDx is the sole laboratory performing hemoglobinopathy and thalassemia investigations for a catchment area of 2 million people in northern Alberta. Samples for hemoglobinopathy and thalassemia testing come from physician request, immigration medicals, and red cell exchange programs for sickle cell anemia patients and as a reflex test generated by the presence of a hemoglobin variant noted on HPLC analysis for HbA1c. We wished to know the frequency of thalassemia and hemoglobinopathies in our catchment area.

Methods EDTA-anti-coagulated blood samples were analyzed by high performance liquid chromatography (HPLC) using the β thalassemia program on the Bio-Rad VARIANT II. Hemoglobin variants found on HPLC were analyzed by electrophoresis at alkaline and acid pH. A complete blood count (CBC) and ferritin tests were requested as part of the hemoglobinopathy/thalassemia investigation.

Results 5562 thalassemia and hemoglobinopathy investigation were physician requested, 1054 HbS screens were requested and 702 hemoglobin variants fortuitously found on HbA1c analysis were analyzed in the period January 1, 2013 to December 31, 2013. 3438 were interpreted as "normal" and 532 were classified as iron deficient

on the basis of their hematology indices (hemoglobin >120g/l, mean cell volume > 80fL), absence of a hemoglobin variant, replete iron status and calculated Mentzer Index and Discriminant Factor. 322 and 370 were classified as α or β thalassemiaa trait respectively. 357 were classified as S trait, 121 as E trait, 63 as D Punjab trait and 62 as C trait. 22 were classified as Homozygous S, 4 as homozygous HbE and 2 as homozygous HbC and 8 as SC disease. 10 were classified as δβ thalassemia trait and 11 as H disease. The remainder was classified as unusual hemoglobin variant or thalassemia.

Conclusion There is a wide diversity of hemoglobinopathies found in northern Alberta. 11% of the hemoglobinopathies were found as a reflex to HbA1c testing.

A-301

Pediatric Reference Intervals for New Reticulocyte and Platelet Parameters

M. O'Leary[1], P. Horn[2], B. Ward[3], P. Steele[2], D. Wright[4]. *[1]Texas Children's Hospital and Baylor College of Medicine, Houston, TX, [2]Cincinnati Children's Hospital and University of Cincinnati, Cincinnati, OH, [3]Cincinnati Children's Hospital, Cincinnati, OH, [4]Abbott Diagnostics, Santa Clara, CA*

Objective: Advances in algorithm development for the Abbott CELL-DYN Sapphire has introduced several new red blood cell parameters describing cell-by-cell size and hemoglobin content for circulating reticulocytes (MCVr, MCHr, CHCr). Reported clinical applications include monitoring RBC production kinetics, distinguishing anemia of chronic disease from iron-deficiency anemia (IDA), and detecting pediatric IDA and erythropoietin-associated functional iron deficiency. Additionally, the reticulocyte channel measures the percentage of platelets that contain residual RNA, reported to correlate with megakaryocytic activity. Potential applications for reticulated platelet (rPLT) values include distinguishing production from consumption and predicting recovery in thrombocytopenic patients. The objective of this study was to determine reference intervals for five pediatric age groups.

Methods: 762 K3-EDTA whole blood surplus samples from previously ordered CBCs were analyzed to collect the reticulocyte channel information. The IRB waived consent for the study samples. Children with clinical and/or laboratory evidence of RBC or platelet abnormalities were excluded. The five age groups included; Group 1 = 1 month - 1 year; 2 = 1 - 3 years; 3 = 3 - 6 years, 4 = 6 - 12 years; and 5 = 12 - 18 years. Reference intervals were calculated (SAS/STAT Software) as per the CLSI guidelines C28-A3C. The non-parametric calculation was used for groups with >120 samples, and the robust method was used for two groups with <120 samples. The RBC indices and platelet count were included in the reference interval calculations to provide a comparison to their reticulated counterparts.

Results: The 95% reference interval limits are in Table 1. Ninety percent confidence intervals for the ninety-five percent upper and lower reference interval limits were calculated, as well (data not shown).

Conclusions: Our results suggest age related trends for the new reticulocyte and reticulated platelet parameters.

Table 1.

Group	1	2	3	4	5
N	52	134	92	216	268
Parameter					
RETC (x10^3/uL)	23.56 - 123.60	18.3 - 101.00	22.58 - 99.86	29.74 - 121.63	27.45 - 105.92
pRETC (%)	0.55 - 3.24	0.41 - 2.24	0.51 - 2.23	0.64 - 2.50	0.55 - 2.21
IRF (fraction)	0.12 - 0.40	0.13 - 0.37	0.10 - 0.30	0.10 - 0.31	0.11 - 0.34
MCV (fL)	74.08 - 101.83	74.13 - 87.56	76.06 - 88.39	78.27 - 91.94	79.62 - 94.29
MCVr (fL)	81.76 - 108.27	80.10 - 100.11	81.78 - 100.51	86.01 - 102.94	89.19 - 107.56
MCH (pg)	24.27 - 35.81	25.14 - 30.22	25.70 - 31.29	26.63 - 32.04	26.59 - 32.51
MCHr (pg)	20.07 - 31.66	22.10 - 30.56	23.38 - 31.50	24.94 - 31.94	25.35 - 32.39
MCHC (g/dL)	32.14 - 36.13	32.55 - 35.77	32.63 - 36.58	32.54 - 36.40	32.48 - 35.97
CHCr (g/dL)	25.57 - 31.39	26.64 - 31.73	27.30 - 32.57	27.51 - 32.17	27.33 - 31.56
PLT (x10^3/uL)	184 - 541	152 - 506	171 - 470	172 - 442	152 - 407
prP (%)	1.31 - 8.16	0.95 - 8.93	0.35 - 6.01	0.26 - 7.33	0.33 - 5.22

A-302

The evaluation of affecting factors on platelet reference ranges in the population of Northeastern China

S. Y. Wu[1], X. Shi[2], J. Sun[3], F. Hassouna[1], C. Wang[3], J. Niu[2]. *[1]Confirmatrix Clinical Laboratory, Lawrenceville,, GA, [2]Internal Medicine, First Affiliated Hospital, Jilin University, Changchun, Jilin, China, [3]Geriatrics , First Affiliated Hospital, Jilin University, Changchun, Jilin, China*

Objective: The purpose of this study is to evaluate the clinical laboratory variables, their correlation to platelet count (PLT), the reliability of established reference ranges and their applications in clinical research and diagnostic test.

Methods: This study was carried out in Chang Chun city and its suburban area in China. 3800 cases were selected during year of 2010 to 2012. Inclusive criteria were as following: 1. No medications within one month; 2. No medical history of thrombosis and hemorrhagic disease. Exclusive criteria were: 1. Female patients are at menstrual period; 2. Female who are pregnant; 3.Patients who have medical history of liver disease or hematologic diseases. Patients who met the inclusive criteria were put in questionnaire survey for past medical history and social history. 5 ml of blood was drawn for clinical chemistry analysis including liver function test (to exclude unknown hepatic disease), blood glucose test and lipid panels. Hematology variables include platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW). SPSS software has been used for ANAOV analysis. P<0.05 was used for statistical significant testing in this study.

Results: This study showed PLT ranged from 147 -363 K/µl. MPV were from 6.8-10.5 fl. PCT were from 0.13%-0.29%. PDW were from 15,5% -18.4%. These variables were affected by the cholesterol and triglyceride level, particularly in the group of 50 years or older. The correlations analysis indicated that the higher the cholesterol, the higher the PLT, MPV, and PCT. The average platelet count was higher in female than male. Smoking, alcoholic, BMI, blood pressure and blood sugar have not effects on platelet variables.

Conclusion: Based on these case studies and the analysis of clinical chemistry variables, we concluded that diet, blood pressure and social behavior do not have impact on platelet values. Age and high cholesterol cause elevated platelets count. This supports the previous study that high cholesterol causes high coagulation diseases including stroke, myocardia thrombosis and deep vein thrombosis.

A-303

Measurement of Serum Ferritin Concentrations in a Japanese Young Population Using a Newly-Developed System, Point Reader™ and Point Strip™ Ferritin

K. Kanamori[1], R. Kubota[2], Y. Kawai[3], N. Sakai[2], K. Shiba[1]. *[1]Bunkyo Gakuin University, Tokyo, Japan, [2]Saitama Prefectural University, Saitama, Japan, [3]USHIO INC., Tokyo, Japan*

Background: Iron-deficiency anemia is usually diagnosed by measuring the level of hemoglobin. However, even if the hemoglobin level is within the reference range, the serum ferritin concentrations may still be <12 µg/L resulting in a latent iron-deficiency state without anemia. Blood donation has a significant impact on the iron levels of the body, especially in blood donors with latent iron deficiency. Therefore, the objective of this study was to evaluate the serum ferritin concentrations in a Japanese young population, using the newly- developed serum ferritin measurement system, Point Reader™ (USHIO, Tokyo, Japan) and Point Strip™ Ferritin (ASKA, Kanagawa, Japan).

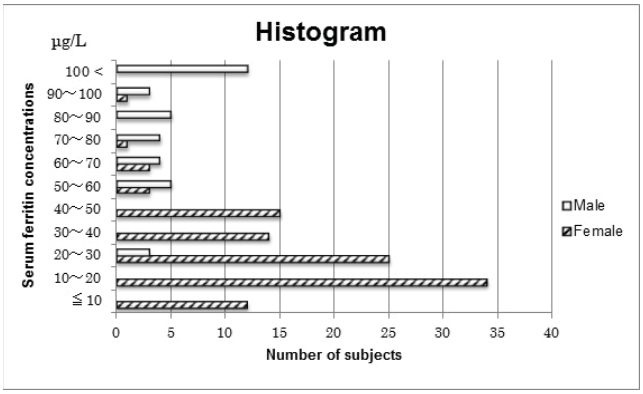
Subjects: Serum and blood samples were obtained from 36 male and 108 female students (age, 20-24 years) from Bunkyo Gakuin University, Tokyo, Japan. The ethical committee of Bunkyo Gakuin University approved this study, and informed consent was obtained from all subjects.

Methods: Serum ferritin concentrations were measured using Point Reader and Point Strip Ferritin based on the principle of immunochromatography. The hemoglobin levels were measured using XE-2100 (Sysmex, Hyogo, Japan).

Result: The serum ferritin concentrations of the young Japanese population were shown in histogram. The serum ferritin concentrations in the 36 male students ranged from 20.2 µg/L to 179.1 µg/L, while that of 12 (11.1%) female students were <10 µg/L. Overall, the serum ferritin concentrations of the male students were observed to be higher than those of the female students. 23 (21.3%) female students showed latent iron deficiency state without anemia.

Conclusions: Out of 108 female students, the 21.3% with latent iron deficiency are at a significant risk of developing iron deficiency anemia from blood loss, such as that

occurring during blood donation. Estimation of the hemoglobin level alone in blood donors may not be adequate; therefore, the estimation of serum ferritin concentrations may also be necessary to detect pre-clinical iron deficiency.



A-304

Prognostic Value of Modest Increases of Plasma D-dimer Concentration in Patients with Previous History of Myocardial Infarction

H. Naruse, J. Ishii, R. Okuyama, T. Hashimoto, F. Kitagawa, T. Kuno, T. Ishikawa, S. Matsui, Y. Ozaki. *Fujita Health University, Toyoake, Japan*

Background: D-dimer can be considered as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system. We prospectively investigated whether modest increases of plasma D-dimer levels might be relevant to prognosis in patients with a previous history of myocardial infarction. Methods: We studied 606 consecutive patients with a previous history of myocardial infarction (median age, 65 years; 508 males) and estimated glomelurar filtration rate (eGFR) ≥15 ml/min/1.73 m2. Blood samples for measurements of D-dimer, total plasminogen activator inhibitor-1 (tPAI-1) and high-sensitive C-reactive protein (hsCRP) were obtained at enrollment. Among these patients, 65% had hypertension, 36% had diabetes, 35% had chronic kidney disease, and 74% had history of coronary revascularization. Results: During a median follow-up period of 43 months, there were 120 cardiovascular events including 20 cardiovascular deaths. Comparably, patients who had cardiovascular event were older (median, 67 vs. 65 years, p = 0.01), had higher levels of D-dimer (0.65 vs. 0.44 μg/ml, p < 0.0001), and displayed a lower level of eGFR (62 vs. 67 ml/min/1.73 m2, p = 0.02) and left ventricular ejection fraction (LVEF, 50 vs. 53 %, p < 0.0001) than those who did not. On Cox regression analyses multivariate including 12 clinical and angiographic variables, D-dimer levels were independently associated with cardiovascular events (relative risk: 1.92 per 10-fold increment, p = 0.02). Clinical characteristics and cardiovascular mortality and cardiovascular event rates according to tertiles of D-dimer were shown in Table. Conclusion: Modest increases of D-dimer may be independently associated with adverse outcomes in patients with a previous history of myocardial infarction. Measurements of D-dimer may be useful for the risk stratification of adverse outcomes in this population.

	Tertiles of D-dimer (μg/ml)			
	1st < 0.35 (n=208)	*2nd 0.35-0.71 (n=197)*	*3rd 0.71 < (n=201)*	*P value*
Age (years)	*61*	*66*	*70*	*< 0.0001*
Male	*89%*	*84%*	*79%*	*0.005*
Diabetes	*31%*	*38%*	*40%*	*0.08*
eGFR (ml/min/1.73m²)	*73*	*67*	*61*	*< 0.0001*
HsCRP (mg/dl)	*0.05*	*0.08*	*0.14*	*< 0.0001*
tPAI-1 (ng/ml)	*14.6*	*13.8*	*13.7*	*0.42*
LVEF (%)	*54*	*51*	*50*	*0.002*
Cardiovascular event rate	*13%*	*19%*	*28%*	*< 0.0001*
Cardiovascular mortality rate	*1%*	*2%*	*7%*	*0.002*

Data are expressed as median or %.

A-306

Acute erythroleukaemia with atypical presentation

L. B. Zuppani[1], S. S. Rodrigues[1], O. Fernandes[1], C. L. Oliveira[1], J. Tabacof[2], O. F. Dias Neto[2], D. P. Jardini[3], E. H. J. T. Dauar[1]. *[1]DASA, São Paulo, Brazil, [2]Clínica Oncológica Paulista, São Paulo, Brazil, [3]Hospital Paulistano, São Paulo, Brazil*

Background: Acute erythroid leukaemias are a group of acute leukaemias characterized by the predominance of erythroid population. It is divided into two groups based on the presence or absence of a significant myeloid component: Erytroleukaemia (Erythroid/ myeloid) and Pure erythroid leukaemia. Erytroleukaemia (Erythroid/ myeloid) is diagnosed when the presence of erythroid precursors in the bone marrow is ≥ 50% of the nucleated cell population and ≥ 20% of myeloblasts in the non-erythroid cell population. Pure erythroid leukaemia is diagnosed when ≥ 80% of cells in the bone marrow are commited with the erythroid lineage (undifferentiated or proerythroblastic in appearance) and there is no significant myeloblastic component.

Clinical case: MCA, a 71 year old woman was admitted to the orthopedic clinic at Hospital Paulistano with bone pain. Her first blood count showed mild anemia (hemoglobin: 10g/dl). On the course of her hospitalization she presented a severe drop in hemoglobin levels, so a bone marrow count was performed. Bone marrow examination (04/16/2013): Slightly hipocelular bone marrow with 18% of cells from erythroid lineage (5% of those cells were proerytroblasts with morfological alterations). Presence of megaloblasts, binucleated red blood cells and altered cytoplasmic features. Neutrophilic series showing assyncronic maturation, 1% of myeloblastic components and megakaryocytic serie without morphological alteration.

Iron stains: 30% of ringed sideroblasts. Cytogenetic analysis: 45, XX, deletion of the long arm of chromosome 5, terminal deletion of the long arm of chromosome 7, monossomy of chromosome 19 and isochromosome of the long arm of chromosome 21. Bone Marrow examination 07/03/2013: Bone marrow with 50,4% of cells of erythroid lineage with predominance of early forms and 21,6% of myeloid blasts.

Conclusion: This case is an example of a rapidly progressive acute erytroleukaemia, that was initially diagnosed as a myelodysplasic syndrome with 5% of proeriytroblasts and absence of a myeloblasts. The cytogenetic abnormalities found in this patient are present in myelodysplasic syndromes (MDS) as well as in erytroleukaemia. Although there are no specific chromosomic abnormalities, the -5/del, -7/del (both present in this case) and chromosome 8 trissomy are the most common cytogenetic abnormalities found in erytroleukaemia. It is known that the presence of complex cytogenetic abnormalities (more than 3 chromosomal abnormalities) is the only statistically significant independent variable that adversely affects survival in the acute erythroid leukaemia group. The present report shows a case that began with a bone marrow count of MDS with 5% proerytroblasts and absence of myeloblasts and rapidly changed its morfological characteristics to a erytroleukaemia. This case highlights the necessity of a biomarker that can precisely differentiate between these two diseases, so that the appropriate treatment can be initiated without delay.

A-307

Evaluation of the Alcor Scientific iSED Erythrocyte Sedimentation Rate Analyzer

J. Dohnal, D. Skale. *NorthShore University HealthSystem, Evanston, IL*

OBJECTIVE: This study evaluates the performance of the Alcor Scientific iSED analyzer. The analyzer measures erythrocyte sedimentation rate using the principles of hemorheology in a photometric based method.

METHODS: Split sample comparisons were performed over a 5-week time period using the manual Westergren Sedimentation Rate method manufactured by LP Italiana Spa as the predicate device. During the study period two levels of Alcor Scientific control material were run each day that sample analysis was performed.

RESULTS: CVs for the commercial control material run during the study were 29.1% at a value of 3 mm/hour and 7.1% at a value of 35 mm/hour. Studies using fresh patient samples yielded similar results and were maintained over time once the instrument was placed into clinical service. There was no evidence of sample carryover. Correlation between the predicate method results and the iSED analyzer results using patient samples was iSED = 0.7006 x (manual method) + 5.113 with an R^2 value of 0.7412. 217 patients were used in the study (61 males and 156 females). The clinical interpretation of the results for the two methods on all 61 male samples and 150 of the 156 female samples correlated. No pattern or explanation for the 6 female patients with disparate clinical interpretations could be found. For the patient samples the concordance coefficient was 0.9423 (substantial agreement) and the weighted kappa coefficient was 0.9424.

CONCLUSIONS: The analytical performance of the iSED analyzer was acceptable with CVs at least as good as those listed in the package insert. The instrument is easy to operate and requires a short training period. Maintenance is minimal and mechanical performance is acceptable. Although some disparate values were found between the two methods no systematic biases were noted. Instrument repairs are affected via a central service center, so instruments needing repairs must be shipped out to the manufacturer. Loaner instruments are not available at the time of this abstract submission. The downtime associated with instrument repair is unacceptable being in the range of 14 - 20 days. Unless a laboratory has multiple instruments in operation, this represents a significant drawback that needs to be addressed by the vendor.

A-308

Elevated Serum Levels of Von Willebrand Factor Antigen are Associated with Poor Prognosis In Patients with Symptomatic Waldenstrom's Macroglobulinemia

I. Papassotiriou[1], E. Kastritis[2], E. Terpos[2], N. Simos[1], E. Eleutherakis-Papaiakovou[2], N. Kanellias[2], K. Pantelaki[1], M. Mazarakis[1], M. Gavriatopoulou[2], M. Roussou[2], M. A. Dimopoulos[2]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Department of Clinical Therapeutics, University of Athens, School of Medicine, Athens, Greece*

Background: Waldenstrom's Macroglobulinemia (WM) is an uncommon malignancy which, is characterized by the infiltration of the bone marrow by lymphoplasmacytic cells, which produce a monoclonal IgM. Recently, it was shown that high levels of von Willebrand Factor antigen (vWF:Ag), are associated with adverse prognosis in patients with symptomatic WM and it was suggested that vWF:Ag levels may reflect interactions between lymphoplasmacytic cells and other cells of their microenvironment such as mast cells and endothelial cells. We aimed to evaluate the prognostic importance of vWF:Ag levels of patients with symptomatic previously untreated WM, in order to validate vWF:Ag as a possible prognostic marker for progression free (PFS) and overall (OS) survival.

Patients and Methods: The analysis included 42 patients with symptomatic WM, and 19 healthy controls of matched gender and age. Anemia (250U/L and 58% had serum albumin <3.0g/dL. Median serum IgM was 3340mg/L (range 246-9563mg/dL). According to IPSSWM, 22% had low, 43% intermediate and 35% high risk WM, respectively. Reasons to initiate therapy included cytopenias in 42% of patients, B-symptoms in 15%, hyperviscosity in 12%, neuropathy in 10% and other reasons in 21%. Primary therapy based on rituximab was given in 93% of the patients and 54% achieved at least 50% reduction of IgM. vWF:Ag levels were measured in serum collected before initiation of therapy by means of a latex particle-enhanced immunoturbidimetric assay (ACL Top 3G, Instrumentation Laboratory, USA).

Results: Median serum level of vWF:Ag was 101U/dL (mean 132.5U/dL, range 19.9-399.0U/dL) and were slightly higher compared to the serum levels of healthy controls (median 85.0U/L, mean 85.0 U/L, range 48.0-124.0U/L). However, 6/42 (14%) had vWF:Ag levels median value was more frequent in patients with beta2-microglobulin >3.0 mg/L (p=0.006) and less frequent in patients with low (11%) vs. patients with intermediate (59%) or high (62%) risk IPSS (p=0.036). There was no correlation of vWF:Ag levels with IgM levels or with the extent of bone marrow infiltration or with other manifestations of the disease. Median follow up of symptomatic patients was 4 years. Patients with vWF:Ag levels within the upper quartile (i.e. vWF:Ag ≥200.0U/dL) had a median progression free survival of 12 months vs. 63 months of patients with vWF:Ag <200.0U/L (p<0.001), while the median survival for patients with vWF:Ag ≥200.0U/dL was 37 months (4-year survival 29%) vs. 4-year survival of 97% for patients with vWF:Ag <200.0U/L (p<0.001).

Conclusion: In conclusion, the serum levels of vWF:Ag provide significant prognostic information in patients with symptomatic WM and patients with levels ≥200.0IU/dL have a very poor prognosis compared to patients with lower levels. vWF:Ag measured in the serum, may become an important prognostic marker in WM and needs further validation.

A-309

Full Automation of Heparin Induced Thrombocytopenia ELISA Assay on Dynex DSX ELISA platform.

C. Cronin, F. Lucas, V. Ricchiuti. *University of Cincinnati Medical Center, Cincinnati, OH*

Background: Heparin induced thrombocytopenia (HIT), with or without thrombosis, is an important cause of morbidity and mortality in patients treated with unfractionated heparin. HIT is invariably characterized by antibodies specific to platelet factor 4 (PF4) and heparin complex. Our goal was to fully automate a HIT ELISA assay (Immucor, Inc., Norcross GA) with the Dynex DSX instrument (Dynex Technologies, Chantilly, VA) for application in clinical laboratories to achieve a less labor intensive approach and high through-put HIT testing.

Method: 37 patient samples were analyzed side by side using the manual HIT ELISA and the automated HIT ELISA on Dynex DSX. Patient samples were added to micro wells coated with platelet factor 4 (PF4) and complexed to polyvinyl sulfonate (PVS) as a PF4:PVS complex. If an antibody was present and recognizes a site on the PF4:PVS complex, binding would occur. Unbound antibodies were washed away and an alkaline phosphate labeled anti-human globulin reagent (Anti-IgG, A, M) was added to the wells and incubated. The unbound anti-IgG/A/M was washed away, and the substrate PNPP (p-nitrophenyl phosphate) was added. After an incubation period, the reaction was halted with a stopping solution and the optical density (OD) was measured by a spectrophotometer at an absorbance of 405 nm using a reference filter of 490 nm. The only changes in the procedure during the programming of Dynex DSX software from the manual HIT ELISA protocol were the removal of the pre-soaking step prior to pipetting reagent and the programming of a longer incubation time which was extended from 30 to 35 minutes. 20 known negative samples for HIT and 17 known positive samples for HIT were analyzed for the presence of PF4 antibodies by the fully automated method on the Dynex DSX, and then manually run by hand. An optical density (OD) greater than 0.4 is used as positive cut-off for PF4 (HIT) antibodies detection.

Results: The qualitative comparison of results obtained with the manual HIT assay and the automated HIT assay showed 100% agreement using the 0.4 OD cut-off. The correlation using the OD from samples was excellent (y(automated)=0.9x(manual)-0.02, r=0.96) with a bias of -0.1. The precision (between days) using the Dynex DSX was above manufacturer expectation (CV<20%) as CV at low level (OD≤0.3) was 19.5% (n=8) and at high level (OD≥1.8) CV was 5.2% (n=8). Precision using manual procedure was 5.5% (n=6) at OD≤0.3 and 16.2% (n=6) at OD≥1.8. The DSX instrument uses disposable pipette tips and performs all pipetting steps for reagents, controls, patient samples as well as washing and rinsing steps. This method is less labor intensive and improves the turnaround time.

Conclusion: The fully automated DSX instrument for HIT testing showed an excellent correlation with the manual procedure. The HIT assay on the Dynex DSX takes less than two hours. It is a fully walk-away solution once the instrument is loaded with reagents and patient specimens. To our knowledge, this is the first fully automated clinical laboratory HIT assay has been described using the Dynex DSX ELISA instrument.

A-310

New policy reduces manual slide reviews on platelet tests

B. R. Morgan[1], D. L. Clark[2]. *[1]Sacred Heart-St. Mary's Hospitals; Ministry Medical Group, Rhinelander, WI, [2]Sacred Heart-St. Mary's Hospitals, Rhinelander, WI*

Implementation of a new platelet manual slide review policy in a small hospital/clinic laboratory serving an oncology service can produce a modest reduction in slide reviews related to platelet count flags, improve turnaround time, and save an estimated $3/slide review (6 min/case). The policy at this institution for performing a Wright stained slide review on specimens tested using a Sysmex XE-2100 Hematology Instrument was <100,000/uL and/or a Sysmex platelet flag (platelet clumps CLP, platelet abnormal distribution PAD, platelet abnormal scattergram PAS). Absolute delta check values set at Δ20,000/uL (<100,000/uL) and Δ50,000/uL (101,000 - 10^6/uL) reflexed a review of clinical history, but not slide review. The goal of the policy revision was to decrease slide reviews related to platelets on blood samples from patients with known histories of low platelet counts. Real time review of LIS/EHR patient information was a necessary component for the new platelet slide review policy and designed as follows:

•No flags- report result

•Slide review performed first time for thrombocytopenia (<100,000/uL), CLP, PAS or PAD; always for CLP flag

•If TCP <100,000/uL and/or PAD, PAS flags present on hemogram:

-Previous slide review performed, same platelet flag, and patient result review consistent with clinical history, finalize result

-Previous slide review performed and patient result review inconsistent with clinical history, perform slide review, then finalize result

-Previous slide check performed and different platelet flag, perform slide review, then finalize result

Three month's platelet count data collected after implementation of the policy yielded 22 patient platelet test results (60% oncology service patients), 0.4% of 5564 platelet count tests showing platelet flags. Nineteen patients had previously documented platelet counts <100,000/uL within a 4 year time span archived by the LIS. Eighty-six subsequent slide checks were avoided by implementation of the policy. On EHR review of patient records, no significant clinical findings appeared to have been missed by the new policy. Median slide check time, defined as start to finalizing result, was 6 min/case; $3/platelet slide check (savings based on $30 labor and benefits). If this sample reflects our patient population and provider ordering patterns, annual savings are modest at approximately $1000/yr. More important are the improved turnaround time of at least 6 min for these specimens, significant given that average turnaround time for platelet count at this laboratory without flags (receipt to verify) is 10min, and decreased technologist disruption by eliminating unnecessary platelet slide checks. Implementation of a new platelet manual slide review policy modestly reduced the number of manual slide reviews performed on patients showing repetitive blood counts with thrombocytopenia <100,000/uL, abnormal platelet distribution or scattergram flags.

A-313

Can Neutrophil/Lymphocyte Ratio be Used in the Differential Diagnosis of Abdominal Pain of Appendicitis and Familial Mediterranean Fever?

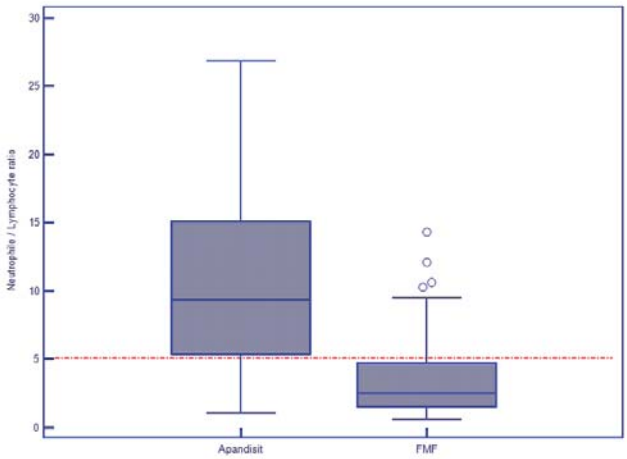
O. Baykan, A. Yaman, G. Gokulu, D. Altinok, O. Sirikci, G. Haklar. *Marmara University School of Medicine, Istanbul, Turkey*

Backround: The neutrophil/lymphocyte ratio (NLR) is proven to be associated with some conditions such as chronic inflammation in cardiovascular diseases, malignancies, ulcerative colitis, gangrenous appendicitis, and amyloidosis. Familial Mediterranean Fever (FMF) is an inherited disorder which is common among individuals of Mediterranean descent. Both FMF and appendicitis may manifest with abdominal pain which is hard to differentiate. This retrospective study aimed to evaluate the ability of the NLR to predict acute appendicitis pre-operatively and to differentiate cases with abdominal pain due to FMF and acute appendicitis.

Methods: A hundred patients who were admitted to emergency unit of Marmara University Pendik E&R Hospital with abdominal pain were included. Fiftysix patients had the diagnosis of appendicitis, were treated operatively and proven as appendicitis histopathologically. FMF group was formed by 44 patients who were monitored at the Rheumatology Clinic with colchicine treatment and without any other diseases.

Results: Median ages (25-75 percentile) of appendicitis and FMF groups were 12 yrs (10-15,75) and 13 yrs (9-17), respectively which were not different significantly (p=0.691). Complete blood counts were measured by LH 780 (Beckman Coulter, USA) and NLR was obtained from the records. Mean NLR values (25-75%) were significantly higher for appendicitis group when compared to the FMF group [2.54 (1,5-4,8) vs. 9.34 (5,32-15,45)] (p<0.001) (Fig 1). The diagnostic performance of NLR to differentiate acute appendicitis from FMF was assessed with a ROC plot and cut-off value of NLR was >4.97. (AUC:0.842, 95%CI: 0.763-0.920, p<0.001) (sensitivity 80% and specificity 77%).

Conclusion: According to the results of our study, NLR of 4.97 seems to be a reliable parameter in discriminating abdominal pain of acute appendicitis from FMF attack. NLR is cost effective, readily available, and can be calculated easily.



A-315

Reticulated Platelets - Towards A Standardized Approach: Results From 'Apparently Disease-free' Subjects In 3 Countries

N. LLEWELLYN-SMITH[1], B. HEDLEY[2], S. LANG[3], D. ROSENFELD[3], N. McNAMARA[3], M. KEENEY[2]. *[1]Abbott Diagnostics, Santa Clara, CA, [2]London Health Sciences Centre, London, ON, Canada, [3]Liverpool Hospital, Liverpool, Australia*

Background Despite obvious potential as a sensitive marker of thrombopoietic activity, the lack of a reference method and standard materials for Reticulated Platelet (RP) counting have hampered widespread adoption. Here we applied a new, highly standardized method (described in a separate poster) to assess typical values in healthy ('apparently disease-free' subjects) at 3 international sites. Our goal was to test whether 3 different laboratories, using the same protocol on 3 different flow cytometers, could recover statistically similar results in apparently disease-free ('normal') subjects.

Methods The 3 sites (instruments) were 1) Abbott Hematology, California (Accuri-C6), 2) Liverpool Hospital, Australia (FACSCanto) and 3) London Health Sciences, Canada (Gallios). K_2EDTA specimens were collected from 71 consented voluntary participants in the Abbott staff donor program, lab staff or surplus CBC specimens from subjects without any hematological abnormality. Briefly: fresh (< 8 hours post draw) whole blood was incubated with the CD61-APC/CD41-APC monoclonal antibody mix and stained with TO before fixation with formaldehyde. A second tube, with PBS in place of TO, served as the control. During analysis, a positive marker was drawn on a TO histogram (gated on the platelet cluster) of the control tube to include 0.1% of the negatives. The instrument settings and this marker were held fixed for the acquisition and analysis of the tube containing the TO-stained platelets. Basic statistics (means, medians, SD and CV) were generated in MS Excel 2010, Analysis of Variance (ANOVA) was used to assess the mean results (males, females, pooled) across the 3 sites.

Results The mean RP results from the 3 sites were as follows: California 5.2% (SD 3.3%, n=21), Canada 4.8% (SD 0.7%, n=21) and Australia 4.6% (SD 3.0%, n=29). Mean for the pooled 71 subjects was 4.9% (SD 2.7%, Range 1.7-17.1%). Differences in the means between the 3 sites were not statistically significant ($p > 0.05$). At one site (California) the mean female RP count was higher (6.1%) than the mean male value (4.1%) though the differences were not statistically significant.

Conclusion We have demonstrated that a standardized flow cytometric protocol, at 3 geographically distinct sites, on 3 different instruments, yielded comparable results in 'apparently disease free' subjects. The major shortcoming of all nucleic acid based methods is the non-specific uptake of the nucleic acid dye by platelet dense and alpha granules. We were able to minimize this by using a dilute TO solution. To further mitigate non-specific dye uptake, we used a simple and objective control strategy (negative control tube) to differentiate specific from non-specific staining. We believe this method is simple and robust enough to form the basis for development of a potential reference method. Further studies to define the ideal dye concentration and characterize inter-lab performance are planned.

A-316

Proteomic profiling of platelets in acute ischemic stroke patients

O. Cevik[1], T. Baykal[2], G. Somay[3], A. Sener[4]. *[1]Cumhuriyet University Faculty of Pharmacy, Sivas, Turkey, [2]Medipol Univesity School of Medicine, Istanbul, Turkey, [3]Hisar Intercontinental Hospital, Neurology, Istanbul, Turkey, [4]Marmara University Faculty of Pharmacy, Istanbul, Turkey*

Background: Platelets are important in the pathogenesis of stroke and ischemic stroke is high level of mortality. Especially antiplatelet agents are useful for prevention and treatment in this patients. Platelets are so easily activated by different stimulants in the circulation which can be affected by activation and apoptosis.

Methods: Using LC-MS based protein profiling, we examined and correlated the proteomic response of stroke patients platelets. Patients were admitted to neurology department who had ischemic stroke within 24 h. Stroke is defined as rapidly developing clinical symptoms/signs of cerebral dysfunction lasting more than 24 h without any cause other than a vascular abnormality. Venous blood was drawn from all patients who had not taken any antiplatelet drugs for the prior 14 days, and was collected into acid citrate dextrose containing tubes for platelet isolation. Platelets tryptic peptides were analyzed in triplicate on the LC-MS/MS system (UPLC-ESI-qTOF-MS). Tandem mass data extraction were done with ProteinLynx Global Server v2.5 (Waters) and searched with the IDENTITYE algorithm against the reviewed database of homo sapiens (www.uniprot.org). Protein Identification detected with PLGS 2.3 and Quantification of the protein expression changes was done with Progenesis LC-MS software V4.0 (Nonlinear Dynamics). In addition, ELISA was used to quantify pro-inflammatory cytokines as TNF-α in the plasma.

Results: Proteomic profiling of platelets obtained from the stroke patients resulted in identification of 500 proteins. Of these, 83 proteins were found to be differentially expressed in patient as compared to control. These differentially expressed proteins were involved in various processes such inflammatory response, cellular movement, immune cell trafficking, cell-to-cell signaling and interaction, hematological system development and function and nucleic acid metabolism. Plasma levels of TNF-α increased in the stroke patients (29.12 ± 1.37 pg/ml) compared with control subjects (5.16 ± 2.84 pg/ml).

Conclusion: This is the first report providing a global proteomic profile of platelets from stroke patients. Our data provide an insight into the proteins that are involved as platelets inflammation response during ischemic stroke. Inflammation caused by stroke changed to platelet cellular proteins interactions in patients.

A-317

Investigating lipid effects on protein C and thrombin-activatable fibrinolysis inhibitor activation by thrombin/thrombomodulin complex

V. Gruzdys, X. Sun. *Cleveland State University, Cleveland, OH*

Background: Thrombomodulin (TM), an endothelial membrane protein, plays central roles in maintaining haemostasis and preventing inflammation by increasing protein C (PC) and thrombin activatable fibrinolysis inhibitor (TAFI) activation by thrombin. APC selectively inactivates coagulation factors Va and VIIIa, thus preventing excessive coagulation. TAFIα modulates inflammatory mediators and reduces plasmin generation. During cell apoptosis, membrane lipids, such as phosphatidylserine and ethanolamine, are enzymatically flipped to the cell surface. Endothelial cell damage often involves lipids changes. The lipid membrane facilitates TM-enhancement of protein C activation by thrombin, however, lipid effect on TAFI activation is unknown. We studied the effects of different phospholipids in generating APC and TAFIα by the TM/thrombin complex in an effort to better understand thrombotic and inflammatory processes for possible implications in diagnosis and treatment monitoring.

Methods: To study the capacity of TM/thrombin to generate APC and TAFIa, liposomes containing TM were generated by extrusion after TM was mixed with a swelled lipid solution of different compositions in Tris-HCl buffer. Liposomal TM was separated from free form by size exclusion chromatography using Sepharose CL-4B. Liposomal TM concentration was verified by first separating lipid and protein components followed by protein content determination by Bradford assay. APC and TAFIα were generated by incubation with liposomal TM and thrombin in otherwise identical conditions. Concentrations of APC and TAFIα were determined by hydrolysis of Spectrozyme PCa and hippuryl-arginine, respectively.

Results: It was shown that incorporation of TM into phoshatidylcholine vesicles increased generation of APC (26.4 pmol ± 0.7 pmol) in comparison to free TM (18.2 pmol ± 0.7 pmol). Phosphatidylethanolamine had decreased APC generation (10.6 pmol ± 0.6 pmol), phosphatidylserine had a large increasing effect (35.9 pmol ± 1.3 pmol) while APC generation by thrombin alone and with phosphatidylcholine vesicles (but no TM) was below detection range. In addition, free TM was added to phosphatidylcholine liposome solution and APC was generated (20 pmol). This data suggested that separate lipid components do not increase APC level as much as the complex between lipids and TM does.Increase in TAFIα generation was observed after TM was reconstituted in phosphatidylcholine (8.34x104 U, where 1 U = hydrolysis of 1 µmol of substrate per min.) and phosphatidylserine containing (9.18x104 ± 0.3x104 U) vesicles when compared to free TM (6.46x104 U). Phosphatidylethanolamine reconstitution resulted in slight elevation of TAFIα, albeit high variation was observed between measurements (7.54x104 ± 1.6x104 U).

Conclusion: We Investigated lipid effects on protein C and thrombin-activatable fibrinolysis inhibitor activation by thrombin/thrombomodulin complex. We found that phosphatidylserine-bound TM dramatically increases the generation of APC and TAFIα in liposome-based systems. This is contrary to phosphoethanolamine, which had a reducing effect on APC generation. This study suggests possible significance of the effects of cell membrane lipids on hemostatic balance and inflammation and could have possible implications related to damaged endothelium situations such as septic shock. Future studies will test this observation with human endothelial cell lines.

A-318

Establishment of reference intervals for HbF and HbA2 in a Northern Alberta population

T. Higgins, K. Rodriguez Capote. *DynaLIFEDx,, Edmonton, AB, Canada*

Background DynaLIFEDx is the sole laboratory performing hemoglobinopathy and thalassemia investigations for a catchment area of 2 million people in northern Alberta. Samples for hemoglobinopathy and thalassemia testing come from physician request, immigration medicals and as a reflex to the presence of a hemoglobin variant noted on HPLC analysis for HbA1c.A review of our data showed that 17% of HbF results exceeded the manufacturer's reference interval of <1.0% so it was necessary to reevaluate the reference interval for Heft. A reference interval for HbA2 was calculated at the same time. **Method** 6616 thalassemia and hemoglobinopathy investigation requests were analyzed in the period January 1, 2013 to December 31, 2013. 3306 were on patients older than 2 years and interpreted as "normal" by a Clinical Chemist based on their hematology indices (hemoglobin >120g/l, mean cell volume > 80fL), absence of a hemoglobin variant, replete iron status and calculated Mentzer Index and Discriminant Factor EDTA-anti-coagulated blood samples were analyzed by high performance liquid chromatography (HPLC) using the β thalassemia program on the Bio-Rad VARIANT II. Total imprecision (CV%) for HbF was 2.2% and 1.2 % at levels of 2.17% and 9.54% respectively. For HbA2 the total imprecision (CV %) was 3.7% and 2.1% at levels of 2.82% and 5.74% respectively. The statistical software package MedCalc® version 11.4.2.0 for Windows was used to analyze the data. **Results** 3306 individuals, median 31 years, range 3 to 92 years were included in the reference interval calculation. As recommended by CLSI a non-parametric method was used to calculate the reference interval as the Kolmogorov-Smirnov test showed that the distribution of HbF and HbA2 values did not follow a normal distribution. Reference Intervals are HbA2 Lower limit (90% CI) 2.3% (2.20 to 2.34), Upper limit(90% CI) 3.4% (3.3 to 3.45); HbF Lower limit (90% CI) 0.2%(0.20 to 0.23) and Upper limit(90% CI) 1.8% (1.80 to 1.90). **Conclusion** The appropriate reference intervals for the Northern Alberta population using the β thalassemia program on the Bio-Rad VARIANT II was up to 1.8% for HbF and up to 3.4% for HbA2.

A-319

Protein C Antigen And Activity In Patients With Sickle Cell Anaemia

S. E. Idogun[1], O. E. Ihenachor[2]. *[1]university of Benin Teach Hospit, BENIN, Nigeria, [2]University of Benin Teaching Hospital, BENIN, Nigeria*

BACKGROUND: Sickle cell disease (SCD) is characterized by vaso-occlusive events and organ damage which form important causes of morbidity and mortality. Protein C is a naturally occurring anticoagulant that has been demonstrated to be deficient in sickle cell anaemia patients and the impact of hypercoagulable states and thrombosis on sickle cell disease still remains uncertain.

AIM AND OBJECTIVES: The objective of this study was to determine the protein C (PC) levels of the sickle cell patients and to explore the relationship between protein C levels and vaso-occlusive events as well as related complications.

SUBJECTS AND METHODS: A cross-sectional study comprising sixty one sickle cell subjects and thirty healthy control subjects. Protein C antigen (quantitative) and

activity (qualitative) levels were assayed using enzyme linked immunosorbent assay and the Protac (photometric) method respectively. Haematological parameters were measured with the haematology automated analyzer. Data were analyzed using SPSS version 16.

RESULTS: The adult SCD subjects had lower values for both PC antigen (68.6 ± 16.0 %; p=0.004) and activity (49.0 ±13.0 %; p=0.005) when compared to the adult control group (PC antigen = 84.8 ± 18.0 %; PC activity = 60.31±10.37 %). Similarly the paediatric SCD subjects had lower PC antigen (54.9 ± 14.9 %; p=<0.001) and activity (48.0 ± 13.1 %; p=0.006) levels compared to their control group (PC antigen = 79.6 ± 16.7 %; PC activity = 58.6 ± 8.0 %). However there was no significant association between PC levels and SCD complications or vaso-occlusive events (p >0.05).

CONCLUSION: There is functional PC deficiency in SCD patients. This supports a hypercoagulable state in the patients. However there was no significant association between PC levels and SCD

complications or vaso-occlusive events. Thus protein C level in SCD patients may not be a good prognostic marker for disease severity.

PROTEIN C SERUM LEVELS

	Adult			**Paediatric**		
Variables	**SCD (n = 30)**	**Controls (n = 15)**	P-value	**SCD (n = 31)**	**Controls (n = 15)**	P-value
Protein C Antigen Mean ± SD (%)	68.6 ± 16.0	84.8 ± 18.0	**0.004**	54.9 ± 14.9	79.6 ± 16.7	**<0.001**
Protein C Activity Mean ± SD (%)	49.0 ± 13.0	60.3 ± 10.0	**0.005**	48.0 ± 13.1	58.6 ± 8.0	**0.006**

***t-test**

A-320

Thrombocytopenia in Plasmodium falciparum Parasitized Pregnant Women

F. A. Botchway[1], W. Ababio[1], P. Bannerman- Williams[2], H. Salifu[3], C. E. Lekpor[4]. *[1]Department of Child Health, Korle Bu Teaching Hospital, Accra, Ghana, [2]Department of Maternity, Korle Bu Teaching Hospital, Accra, Ghana, [3]Morehouse School of Medicine, Atlanta, GA, [4]Department of Medical Laboratory, Kwame Nkrumah University Of Science and Technology, Kumasi, Ghana*

Background: Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions of the world. Hematological changes associated with malaria in pregnancy are not well documented, and have focused predominantly on anemia.

The aim of this study was to determine the impact of Plasmodium falciparum parasitaemia on the platelet count of pregnant women at the maternity department of Korle Bu Teaching Hospital, Ghana

Methods: This case control study evaluated the effect of malaria parasitemia on the platelet count of Sixty (60) plasmodium parasitized pregnant subjects. Sixty non- malaria parasitized pregnant women and sixty non-pregnant and non-malaria-infected subjects served as control. 1.0 ml of blood sample was taken into EDTA bottle for Full Blood Count using Mindray BC 5300. Thick and thin film prepared and stained with Giemsa for the determination of P. falciparum parasite species and density using light microscopy. A platelet count of 100 × 109 /L was the threshold at two standard deviations below the mean for healthy Ghanaian pregnant women used to indicate thrombocytopenia. Differences in platelet counts were compared based on malaria species and parasitemia in matched non-pregnant and pregnant women.

Results: The mean platelet counts (×109 /L) were significantly lower in pregnant subjects with an episode of Plasmodium falciparum malaria 101.3 ± 9.2 × 109 /L compared to non-parasitized and healthy non-pregnant controls (245.09 ± 23.10 and 260 ± 50.5 × 109 /L) respectively. Platelet count values were 102.5 ± 9.58 ×109 /L and 116.3 ± 15.7 × 109 /L for the primigravidae and multigravidae respectively. (χ= 10.36; P = 0.05). Parasite density was significantly higher among Plasmodium falciparum parasitized primigravidae compared to multigravidae 2140 (1628-2652) parasites/µL in primigravidae women compared to 1816 (1420-2212) parasites/µL in multigravid women. The mean parasite count in Plasmodium falciparum parasitized subjects was 2610 ± 224 parasites/µL, 95% confidence interval (2082-3108). Malaria parasite was found to exert a significant reduction in platelet density in parasitized subjects. This reduction was more pronounced in primigravidae and multigravidae. An inverse relationship was established between parasite density and platelet count (y =-0.020 ×+86.2, r =-0.3).

Conclusion: There is need for a strengthened antenatal care system with increased awareness of the problem among communities most affected by malaria. Preventative strategies including regular chemoprophylaxis, intermittent preventative treatment with antimalarials and provision of insecticide-treated bed nets should be implemented as well as integration of malaria control tools with other health programmes targeted to pregnant women and newborns.

A-321

Risk Stratification and Progression follow-up of MGUS Patients: value of the sFLC and Heavy Chain/Light Chain Pairs markers

J. Jimenez[1], L. Campos[2], T. Pais[2], N. Barbosa[2], C. H. Larramendi[1]. *[1]University Hospital Severo Ochoa, Madrid, Spain, [2]The Binding Site, Barcelona, Spain*

Background: MGUS is the most frequent MG, usually considered a benign MG, and defined by a serum monoclonal protein (MP) <3 g/dL, less than 10% of plasma cells in the bone marrow, no related organ or tissue impairment and no evidences of other B-cell proliferative disorder. The rate of progression towards Multiple Myeloma (MM) is of 1-2% however, the designation itself highlights the current difficulty in precisely identifing patients that will progress towards malignant MG. Based on the MP size and type and the serum Free Light Chains (sFLC) ratio at diagnosis it is possible to stratify the patients according to probability of progression allowing a better management of the MGUS patients, according to the IMWG guidelines. We have been studying the frequency of specific immunoglobulin heavy/light chain pair's alterations in a cohort consisting of MGUS patients with different risk of progression. To validate the significance of our preliminary findings we have increased the cohort in all risk groups and included the follow-up of patients that have progressed to MM.

Methods: 308 samples from 248 MGUS patients were included, both newly and previously diagnosed. All patients were risk stratified according to the IMWG guidelines serum M-protein levels and type by SPE and IF, and sFLC and HLC by nephelometry (Freelite™ and Hevylite®, respectively). Total immunoglobulin levels were also determined to establish the frequency of classic immunoparesis (BNII. Siemens).

Results: The Hevylite assay allows the determination of an imbalance on the immunoglobulins of the same isotype (i.e., IgG-K/IgG-L ratio) identifying the presence of a MP in serum. It also allows to observe the immunoparesis within the same isotype of immunoglobulin (i.e., suppression of the uninvolved HLC pair IgG-L in a IgG-K monoclonal gammopathy). The frequency of HLC immunoparesis is significantly superior to the classic immunoparesis for low-intermediate (p<.0005) and intermediate-high risk groups(p<.001). Besides, the frequency increases in the higher risk-of-progression groups, although only significantly for the HLC immunoparesis type(p<.01 HLC vs p<.27). In IgM cases the differences between classic and HLC immunoparesis did not reach significance, possibly due to the size of the population. Furthermore, for IgG MGUS patients, both the HLC ratios and the uninvolved HLC immunoglobulin levels show a significant trend towards more extreme values as the risk of progression increases. 3 IgG MGUS patients with low-intermediate risk progressed towards MM. For these particular cases an abnormal sFLC ratio was the only established criteria that indicated the risk for progression: 1) While normal at presentation, the HLC ratio became abnormal during follow-up, initially due to the suppression of the uninvolved HLC pair; 2) both FLC and HLC ratios were abnormal prior conversion and those abnormalities became more extreme with active MM; 3) HLC normal at presentation, the patient evolved into an oligosecretory disease.

Conclusion: The sFLC ratio is a relevant indicator of risk for progression in this population of MGUS patients. The frequency and distribution of HLC alterations (both ratio and HLC-immunoparesis) within the specific MGUS risk-groups is suggestive of its utility as a marker for progression.

Tuesday, July 29, 2014

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

Immunology

A-323

Evaluation of pre eclampsia markers in pregnant women with chronic hypertension and pregnant women without hipertension

C. E. Ferreira[1], P. R. S. Ferreira[1], L. G. Oliveira[2], R. C. Sanches[1], A. C. L. Faulhaber[1]. *[1]Hospital Albert Einstein, São Paulo, Brazil, [2]Hospital Vila Nova Cachoeirinha, São Paulo, Brazil*

Background: The relationship between two biomarkers, soluble tyrosine kinase (sflt1) and placental growth factor (PIGF) has been described as useful for precocious identification of pregnant women at risk for pre eclampsia

Methods: 20 hypertensive pregnant women were included among the twenty- and thirty-sixth weeks of pregnancy and a control group of non hypertensive pregnant women was the comparison group. All serum samples were frozen at -80C , and processed simultaneously. The markers were processed Cobas 6000 analyzer from Roche Diagnostics.The cut- off ratio for sFlt-1/PLGF exclusion of preeclampsia described in the package insert of the kit is 33 , with 95% sensitivity and 94% specificity . Values greater than 85 are suggestive of disease.

Results: In the group of pregnant women without hypertension, the average ratio was 22 and in the hypertensive group was 284 . There was a significant difference between the groups evaluating the paired t-test ($p < 0.0001$) . Group of hypertensive pregnant women showed no relation to the lower cut- off suggested for exclusion of pre -eclampsia . In the group of healthy patients only 2 patients showed higher values compared to the deleting 33 does not pre- eclampsia are possible. In the group of chronic hypertensive pregnant women 70 % (13/20) had higher values at 85 .

Conclusion: In our sample, the use of markers of preeclampsia was found to be of the most value to clinical practice in the evaluation of patients with potential risk for progression to pre - eclampsia . Clinical trials are necessary to follow up in this group of patients

A-324

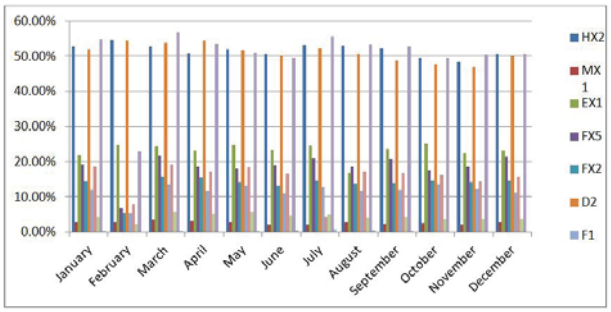
Seasonal frequence of the most requested specific IGE

N. Z. Maluf, S. A. D. Mendonça, L. B. Faro, F. R. M. Abreu, M. D. C. Freire. *DASA, Barueri, Brazil*

Background: Allergic asthma, rhinitis, and atopic eczema are among the commonest causes of chronic ill health in the world. Asthma is one of the most common chronic conditions affecting both children and adults, yet much remains to be learned of its aetiology. Although genetic predisposition is clearly evident, gene-by-environment interaction probably explains much of the international variation in prevalence rates for allergy and asthma. In our laboratory the ten most requested specific IgE are: IgE specific for dust and mites (HX2), IgE specific to fungi (MX1), IgE specific for epithelial animals (EX1), IgE specific for baby food (FX5), IgE specific for seafood (FX2), IgE specific for D. farinae (D2), IgE specific to egg white (F1), Milk specific IgE (F2), IgE specific to soybean (F14), IgE specific to D. pteronyssinus (D1) and IgE specific for cocoa (F93). Analyse the seasonal frequence of the most requested specific IgE.

Methods: We analyse the frequence of the IgE specific for dust and mites, IgE specific to fungi, IgE specific for epithelial animals, IgE specific for baby food, IgE specific for seafood, IgE specific for D. farina, IgE specific to egg white, Milk specific IgE, IgE specific to soybean, IgE specific to D. pteronyssinus and IgE specific for cocoa throughout the year 2012. The informatics system has given the data and for the statistical analysis we used the dispersion

Results: The results of this data are on the graph.



Conclusion: We conclude that there is no seasonal fluctuation in the incidence of IgE specific studied.

A-325

A Rapid and Effective Tool for Monitoring Monoclonal Antibody Production

B. Bhullar, C. Hui, M. Hariharan, J. Hewitt, N. Vats. *MedMira Laboratories, Halifax, NS, Canada*

Background: Monoclonal antibodies (Mab) are used in a variety of fields from diagnostics to therapeutics. We examined the effectiveness and utility of tools created with MedMira Miriad RVF Toolkit to assess Mab post-production functionality.

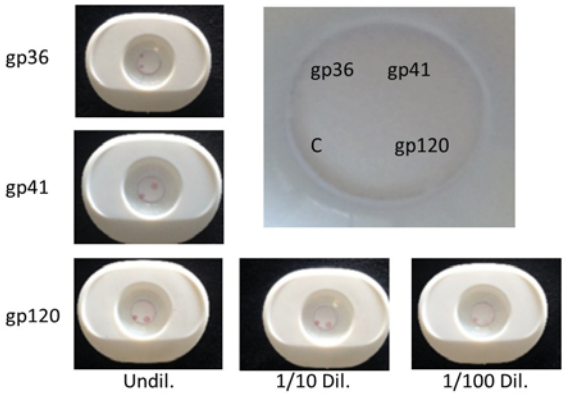
Methods: Six hybridoma supernatants, developed against unique peptide sequences derived from HIV and HCV proteomes, were obtained from a supplier. Miriad RVF Toolkit was used as per manufacturer instructions, with 0.5µL (1mg/ml) of each peptide or mouse IgG (control) spotted on to the cartridges using a micropipettor. Cartridges contained multiple test spots, one spot per peptide and one control spot (see Fig 1 for illustrative example for HIV peptides). Two sets of cartridges, one each for HCV and HIV peptides, were prepared and allowed to dry at room temperature for 30 minutes. Samples of neat or PBS diluted hybridoma supernatants were added during procedural steps.

Results: Reactive results, shown in Fig 1, were scored on a one to three grading scale, three being the highest. Increasing dilutions resulted in decreased reactivity. Two antibodies HCV MDL-1 and 3 became non-reactive at a 1:100 dilution, all others yielded reactive results at the 1:100 dilution. For example, anti-gp 120 antibodies were reactive down to a 1:100 dilution as illustrated in Fig 1. The specificity of the antibodies was evidenced by lack of cross reactivity of each Mab applied to cartridges containing multiple spots.

Conclusion: Testing with the Miriad RVF Toolkit was completed in less than one hour following receipt of antibodies. Miriad RVF Toolkit therefore represents a tool that can be used to efficiently assess production levels, functionality, and specificity of Mab. The time to results also allows Miriad RVF Toolkit to be used as an in-process monitoring tool during production. Results can be documented by recording of visual interpretation or by photographs as illustrated in Fig 1.

Fig 1.

Capture Peptide	Graded Miriad RVF Results/scores at various dilutions		
	neat	1:10	1:100
HCV MDL-1	3+	1+	-
HCV MDL-3	3+	2+	-
HCV MDL-4	3+	3+	2+
HIV gp36	3+	3+	2+
HIV gp41	3+	3+	2+
HIV gp120	3+	3+	1+



A-326

Usefulness of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinima

K. Young Jae. *Samsung Changwon Hospital, Changwon, Korea, Republic of*

Background: Alpha-fetoprotein(AFP) has been used as a diagnostic marker for hepatocellular carcinoma(HCC), and fucosylated fraction of AFP(AFP-L3) has been proposed as a marker for HCC.

M**ethods:** We evaluated performance of the micro-total analyzer system(μ-TAS), on-chip immunoassay analyzer for AFP-L3. The linearity, precision and carry-over rate of μ-TAS were evaluated, and we compared the AFP-L3% levels between patients with early HCC and control group with benign liver diseases.

Results: The linearity was good(R2=0.9995) and coefficient of variation(CVs) of between-day precision in high and low concentration were 0.2% and 0.18%, respectively. AFP-L3% levels were higher in patients with early HCC than in control(13.4%±16.9% versus 4.6%±3.4%). The sensitivity and specificity with AFP-L3% were 57% and 67% at a cut-off value of 5%, and 43% and 83% at a cut-off value of 7%, respectively.

Conclusion: μ-TAS showed good performance of linearity, precision and carry over rate, and AFP-L3% could be a suitable serologic marker for evaluating early HCC.

A-327

What is the Optimal Threshold for an ANA ELISA?

J. Parikh[1], U. Smith[2], L. Ingram[2], E. S. Pearlman[2]. *[1]University of Tennessee Health Sciences Center, Memphis, TN, [2]Veterans Affairs Medical Center, Memphis, TN*

Context: ELISA ANA screening [Seradyne; Indianapolis, IN] was brought in-house at the VAMC in 2013 using an automated Triturus processor [Grifols; Los Angeles, CA]. Rheumatologists had previously indicated dissatisfaction with excess false positive screens and initial validation studies had suggested improved specificity using a threshold optical index [OI] of 2.5 in place of the manufacturer's suggested value of 1.0. We evaluated this change using clinical data. **Methodology**: Reference lab [RL] confirmation (Lab Corp; Raleigh, NC) was obtained in 34(group 1) and 23 (group 2) consecutive specimens (33 and 21 patients) with OI values 2.5 and between [1.0, 2.5) respectively with adequate specimen volume. RL confirmatory testing entailed either a panel of specific antigens (if ordered) or a lab ordered IFA. Positive results on one of more panel antigens or an IFA titer 1:80 was considered confirmatory. The electronic health record was reviewed for clinical data. **Results**: Median age was 64 [27-91] YO with11 (20%) females [twice the female proportion in our general population]. 26 (76.5%) and 9 (39%) specimens confirmed in groups 1 and 2 respectively [chi sq. = 8.16, p<0.005]. Review of the 7 patients (9 specimens) with false negative screens using the higher threshold revealed 2 with hepatitis C and 2 who were ≥80 YO. The appearance of aberrant autoantibodies in these instances is well known. The fifth patient was status post renal transplant for IgA nephropathy. This patient had two specimens sent 4 months apart with OIs of 2.91 and 1.46 and IFA titers of 1:640 (homogeneous pattern)] and 1:320 (speckled) respectively. A multi-antigen panel done with the second specimen was negative. The sixth patient had been diagnosed with Rheumatoid arthritis with high titer of anti-CCP antibodies. The last patient had been diagnosed with SLE elsewhere in the 1990s based on renal biopsy. RL ELISA screens in 2008 and 2010 were negative. It did not appear that results of ANA screening affected clinical management in any of these patients. **Conclusions**: Our study, although small, suggested that improvement in specificity with a decreased number of workups for false positive ANA screens was possible through adjustment of the OI threshold without negative clinical consequences.

A-328

Evaluation of an Anti-Streptolysin O assay for use on the Binding Site Next Generation Protein Analyser

S. Ayub, A. Kaur, M. Solanki, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Elevated blood serum concentrations of human Anti-Streptolysin O (ASO) can be used to provide serologic evidence of past or present infection by β-Haemolytic Streptococci bacteria. Increasing serum concentration of ASO antibodies are produced in response to Streptolysin-O exotoxins secreted by the bacteria. Measurement of ASO levels in serum can be used as an aid in the diagnosis of diseases such as glomerulonephritis, rheumatic fever, bacterial endocarditis, tonsillitis, and scarlet fever. Here we evaluate an ASO assay designed for the quantitative *in vitro* measurement of human ASO in serum using the Binding Site's next generation protein analyser. The instrument is a continuous throughput, bench top turbidimetric analyser capable of automatic sample dilutions up to 1/10,000 and having a throughput of up to 120 tests per hour. Analyser precision is promoted by single-use cuvettes, whilst the user interface is enhanced through bi-directional communication capability, primary sample ID and fully bar coded reagent management systems. Evaluation of the assay on the next generation protein analyser demonstrated an overall assay time of 12 minutes which was read at end point. The assay auto dilutes a single serum based calibrator to produce a measuring range between 50 - 800.00 IU/mL at the standard (1/10) sample dilution. Samples outside the standard range auto re-dilute to neat (1/1) or a secondary dilution (1/20) as appropriate. Results which are still outside the measuring range following auto dilution are reported as <5.00 IU/mL, or >1600 IU/mL. Total precision studies performed at 5 different levels across the measuring range were assessed in duplicate over 21 working days, using a single kit lot on three analysers. Levels assessed were at 731 IU/mL (SD = 33.696, %CV = 4.6%), 427 IU/mL (SD = 16.403, %CV = 3.7%), 82 IU/mL (SD = 4.575, %CV =5.8%), 236 IU/mL (SD = 9.285, %CV =3.9%), and 151 IU/mL (SD = 6.586, %CV = 4.5%). The assay gave a linear response over the measuring range of 50 - 800.00 IU/mL at the standard 1/10 sample dilution and over a range of 5.00 IU/mL- 80.00 IU/mL at the minimum 1/1 dilution. A linear regression of $y = 0.981x - 16.2$ and $R^2 = 0.997$ was demonstrated at 1/10, whereas a regression of $y= 1.032x - 0.411$ and $R^2 = 0.998$ was demonstrated at 1/1 dilution. No significant interference was seen when the assay was challenged with haemoglobin (500mg/

dL), bilirubin (200 mg/L) and chyle (1500 FTU). Comparison comprising normal and clinical samples (n =121) was carried out against the Binding Site SPA PLUS analyser, covering between 52.000 IU/mL - 822.000 IU/mL. Analysis by Passing-Bablok regression demonstrated a linear fit of y = 0.96x + 0.92. We conclude that the ASO assay for the Binding Site Next generation protein analyser is reliable accurate and precise and shows good agreement with existing assays.

A-329

Cytokine and IFN-γ-Induced Chemokine mRNA real-time PCR Taqman Probe Assay After Mycobacterium tuberculosis Specific Antigen Stimulation in Whole Blood from Infected Individuals

H. Kim[1], S. Kim[2], J. Cho[3], S. Hong[1], Y. Kim[1], G. Kim[1], S. Ahn[1], S. Park[1], D. Lee[4], H. Jin[5], S. Park[6], S. Cho[7], H. Lee[1]. *[1]Yonsei University, Wonju, Korea, Republic of, [2]Institute for Life Science and Biotechnology, Seoul, Korea, Republic of, [3]Daegu Health College, Daegu, Korea, Republic of, [4]Hyejeon College, Hongseoung, Korea, Republic of, [5]Catholic University of Pusan, Busan, Korea, Republic of, [6]Daegu Hanny University, Daegu, Korea, Republic of, [7]Yonsei University, Seoul, Korea, Republic of*

Background: Recently, the interferon gamma (IFN-γ) release assay (IGRA) was introduced as an alternative immunodiagnostic method to the tuberculin skin test (TST) for detecting latent tuberculosis infection (LTBI). However, IGRAs are known to have limited sensitivity and cannot differentiate between active pulmonary tuberculosis (PTB) disease and LTBI. Numerous cytokines and regulator factors have been implicated in the pathogenesis and control of *Mycobacterium tuberculosis* (MTB) infection. Therefore, additional cytokines including T helper 1 (TH1)-type and T helper 2 (TH2)-type cytokines and chemokines associated with MTB infection may improve the performance of IGRAs. **Methods:** In the present study, a molecular diagnostic method using the real-time RT-PCR TaqMan® assay, which is able to quantitate mRNA expression levels, was developed for eight human targets (IFN-γ, TNF-α, IL-2R, IL-4, IL-10, CXCL9, CXCL10, and CXCL11) and evaluated with three different patient groups (active PTB, LTBI, and healthy non-TB groups). **Results:** Results revealed that positivity of TNF-α, IL-2R, and CXCL10 in the active PTB group was 96.43%, 96.43%, and 100%, respectively. The positivity of IL-2R and CXCL10 in the LTBI group was 86.36% and 81.82%, respectively. Statistical results revealed that TNF-α and CXCL9 (both $p<0.0001$) were the best individual markers for differentiating between the three different MTB infection groups. For optimal sensitivity, the simultaneous detection of multiple targets was attempted. The combination of IFN-γ, TNF-α, and IL-2R and the combination of TNF-α, IL-2R, CXCL9, and CXCL10 showed the best performance for detecting active PTB (both 100% positivity) and LTBI (86.36% and 81.82% positivity). **Conclusion:** These results imply that the combination of suitable single markers is very useful for the efficient diagnosis of MTB infection and the differentiation of MTB infection status.

A-330

Toxoplasma gondii IgG avidity among Brazilian IgG+/IgM+ women

V. V. Frade, I. C. B. Almeida, D. Panisa, V. T. Alástico, C. F. A. Pereira, N. Gaburo. *DASA, Sao Paulo Brasil, Brazil*

Background: *Toxoplasma gondii* is prevalent in Brazil and and IgG and IgM is routenly requested for pregnant women. The IgG avidity test analyzes the bonding strength of the antigen-antibody complex after treatment with urea. In the acute phase of the disease, the binding of antigen-antibody complex is easily dissociated because the IgG avidity is reduced. This test maybe used as a supplemental assay for samples that are IgM and IgG positive in order to indicate recent infection.

Objective: To describe the results obtained by the IgG avidity test in women that is IgG and IgM positive for *Toxoplasma gondii.*

Methods: We have selected 413 samples from our routine that were IgM positive with Toxo IgM kit (Roche, Mannheim, Germany) and presented IgG level higher than 50 IU/mL by the Toxo IgG kit (Roche). The *Toxoplasma gondii* avidity test was performed using TSI Toxok -G - Plus kit (DiaSorin, Saluggia, Italy) which is an ELISA test. The avidity index was calculated by the ratio of the optical density of the urea buffer treated sample divided by optical density of the sample without urea treatment. Results below 30%, were considered recent infection (less than 12 weeks), results higher than 60 % infection were considered more than 12 weeks of infection. Between 30-60% the result was considered inconclusive.

Result: The results are shown on Table 1. Only 1% of the females were considered recent infected and 2.7% inconclusive

Conclusion: We conclude that the IgG avidity test for toxoplasmosis may be an important tool for the interpretation of IgG/IgM results in pregnant women.

Avidity	Number of samples	%
>60%	398	96,4
30-60%	11	2,7
<30%	4	1,0
Total	413	100

Table 1- IgG avidity detected in the studied samples.

A-331

Neopterin ELISA kit: Analytical evaluation and verification of the reference interval in native population in Argentina.

L. E. D'Agostino[1], F. D. Ventimiglia[2], J. A. Verna[1], J. J. Bruno[1], A. L. Capparelli[3]. *[1]Laboratorio de Análisis Clínicos e Inmunológicos, La Plata, Argentina, [2]Universidad Nacional de La Plata, La Plata, Argentina, [3]INIFTA- Universidad Nacional de La Plata, La Plata, Argentina*

Background: Neopterin, 2-amino-4-hydroxy-6-(D-erytrhro-1′,2′,3′-trihydroxypropyl)-pteridine, is a low-molecular compound synthesized from guanosine triphosphate (GTP) by macrophages. The clinical utility is like specific marker for the activation of cellular immunity. Measurement of plasma neopterin concentrations has been proposed as a test for monitoring the activity of infectious, autoimmune and malignant diseases.

Objective: The purpose of this study was to evaluate GenWay Neopterin ELISA kit performance and verification of the reference interval in native population in Argentina.

Materials and methods: Plasma samples were obtained from 38 healthy volunteers (aged 17-60 years). Neopterin was determined by ELISA GenWay Biotech Inc. The verification of the reference interval was calculated using the CLSI guidelines C28-A3. Bias and imprecision, were calculated using the protocol EP-10 from CLSI, with three different standard concentrations (nmol.L^{-1}): 4; 12 and 37. The results were compared with the specifications of folate, due to neopterin desirable specifications are not yet established. *Intra-assay and inter-assay variability were determined in* ten normal plasma samples and ten samples with elevated neopterin concentrations from rheumatoid arthritis patients (RA), QC plasma provided by the manufacturer and compared with literature data. The criteria for acceptable performance were the target value plus or minus 2 standard deviations. To study behavior among different sources of specimens, variance analysis ANOVA was used.

Results: The limits of references values of neopterin were verified (< 10.0 nmol.L^{-1}). The lower and higher limits were 1.36 (percentile 2.5) and 9.93 (percentile 97.5) nmol.L^{-1} respectively. Bias and imprecision, encountered in the standard concentrations were: 13.6 % and 11.2%; 9.8 % and 3.1%; 7.6% and -1.4% respectively. Intra-assay variability in normal and elevated samples was 7.9% and 11.4% respectively; in QC samples (normal and elevated) were 9.8% and 6.1% respectively. Inter-assay variability for the same group of samples was 8.2%; 13.8%; 10.4% and 7.8% respectively. The lot to lot CVs for the two samples used for Quality Control of three lots of the neopterin ELISA kit were 13.1% and 7.4% respectively. Total error allowable (TE_a) for neopterin was calculated for the three standard concentrations: 33.64%; 19.27%; 13.94%, TE_a for folate is 39.0%.Variance analysis had not shown statistical significance among the processed samples.

Conclusions: The verification of neopterin reference interval on healthy volunteers in native population is consistent with literature data. This interval allowed distinguishing between healthy and RA patients. The precision was verified by the good intra and inter-assay coefficients of variation. The lot to lot CV was < 13.5%. Although desirable specifications for neopterin are not yet established, we used folate in terms of comparison; folate is a pterin derivative. We found that the mean neopterin's TEa was 22.3% and the highest value obtained, 33.64 %, was smaller than the folate. We conclude that the results with GenWay ELISA on imprecision and bias were acceptable when were compared with the well-known folate's desirable specifications. Variance analysis had not shown statistical significance between neopterin measurements among different sources. In summary, GenWay ELISA kit characterized here is valuable for clinical applications, is simple and rapid for neopterin determinations in serum or plasma.

A-332

Assessment of a Particle Immunofiltration Assay [PIFA] for Antibodies Associated with Heparin Induced Thrombocytopenia (anti-HIT)

C. Morris[1], K. Ballard[2], C. Finch Cruz[2], E. S. Pearlman[2]. *[1]University of Tennessee Health Sciences Center, Memphis, TN, [2]Veterans Affairs Medical Center (VAMC), Memphis, TN*

Background: The Department of Surgery at VAMC queried the clinical laboratory about the feasibility of implementing a rapid assay for anti-HIT. The only assay satisfying their specifications was a PIFA [Akers Biosciences; Thorofare, NJ] The question was how to evaluate this assay given the low incidence of true positive (TP) cases of HIT as indicated by a positive result on a functional assay e.g. the serotonin release assay [SRA]. **Methods**: To circumvent the low incidence of TPs we pooled serum specimens from five patients that had been received for Vitamin D assay. PIFA was negative on the pooled serum that was then used to serially dilute the positive control material supplied by the manufacturer between 1:2 and 1:32. The diluted control material was run using PIFA and the dilutions and the negative pooled serum were then sent to a reference lab (RL) [Lab Corp; Raleigh, NC] where the material was tested using an ELISA assay with a threshold index of 0.4. The entire procedure was repeated using a different lot of positive control material (total of 12 determinations sent to the RL). **Results**: Of the 12 simulated specimens, 5 were positive by both PIFA and ELISA and 4 negative by both methods [overall concordance=75%] Three specimens were positive by PIFA and negative by ELISA. All discordant specimens had ELISA indices >0.3 but less than 0.4. **Conclusions**: The study was small and SRA results were not available on the simulated samples. However it would appear that FPs already known to be a problem when employing ELISA would be increased when using PIFA as the latter apparently begins generating positive results when the ELISA index is >0.3. A negative PIFA result is however concordant with ELISA. PIFA may therefore be usable as the first step in an algorithm insofar as a negative result would suggest that anti-HIT is unlikely and a positive result could be referred for additional testing.

A-333

New immunological assays for diagnosis of Schistosoma mansoni for clinical acute and/or chronic forms

R. F. Q. Grenfell[1], V. Silva-Moraes[1], D. Harn[2], P. Z. Coelho[1]. *[1]Fundação Oswaldo Cruz, Belo Horizonte, Brazil, [2]University of Georgia, Athens, GA*

Background: Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for Schistosoma mansoni infection, with 4 endemic areas together with 80 travelers infected in a freshwater pool.

Methods: Sera, whole blood, urine and saliva samples from the patients were used for the standardization of innovative diagnostic methods. Comparisons were performed with eggs in feces, IgG titers, encephalomyelitis by NMR and clinical symptoms. The new methods used were immunochromatography (dipstick), Immunomagnetic Separation and ELISA with highly purified monoclonal antibodies.

Results: We could diagnose acute patients 10 days post-infection, also more than 95% of positive cases from chronic and low endemicity patients. New methods for IgG detection using purified glycoprotein or recombinant protein or peptides (10 aminoacids) were superior to conventional ELISA.

Conclusion: Best results were seen for recombinant protein with 100% of sensitivity. Data showed 100% of sensitivity of chronic patients and 98% of acute patients.

Financial support: Fapemig, CNPq, Capes, Fiocruz, Fiotec, PDTIS (Brazil). Fulbright, NIH, University of Georgia (USA).

A-334

Audit of Autoimmune Neurological Antibodies requesting in a Singapore institution

M. Tan, L. Ong, B. Saw, A. Tan, Y. Tan, M. Voong, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore*

Background: There is increased testing of autoimmune neurological antibodies for evaluation of neurological diseases, but there is limited information on its prevalence in our local population. We aimed to determine the neurological antibody requesting patterns and seropositivity rates in patients presenting with neurological disorders in our institution.

Materials and Methods:

Autoimmune neurological antibodies Anti-NMDA, Anti-VGKC, Anti-AQP4, Anti-Hu, Anti-Yo and Anti-Ri requested from January 2010 to December 2013 in our institution were reviewed; only the first positive antibody result was considered for patients with multiple requests. For Anti-AQP4, cerebrospinal fluid oligoclonal bands (OCB) and pleocytosis information were reviewed as well.

Statistical analysis was performed using SPSS Version 17.0.

Results:

There were 443 requests of neurological antibodies for 203 patients, of which three most common presenting complaints were seizures, altered mental state and encephalitis.

The median age was 48.4 years old, with male to female ratio of 1.09, and 118 Chinese, 19 Malays, 17 Indians and 49 belonging to other ethnic groups. The median age and ethnic breakdown for each antibody is shown in the table.

7.6% of these patients were positive for any of the neurological antibodies. Anti-AQP4 had the highest seropositivity at 23.4%, followed by Anti-VGKC(9.9%), Anti-NMDA(9.2%), Anti-Yo(3.9%), Anti-Hu(1.3%) and Anti-Ri(0%). No patients were positive for more than 1 antibody.

Amongst patients with positive Anti-AQP4, they are older (55.6 versus 36.9 years old in negative patients) and less likely to have a positive OCB or pleocytosis. Young females were more likely to be positive for Anti-NMDA compared to older females or males, as shown in the table.

Indian ethnicity was positively associated with Anti-VGKC positivity.

Conclusion:

Our data showed that autoimmune neurological antibody positivity were not uncommon, however this could be due to requesting bias. Further studies will be helpful to identify their prevalence in patients with different neurological complaints.

Type of neurological autoantibody and characteristics	All patients	Patients with positive result	Patients with negative result
Anti-Aquaporin 4 Antibody (AQP4)			
Number of patients (%)	47	11 (23.4)	36 (76.5)
Median age (years)*	44.6	55.6	36.9
Ratio of chinese:malay:indian: other ethnic groups	22:5:6:14	8:0:0:3	14:5:6:11
Anti-N-methyl-D-aspartate Antibody (NMDA)			
Number of patients (%)	120	11 (9.2)	109 (90.8)
Median age (years)*	42.5	26.5	44.9
Ratio of chinese:malay:indian: other ethnic groups	71:13:6:20	5:1:0:5	66:12:6:25
Anti-Voltage gated K channel (VGKC)			
Number of patients (%)	81	8 (9.9)	73 (90.1)
Median age (years)	53.8	50.1	54.1
Ratio of chinese:malay:indian: other ethnic groups	53:3:8:17	3:1:3*:1	50:2:5:16
Anti-Yo			
Number of patients (%)	76	3(3.9)	73 (96.1)
Median age (years)	57.3	56.7	57.5
Ratio of chinese:malay:indian: other ethnic groups	53:5:6:12	2:1:0:0	51:4:6:12
Anti-Hu			
Number of patients (%)	77	1 (1.3)	75 (97.4)
Median age (years)	57.3	73.4	57.2
Ratio of chinese:malay:indian: other ethnic groups	53:7:4:12	0:1:0:0	53:6:4:12
* Difference between patients with positive antibody compared to those with negative antibody is significant with p value <0.05.			

A-335

Deficiency of CD16 in polymorphonuclear neutrophils. Myelodisplastic disorder, paroxysmal nocturnal hemoglobinuria or neutrophilic FcGRIIIB gene deficiency?. A case report.

B. Alvarez[1], A. González[1], A. Lafuente[2], E. Ruiz[2], L. Conejo[1], M. Medrano[1], A. Vlagea[1], J. Alonso[1], N. Del Amo[1], C. González[1], D. Velasco[1], J. Villarrubia[1], R. Guillén[1], F. Cava Valenciano[1]. *[1]Laboratorio Central BRSalud, Madrid, Spain, [2]Hematology department. Hospital del Tajo, Madrid, Spain*

Background CD16 antigen, the human Fcγ receptor III (FcgRIII), is a protein constitutively expressed on polymorphonuclear neutrophils (PMNs), monocytes and NK-cells. CD16 participates in phagocytosis and in antibody-dependent cellular cytotoxicity. It exists in 2 different forms encoded by 2 nearly identical linked genes, FcgRIIIA and FcgRIIIB that generate alternative membrane anchored molecules: FcgRIIIA (50-65 kd) is a transmembrane form expressed on NK cells

and macrophages; FcγRIIIB (48 kd) is anchored through a phosphatidylinositol (PI) linkage and expressed only on neutrophils.

Abnormal CD16 expression in cell surface might indicate myelodysplastic disorder or indicate an acquired clonal hematologic disorder such as paroxysmal nocturnal hemoglobinuria (PNH).

In the present study we report a case of total CD16 deficiency on PMNs.

Methods We studied the peripheral blood from a 72-year-old male that only presented neutropenia. A 6-color flow cytometry tube was used including the markers CD16, CD24, CD14, FLAER, CD33 and CD45. The cells were run on a 3-laser FACSCanto II (BD) with FACSDiva software (BD) and analyzed using Infinicyt software (Cytognos).

Results Immunophenotypic analysis of peripheral blood sample from patient showed a lack of CD16 expression on neutrophils, but NK cells were CD16+. The GPI-anchored proteins such as FLAER, CD24, and CD14 and all other myeloid antigens were expressed normally in neutrophils and monocytes.

Conclusion These results suggest a neutrophilic FcGRIIIB gene deficiency and reject a myelodysplastic disorder and paroxysmal nocturnal hemoglobinuria. Previous studies indicate deficiency of CD16 does not compromise the host defence. Apparently, the other receptors for IgG, CD32 and CD64, can compensate for the lack of CD16 Eur J Clin Invest. 2004 Feb;34(2):149-55.

A-336

Diagnostic efficacy evaluation of IL-1RI, IL-1 β and CDK2 in peripheral blood and synovial fluid with rheumatoid arthritis

X. Huang, X. Chen, J. Zhuang. *Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China*

Background: To explore the diagnostic efficacy of interleukin 1 receptor typeI(IL-1RI), interleukin 1β(IL-1 β) and cyclin dependent kinase 2(CDK2) in peripheral blood and synovial fluid with rheumatoid arthritis (RA).

Methods: There were selected 94 cases with RA patients in rheumatology outpatient and inpatient department, 40 cases with systemic lupus erythematosus (SLE) patients in outpatient department, 20 cases with acute upper respiratory tract infection(AURTI) patients in emergency department, 20 healthy persons. All subjects were eligible for inclusion criteria. All subjects were drew vein blood 3 ml besides 1 ml knee joint fluid from 24 patients with active RA patients. The level of IL-1RI, IL-1β and CDK2 were detected in serum and synovial by quantitation ELISA, then the three items were evaluated diagnostic efficacy.

Results: There were all significant differences among experimental groups on either IL-1RI, IL-1β or CDK2 by square variance analysis(P<0.001), respectively. On IL-1RI, there were significant differences between RA patients group and RA patients joint synovial fluid group or SLE patients group or acute upper respiratory tract infection group and control group (P<0.001), respectively. On CDK2, there were significant differences between RA active phage and RA relieve phage, between RA patients group and RA patients joint synovial fluid group or SLE patients group or acute upper respiratory tract infection group or control group (P<0.001) , respectively. On IL-1β, there were significant differences between RA active phage and RA relieve phage, between RA patients group and RA patients joint Synovial fluid group or healthy people group (P<0.001), respectively.

Compared RA patients active phage with RA relieve phage, area under curve of ROC in CDK2 was largest, followed IL-1β andIL-1RI. Compared RA patients group with SLE patients group, area under curve of ROC in CDK2 was largest, followed IL-1RI. Compared RA patients group with acute upper respiratory tract infection patients group, area under curve of ROC in IL-1RI was largest, followed CDK2. Compared RA patients group with control group, area under curve of ROC in CDK2 was largest, followed IL-1RI.

Conclusions:IL-1RI had low diagnostic efficiency next to CDK2, but it could efficiently differentiate RA and acute upper respiratory infection (AURI). CDK2 had higher diagnostic efficiency, which could efficiently differentiate active phase and relieve phase of RA, and differentiate RA and SLE, but had low diagnostic efficacy next to IL-1RI, in differentiating RA and AURI. CDK2 plus IL-1RI plus IL-1β paralleling joint diagnosis may increase diagnostic value of RA. CDK2 plus IL-1RI plus IL-1β tandem joint diagnosis may increase early diagnostic value of RA.

A-337

Evaluation of an IgG3 assay for use on The Binding Site Next Generation Protein Analyser

K. A. Samuels, J. R. Kerr, L. D. Southan, A. Kaur, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. Deficiency of IgG3 has been reported to be associated with viral infections of the urinary tract. Here we describe the evaluation of an IgG3 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 55 - 2200 mg/L at the standard 1/20 sample dilution. Samples lower than the bottom of the standard measuring range are automatically retested at the lower 1/2 sample dilution, providing a measuring range of 5.5-220 mg/L. Precision studies (CLSI EP05-A2) were performed at five levels, over 21 days with 2 runs per day. Antigen levels of 8.9mg/L, 117.8 mg/L, 159.85mg/L, 258.3 mg/L and 1774.5mg/L were assessed for total and within run precision over 3 reagent lots on 3 analysers. The coefficients of variation were 12.1% and 8.4% for the 8.9mg/L sample, 8.3% and 5.1% for the 117.8mg/L sample, 9.9% and 8.2% for the 159.8mg/L sample, 6.6% and 3.8% for the 258.3mg/L sample and 5.8% and 2.0% for the 1774.5mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (55-2200mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 1.0136x - 11.368, R^2 =0.9971). No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 FTU) when spiked into a sample with known IgG3 concentration. Correlation to the Binding Site IgG3 assay for the SPA PLUS was performed using normal and clinical samples (n=102, range 102.10-1836.90 mg/L). Good agreement was observed between assays when anlysed by Passing-Bablok regression; y=1.07x -20.80. We conclude that the IgG3 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-338

Validation of a multiplex electrochemiluminescence assay for quantitation of synovial fluid cytokines and establishing reference interval in non-infected arthroplasty patients

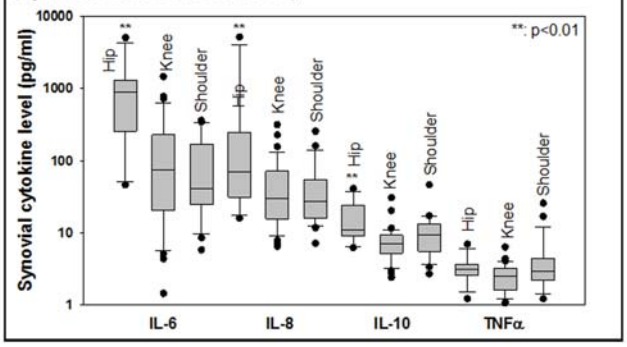
X. Zhang, B. Gibson, S. J. Frangiamore, A. Saleh, M. J. Grosso, M. F. Kovac, T. W. Bauer, E. T. Ricchetti, J. P. Iannotti, T. M. Daly. *Cleveland Clinic, Cleveland, OH*

Background: Periprosthetic joint infection (PJI) is a severe complication following arthroplasty. Emerging studies suggest that proinflammatory cytokines in synovial fluid are promising markers for PJI diagnosis. However no assay has been validated for measurement of cytokines in synovial fluid and baseline levels have not been established in non-infected artroplasty patients. We developed and validated an electrochemiluminescence (ECL) multiplex assay for quantification of 9 cytokines in human synovial fluid and established reference intervals in artroplasty patients. Method: The human ultrasensitive cytokine assay (Meso Scale Discovery. Rockville, MD) was modified to measure IL-1β, IL-2, IL6, IL-8, IL-10, IL-12p70, GM-CSF,IFN-γ, TNF-α in synovial fluid. The assay was validated following CLSI guidelines. Patients who underwent primary arthroplasty or arthroscopic rotator cuff repair in hip, knee or shoulder, were enrolled prospectively. These patients were known not have infections and thus were ideal controls for PJI. Synovial fluid was collected intraopratively. Results: The intra- and inter-assay imprecision (n=20) for all the cytokines was less than 15%. Assay accuracy was evaluated by spiking recombinant cytokines into synovial fluid. Percent recovery was within 100±20%. The assay sensitivity and linear range was summarized in the table. No difference was observed between different genders or the two surgical groups. However, patients >=70 years old had significantly higher synovial IL-6 and IL-8 levels than other age groups. Knee and shoulder joints showed similar cytokines levels while hip synovial fluid contained significant increases in IL-6 and IL-8(Fig1). Reference intervals were established for synovial cytokines of knee/shoulder joints in non-infected arthroplasty

patient (<70 years old, see table). Conclusion: The ECL assay provides a reliable method for quantitation of multiple cytokines in less than 25µL synovial fluid. IL-6 and IL-8 are the major cytokines in synovial fluid. Age and joint specific reference interval was necessary for diagnosis of PJI using synovial cytokines.

Analytes	Limit of detection (pg/ml)	Limit of quantitation (pg/ml)	Linear range	Reference interval n=52(pg/ml)
IL-1β	0.9	5.5	0.9 - 50000	<5.5
IL-2	0.7	2.1	0.7 - 10000	<3.1
IL-6	0.6	0.9	0.6 - 50000	<793.1
IL-8	0.3	0.3	0.4 - 50000	<192.7
IL-10	1.0	4.8	0.4 - 50000	<21.9
IL-12p70	1.1	11.5	1.1 - 50000	<11.5
IFNγ	1.3	4.8	0.4 - 50000	<9.4
TNFα	0.9	2.1	0.9 - 50000	<9.6
GM-SF	0.8	3.0	1.6 - 50000	<3.0

Cytokine levels in synovial fluid in non-infected arthroplasty patients
(hip: n=11; knee: n=36; shoulder: n=26)



A-339

Evaluation of the immunoassay reagent kit for high sensitive troponin-I (ARCHITECT® *STAT* high sensitive Troponin-I) with fully-automated chemiluminescent immunoassay analyzer, and the clinical trials in Japan using medical checkup examinees

I. Tanaka[1], K. Sakamaki[2], T. Kasumi[2], K. Nagashima[2], M. Akuzawa[2], K. Nakajima[3], Y. Shimomura[3], N. Nagano[3], Y. Ando[4], H. Kinukawa[1], T. Yoshimura[1]. *[1]Abbott Japan Co., Ltd., Chiba, Japan, [2]Hidaka-kai, Hidaka Hospital, Health Care Center, Gunma, Japan, [3]Hidaka-kai, Hidaka Research Center, Gunma, Japan, [4]Hidaka-kai, Gunma, Japan*

Background: Troponin-I is widely used as an aid in the diagnosis of acute myocardial infarction because of its cardiac specificity. In Japan, the measurement of troponin-I belongs to class-I in Guidelines for Management of Acute Coronary Syndrome without Persistent ST Segment Elevation(Japanese Circulation Society (JCS) 2007)and Class-II in Guidelines for Treatment of Chronic Heart Failure (JCS 2010). Use of a high sensitivity troponin assay is recommended due to improved analytical performance. An assay is considered to be high sensitive if the %CV value is 10% or less at the 99th percentile and if at least 50% of normal subjects are detectable. The goal of this study was to evaluate the analytical performance characteristics of ARCHITECT *STAT* high sensitive Troponin-I assay on the fully automated chemiluminescent ARCHITECT analyzer and to determine the 99th percentile upper reference limit and the factors which affect troponin-I in Japanese populations undergoing routine health checks.

Methods: The ARCHITECT *STAT* high sensitive Troponin-I assay is a double monoclonal antibody sandwich assay. This assay has an assay time of approximately 18 minutes and an analytical range of 0.0 to 50,000.0 pg/mL. Precision was evaluated with 3 different sera in duplicate, twice a day for 5 days (n=20). The limit of blank (LoB) and limit of quantitation (LoQ) were evaluated with 20 consecutive measurements of calibrator A (zero concentration) and 5 different patient sera in duplicate for 5 days. Dilution linearity was determined by diluting 3 serum samples with the dedicated diluted solution. Correlation was performed with 50 samples spanning the range of 32-35,389 pg/mL on ARCHITECT *STAT* Troponin-I assay (the conventional troponin-I assay). The 99th percentile of Japanese healthy population and the factors which affect troponin-I were evaluated with 283 patients undergoing routine health checks.

Results: Precision showed %CVs of 2.6-5.9 over the range of 34.18 to 3799.79 pg/mL. LoB and LoQ were 0.35 pg/mL and 3.11 pg/mL, respectively. Method comparison showed a correlation coefficient of r = 0.98 and a slope = 0.98. The 99th percentile value of 166 Japanese apparent healthy subjects was 22.4 pg/mL. This value was equivalent to the 99th percentile value reported in the package insert from Abbott Laboratories. The multiple regression analysis revealed that male sex and age were independent factors for increased cardiac troponin-I levels. Cardiac troponin-I levels were significantly higher in males and positively associated with the age.

Conclusion: The ARCHITECT *STAT* high sensitive Troponin-I assay demonstrated good analytical performance and improved imprecision at low concentration in comparison to the conventional ARCHITECT *STAT* Troponin-I assay. ARCHITECT *STAT* high sensitive Troponin-I meets the definition of high-sensitivity troponin reagent proposed by the IFCC Task Force. The 99th percentile value which was established by the manufacturer may be used in Japan, but it would be necessary to consider the effects of age and gender.

A-340

A Lyophilized Quality Control Material for Human Allergen Specific IgE Testing.

Z. Bradic, J. Cole, A. Riviere. *Bio-Rad Laboratories, Inc., Irvine, CA*

In vitro measurements of circulating IgE antibodies specific for allergens (sIgE), such as pollens, foods, drugs, venoms, insects, mites, molds and epidermals, are useful for the diagnostic assessment of a patient's allergies. Lyphochek® Allergen sIgE Control Negative and Panel A is a lyophilized, human serum based third party quality control material to monitor the precision of laboratory testing procedures for specific IgE antibodies.

Procedure: Control material was tested for fifteen sIgE on various testing platforms for recoveries, precision, opened vial stability, and accelerated temperature studies for shelf-life prediction.

Results: The control material was assayed to determine sIgE recovery values using several test platforms. The Negative control results were all below the detection limit of the test method used. The table below lists results for the sIgE present in the Panel A control.

Allergen		Specific IgE		
		Siemens Immulite 2000 (KU/L)	Thermo Fisher Phadia® ImmunoCAP (KU/L)	Hycor HYTEC 288 (IU/mL)
D1	D. pteronyssinus	25.01	16.70	8.73
D2	D. farinae	29.38	22.15	16.78
E1	Cat dander	33.93	17.80	8.17
E3	Horse dander	17.99	7.15	3.69
E5	Dog dander	32.95	55.44	14.81
F1	Egg white	3.74	1.20	1.19
F2	Cow's milk	10.29	5.92	2.29
F13	Peanut	3.20	4.15	1.37
G2	Bermuda grass	19.64	15.99	7.57
G3	Orchard grass	29.33	17.63	13.25
G6	Timothy grass	24.81	13.97	12.86
M3	Aspergillus fumigatus	2.76	3.82	0.35
M6	Alternaria tenuis	10.96	8.45	3.69
T3	Birch	20.55	10.10	3.13
W6	Mugwort	2.33	6.12	0.54

Open vial stability studies showed that the sIgE in the control is stable for a minimum of 28 days at 2-8°C. Accelerated temperature studies predicted a shelf-life of over 3 years when stored in lyophilized form at 2-8°C.

Conclusion: Lyphochek Allergen sIgE Control, Negative and Panel A levels are suitable to monitor the precision of laboratory testing procedures for sIgE in human serum or plasma.

A-341

Effect of CD95 on inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes

X. Huang. *Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou,Guangdong, China*

Background: Many CD95-expressing cells don't always undergo apoptosis after stimulation with CD95 ligation. To investigate the role of expression of CD95 (Fas/

Apo1) on inflammatory response in fibroblast-like synoviocytes(FLS) obtained from rheumatoid arthritis(RA) and to evaluate the role of phosphatidylinositol 3-kinase(PI3K)/protein kinase B(PKB or Akt) pathways within this process.

Methods: The expression levels of CD95 were measured by immunohistochemistry and reverse transcription polymerase chain reaction(RT-PCR). Apoptotic cells were detected by in situ apoptosis detection (TUNEL) assay. The RA-FLS were treated with agonistic anti-CD95 antibody or CD95 siRNA, then the proliferation was detected by CCK-8,and mRNA level of inflammatory cytokines were detected by RT-PCR. After the RA-FLS were treated with agonistic anti-CD95 antibody, the total Akt and pAkt protein expression was analyzed by western blot, and the changes mentioned above were observed while incubated with the PI3K inhibitor LY294002. **Results:** A significant increase of CD95 antigen was found in RA compared with osteoarthritis (OA) samples, while apoptosis in RA synovial tissue were not obvious. Low concentrations of agonistic anti-CD95 antibody could promote RA-FLS growth and IL-6 mRNA expression, while high concentrations could induce apoptosis and both of these phenomenons were inhibited by CD95 siRNA. Agonistic anti-CD95 antibody could stimulate the expression of pAkt, and PI3K specific inhibitor LY294002 could induce opposite change.

Conclusion: Stimulation of CD95 could promote RA-FLS proliferation and inflammation, and activation of the PI3K/Akt signaling pathway might be the potential mechanism.

A-342

Quantitative detection of Plasmodium falciparum Histidine Rich Protein 2 in saliva

F. A. Botchway[1], C. E. Lekpor[2], R. Ansah[3]. *[1]Department of Child Health, Korle Bu Teaching Hospital, Accra, Ghana, [2]Department of Medical Laboratory, Kwame Nkrumah University Of Science and Technology, Kumasi, Ghana, [3]University of Ghana, Legon, Ghana*

Background: Malaria is a global health priority with a heavy burden of fatality and morbidity. Improvements in field diagnostics are needed to support the agenda for malaria elimination. Saliva has shown significant potential for use in non-invasive diagnostics, but the development of off-the-shelf saliva diagnostic kits requires best practices for sample preparation and quantitative insight on the availability of biomarkers and the dynamics of immunoassay in saliva. This study measured the levels of the PfHRP2 in patient saliva.

Methods: Matched samples of blood and saliva were collected between March and August, 2011 from forty patients at the ER and OPD of the pediatric unit of Korle Bu Teaching Hospital. Parasite density was determined from thick-film blood smears. Concentrations of PfHRP2 in saliva of malaria-positive patients were measured using a custom chemiluminescent ELISA in microtitre plates. Forty negative-control patients were enrolled. Saliva samples were stabilized with protease inhibitor

Results: Of the forty patients with microscopically confirmed P. falciparum malaria, thirty seven tested positive for PfHRP2 in the blood using rapid diagnostic test kits, and forty for PfHRP2 in saliva. All negative-control samples tested negative for salivary Pf HRP2. The ELISA greed with microscopy with 100 % sensitivity and 100 % specificity. Salivary levels of PfHRP2 ranged from 15 to 1,162 pg/mL in the malaria-positive group.

Conclusion: Saliva is a promising diagnostic fluid for malaria when protein degradation and matrix effects are mitigated. Systematic quantitation of other malaria biomarkers in saliva would identify those with the best clinical relevance and suitability for off-the-shelf diagnostic kits.

A-343

Evaluation of an Alpha 2-Macroglobulin assay for use on the Binding Site Next Generation Protein Analyser

S. L. Stone, A. Kaur, A. J. Alvi, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Alpha 2-Macroglobulin is a 725 kDa protein which, due its high molecular weight, is distributed almost exclusively in the intravascular pool. Increased levels of Alpha 2-Macroglobulin are associated with nephrotic syndrome, liver cirrhosis and diabetes mellitus. Measurement of Alpha 2-Macroglobulin can also aid in the diagnosis of blood-clotting or clot lysis disorders. Here we describe the evaluation of a serum Alpha 2-Macroglobulin assay for use with the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.1928 - 6.17 g/L at the standard 1/10 sample dilution. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days. Five antigen levels were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 3.2%, 1.0%, 1.2% and 2.8% for the 5.3g/L sample, 2.8%, 0.7%, 1.0% and 2.5% for the 4.1g/L sample, 3.1%, 1.1%, 1.1% and 2.7% for the 3.5g/L sample, 2.4%, 0.9%, 0.9% and 2.1% for the 2.3g/L sample and 3.5%, 2.1% 1.3% and 2.5% for the 0.34g/L sample. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the extended measuring range (0.184 - 6.363 g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=1.002x - 0.01903, R^2 = 0.9998). No significant interference was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into samples with known Alpha 2-Macroglobulin concentrations at the standard sample dilution. Correlation to the Binding Site Alpha 2-Macroglobulin assay for the SPA PLUS analyser was performed using normal and clinical samples (n=146, range 0.233-5.554g/L). Good agreement was demonstrated by Passing-Bablok regression; y=1.03x - 0.01g/L. We conclude that the Alpha 2-Macroglobulin assay for the Binding Site Next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-345

Evaluation of the performance characteristics of a new assay for Ferritin on Toshiba 2000FR

A. Shimakawa[1], M. Kano[2], R. Tachibana[2], Y. Ito[2]. *[1]JA Niigata Kouseiren Sado General Hospital, Niigata, Japan, [2]Denka Seiken Co.,Ltd., Tokyo, Japan*

Background:

Ferritin has been a well known marker over the past 20 years as its level in blood is abnormally increased with certain disorders. Furthermore, more recently high sensitivity is increasingly required to diagnose the iron deficiency anemia, for which a lower threshold is set at 12 µg/L or below. On the other hand, serum Ferritin levels may become very high in some disorders such as hemochromatosis, hemosiderosis, etc. Thus, assays for Ferritin levels in serum and plasma are required to have a wide assay range with good prozone (high dose hook effect) tolerance and with a high sensitivity for accurate and reliable measurements. We evaluated the FERNX, a new latex particle-enhanced turbidimetric immunoassay for serum and plasma Ferritin levels with ultra-sensitivity and good prozone tolerance, available from Denka Seiken Co., Ltd. We evaluated the FERNX comparing to the current reagent and also monitored the on-use stability at lower end.

Methods:

We carried out the study on a TBA-2000FR automated clinical chemistry analyzer (Toshiba). The performance data was compared to the current reagent available from Denka Seiken by testing lower detection limit, precision, linearity, prozone, interferences and correlation. The on-use stability was assessed by testing lower detection limit every week with the reagent which was set on the analyzer during the whole study.

Results: The FERNX showed the correlation coefficient 0.99 against the current reagent with 170 clinical samples. No interferences were observed with hemolysis (hemoglobin: 487 mg/dL), icterus (Bilirubin: 20 mg/dL) or lipemia (1410 FTU) with both reagents. CVs (SDs) of the new assay from within-run imprecision with 3 different samples (10 µg/L, 60 µg/L and 310 µg/L) were smaller than the current reagent. The new assay showed prozone tolerance better than the current reagent. Recoveries by the new assay were higher than the upper limit of the measuring range (1,000 µg/L) with samples containing actual Ferritin concentrations up to 50,000 µg/L, on the other hand recoveries by the current reagent were lower than the upper limit with the samples with actual Ferritin concentrations at 6,000 µg/L or higher. Lower detection limit was 3-fold smaller than the current reagent (2 µg/L and 6 µg/L, respectively). The lower detection limit remained at the sample level (2-3 µg/L) during the on-use stability study up to 5 weeks.

Conclusion: The FERNX showed better performance compared to the current reagent. The FERNX showed excellent prozone tolerance and also excellent sensitivity even in the on-use stability study. The FERNX, a new latex particle-enhanced turbidimetric immunoassay reagent for serum and plasma Ferritin, is useful for diagnosis of wide variety disorders including the iron deficiency anemia.

A-346

Development of a latex-enhanced immunoturbidimetric assay for the measurement of MMP-3 levels on automated clinical chemistry analyzers

A. Hirayama, C. Kaneko, M. Asami, K. Okuyama, S. Kitahara, T. Shimizu, Y. Nakamura, H. Takahashi, N. Fujita, H. Yago, K. Saito. *SEKISUI MEDICAL CO., LTD, Ryugasaki, Ibaraki Pref., Japan*

Matrix metalloproteinase-3 (MMP-3) is a proteolytic enzyme produced by synovial cells and which participates in joint destruction. The concentration of serum MMP-3 in normal subjects is reported to range from approximately 17.3 to 121 ng/mL, while patients with rheumatoid arthritis (RA) display significantly increased serum MMP-3 levels as the condition worsens. It has been demonstrated that measuring serum MMP-3 levels is a useful marker for evaluating inflammatory activity, prognostic outcome and therapeutic effect in RA.

We have developed a new method for measurement of serum and plasma MMP-3 levels for use in clinical chemistry analyzers. This method is based on latex-enhanced immunoturbidimetry, using anti-human MMP-3 mouse monoclonal antibodies. The concentration is determined by measuring the change in absorbance that results from agglutination of latex particles.

The reagents are supplied ready-to-use, and the assay can be completed within 10 min. Using a Roche/Hitachi 917 auto analyzer, 2.4 µL of human serum or plasma was mixed with 120 µL of the first buffer solution and incubated for 5 min at 37ºC. Subsequently, 40 µL of the second reagent, which contains the monoclonal antibody-coated latex particles, was added and the absorbance was monitored at 570 nm/800 nm (main/sub wavelengths) for 5 min.

The lower detection limit for MMP-3 was 10 ng/mL, and the upper quantitation limit was 1,600 ng/mL. No prozone effect was observed in MMP-3 samples of concentrations from 1,600 through 2,500 ng/mL. The within run C.V. (n=10) at 100 ng/mL, 200 ng/mL, and 400 ng/mL was 1.2 %, 0.6 %, and 0.9 %, respectively. The between run C.V. (n=10) at 100 ng/mL, 200 ng/mL, and 400 ng/mL was 1.7 %, 2.1 %, and 2.6 %, respectively. Interference studies showed no effect from bilirubin, hemoglobin, rheumatoid factor (RF), or chyle at concentrations of 20 mg/dL, 500 mg/dL, 500 IU/dL, and 2,000 formazin turbidity units, respectively.

Comparison of our assay kit with the approved IVD reagent, the principle of which is enzyme immunoassay, yielded a correlation coefficient of 0.980 and an equation of Y (present method) = 0.95X (the ELISA kit) + 10.53 (n = 115 serum samples). We concluded that this assay reagent provides an accurate, precise, and simple method for routine measurement of MMP-3 levels in serum and plasma samples.

A-347

Development of a Duplex Assay for the Simultaneous Detection of Anti-Thyroglobulin and Anti-Thyroid Peroxidase Autoantibodies Employing Biochip Array Technology.

T. McFadden, S. Deegan, C. Richardson, S. Brockbank, J. Lamont, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Autoantibodies (aAb) are established clinical markers of autoimmune disease; in particular, they aid in the diagnosis of autoimmune thyroid disease (AITD) and distinguish it from other forms of thyroiditis. AITD causes cellular damage and alters thyroid gland function by humoral and cell mediated mechanisms. A characteristic feature of AITD is the production of aAb to thyroglobulin (Tg) and thyroid peroxidase (TPO), key regulatory proteins in the synthesis of the hormone thyroxine. Elevated serum levels of Tg aAb and TPO aAb have been shown to be associated with chronic thyroiditis such as Hashimotos' thyroiditis (which results in hypothyroidism) and Grave's disease (which results in hyperthyroidism). TPO aAb are considered a more sensitive marker of thyroid autoimmunity; however, depending on the patient, TPO aAb may be low while Tg aAb are elevated, thus dual measurement of TPO aAb and Tg aAb will facilitate a more accurate diagnosis of thyroid autoimmunity. The aim of this study was to develop a duplex detection system that will allow for the simultaneous detection of Tg aAb and TPO aAb from a single serum sample using biochip array technology. This represents a useful analytical tool for applications in clinical settings. **Methods:** Human TPO and Tg proteins were immobilized to discrete testing regions (DTR) on a biochip surface using an indirect sandwich assay format. Chemiluminescent signal from each DTR was detected by digital imaging technology on the Evidence Investigator analyser. The multi-analyte calibrators for the standard curve were developed from serum samples containing high levels of TPO aAb with low levels of Tg aAb and vice versa. The calibrators were then standardised using NIBSC reference material 65/93 and 66/387 for Tg aAb and TPO aAb respectively. A correlation study was conducted with a cohort of 236 clinical serum samples, which were assessed for both Tg aAb and TPO aAb, using the biochip array technology and commercially available immunoassays. **Results:** Cross-reactivity and interference testing demonstrated that each individual assay was specific for its target analyte. Both assays demonstrated high sensitivity with detection levels of 0.08 IU and 0.002 IU for Tg aAb and TPO aAb respectively. Mean %recovery for the reference material was 107% with a %CV of 5.22 for Tg aAb and 103% with a %CV of 18.63 for TPO aAb. Correlations to the assigned values resulted in a correlation coefficient of 0.968 and a slope of 0.9372 for Tg aAb and a correlation coefficient of 0.918 and a slope of 0.8446 for TPO aAb. **Conclusion:**This study reports on the development of a clinical diagnostic product for the simultaneous measurement of TPO aAb and Tg aAb in the detection of AITD. Using biochip array technology, this duplex assay simultaneously measures levels of both Tg and TPO aAbs from a single sample, offering advantages over current diagnostic tools which use individual tests for the measurement of these aAbs. This newly developed assay uses low sample volume and will provide a highly sensitive and specific test for the detection of each analyte in a clinical setting.

A-349

Comparison of the AESKU HELIOS IFA system with another ANA Screening Method

K. Tokoro[1], M. Thunø[1], B. Larida[1], T. Matthias[2], B. Rabin[3]. *[1]AESKU.INC, Oakland, CA, [2]AESKU.KIPP Institute, Wendelsheim, Germany, [3]University of Pittsburgh Medical Center, Pittsburgh, PA*

Background: This study compares the AESKUSLIDES® HEp-2 cell line processed on the fully automated HELIOS IFA analyzer (AESKU.SYSTEMS) to the NOVA Lite® HEp-2 cell line (INOVA) processed on the PhD system (Bio-Rad Laboratories).

Methods: 82 de-identified serum samples were tested on the HELIOS automated analyzer at AESKU, Oakland and processed on the PhD system at University of Pittsburgh Medical Center (UPMC). Base dilutions were performed at 1:80 and titrated to an end-point dilution of 1:1280. Several discrepant positive samples were subsequently run in the BioPlex® 2200 system using the BioPlex 2200 ANA screen (Bio-Rad Laboratories). Two independent clinical laboratory scientists evaluated the results at both locations.

Results: Qualitatively, 80/82 (97.6%) HELIOS results were concordant with the PhD system. Titration results also correlated well: 19 samples were double negative, 10 were borderline (1:80 or negative), 41 within 1 titer, 7 within 2 titers, 4 within 3 titers, and 1 within 4 titers Borderline outcomes were treated as comparatively equal when paired results were 1:80 and negative.

5 out of 7 samples within two titers of the PhD/INOVA results had higher HELIOS end-point titrations (range 1:320-1:1280). 3 out of 4 samples with a distance of 3 titers had higher HELIOS end-point titrations. Clinical data and BioPlex results of these 3 samples indicated autoimmune hepatitis or Sjgrens syndrome and an SSA or Centromere result >8 (BioPlex cutoff <1.0). Two additional samples with no clinical history of connective tissue diseases (1:320/negative and 1:80/1:1280, PhD:HELIOS) had a BioPlex result of negative and SSA/SSB >8 respectively. The clinical and diagnostic accuracy of the AESKUSLIDE/HELIOS reagent system is favorable based on higher end-point titrations and confirmatory data.

Conclusion: Many laboratories allow a comparative titer discrepancy of 1 dilution as a diagnostic convention when determining precision. Therefore, the combination of AESKUSLIDES® and HELIOS analyzer has a higher sensitivity and specificity than the INOVA/PhD system. AESKU ANA IFA reagent systems are designed to be more clinically relevant to disease state individuals and are therefore more diagnostically significant.

A-350

Evaluation of an IgG1 assay for use on the Binding Site Next Generation Protein Analyser

E. L. Freeman, J. R. Kerr, L. Southan, A. Kaur, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. Reduced IgG1 levels are often indicative of general immunodeficiency. Here we describe the evaluation of an IgG1 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser

capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 1500-36,000mg/L at the standard 1/10 sample dilution, with sensitivity of 150mg/L. High samples were remeasured at a dilution of 1/40 with a measuring range of 6000-144,000mg/L. Precision studies (CLSI EP5-A2) were performed at nine levels in duplicate over 21 working days and were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 3.2%, 1.7%, 2.4% and 1.1% for the 522 mg/L sample, 5.5%, 2.6%, 2.5% and 4.2% for the 2871mg/L sample, 3.0%, 1.6%, 1.4% and 2.1% for the 3083mg/L sample, 3.3%, 1.8%, 1.8% and 2.1% for the 4869mg/L sample, 5.5%, 1.2%, 5.4% and 0% for the 7179mg/L, 4.1%, 1.5%, 3.6% and 1.4% for the 12,131mg/L sample, 3.4%, 1.2%, 3.0% and 1.1% for the 14,542mg/L sample, 5.4%, 2.0%, 3.6% and 3.5% for the 13,847mg/L sample and 4.7%, 3.1%, 2.5% and 2.5% for the 28,132mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (1500-36,000mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 0.97x + 101.70, R^2 = 1.00). No significant interference (within ±10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgG1 concentrations when run using the minimum sample dilution. Correlation to the Binding Site IgG1 assay for the SPA PLUS was performed using 142 samples (range 1064 - 16,822mg/L). Good agreement was observed between assays (mean 6273mg/L; range 1064 - 16,822mg/L v mean 6199mg/L; range 1055-15,177g/L), Passing-Bablok regression; y=1.05x - 228.61. We conclude that the IgG1 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-351

Evaluation of an IgG2 assay for use on the Binding Site Next Generation Protein Analyser

L. D. Southan, E. L. Freeman, A. Kaur, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Assays for measurement of IgG subclasses in serum are routinely used in many immunology laboratories in the diagnosis of IgG deficiencies. Abnormal levels of one or more subclass may be associated with conditions including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia. In particular, reduced production of IgG2 in children is associated with recurrent infection. Here we describe the evaluation of an IgG2 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 194-7000mg/L at the standard 1/10 sample dilution, with sensitivity of 2.96mg/L. High samples were remeasured at a dilution of 1/40 with a measuring range of 776-28,000mg/L. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days and were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 4.8%, 1.5%, 2.4% and 3.9% for the 80mg/L sample, 5.7%, 3.3%, 1.6% and 4.4% for the 390mg/L sample, 3.5%, 1.0%, 1.3% and 3.0% for the 1828mg/L sample, 2.6%, 1.0%, 1.4% and 2.0% for the 2982mg/L sample and 2.9%, 1.2%, 1.4% & 2.3% for the 5855mg/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (194-7000mg/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values (y= 0.9612x + 0.0298, R^2 = 0.9878). No significant interference (within ±10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgG2 concentrations when run at the minimum sample dilution. Correlation to the Binding Site IgG2 assay for the SPA PLUS was performed using 143 samples (range 386 - 8031mg/L). Good agreement was observed between assays when analysed by Passing-Bablok regression; y=0.99x - 0.02. We conclude that the IgG2 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-353

Examining the Frequency of Autoantibodies in the Brazilian Population in 2013 Using the Multiplex Technique

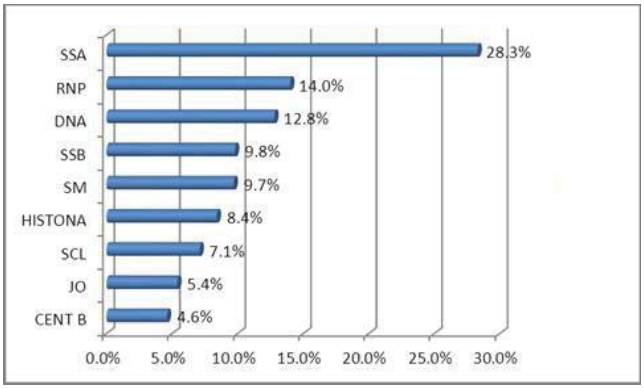
F. B. Flumian, F. O. Paiva, D. C. B. Carvalho, M. A. Rigone, T. N. Carvalho, O. P. Denardin, N. Gaburo. *DASA, Sao Paulo, Brazil*

Background: Autoantibodies are antibodies that react against the organism's own components. The appearance of a large number of autoantibodies is a pathological condition that can occur in a number of diseases, such as diabetes mellitus type 1, systemic lupus erythematosus, Sjögren's syndrome, Hashimoto's thyroiditis, Graves' disease and rheumatoid arthritis, among others.Objective: Describe the frequency of autoantibodies in Brazilian samples using the Luminex technology.

Methods: In the period from January to December 2013, 42,249 serum samples of patients from all over Brazil were analyzed by DASA's Manual Immunology laboratory. The samples were tested for the following autoantibodies: dsDNA, SSA, SSB, Sm, RNP, Scl-70, Jo-1, Centromere B and Histone, using the Athena Multi-Lyte ANA II Plus Test System (Zeus Scientific, Raritan, NJ) and the Luminex 200 IS equipment, version 2.3 (Luminex, Austin, TX). The results were interpreted using the Luminex 200 software, version 2.3, according to the reference value indicated by the kit's manufacturer.

Results: Of the samples tested for autoantibodies using the Luminex method, 27,884 (66%) presented a negative result and 12,674 (30%) presented a positive result, while 1,689 (4%) presented an inconclusive result. Among the samples that presented a positive result, the most prevalent autoantibody observed was SSA, present in 28.3 % of the samples analyzed. SSA is related to diseases such as Sjögren's syndrome, Systemic Lupus Erythematosus, Rheumatoid Arthritis, SS/SLE Overlap Syndrome, Subacute Cutaneous LE (SCLE), Neonatal Lupus and Primary Biliary Cirrhosis, among others. Graph 1 represents the autoantibodies detected and their respective frequencies in the population studied.

Conclusion: The Luminex xMAP technology enables the detection of multiple analytes in a single reaction well, reducing operating costs and physical space, and may be widely applicable to various processe Graph 1 - Frequency of the autoantibodies detected.



A-354

Evaluation of a Cystatin C assay for use on the Binding Site Next Generation Protein Analyser

H. Johnson, F. Murphy, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Cystatin C has been shown to be superior to creatinine as a marker of glomerular filtration rate and is increasingly used in the diagnosis of renal dysfunction. Here we describe the evaluation of a Cystatin C assay for the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to automatically dilute a single calibrator to create a 6-point calibration curve with a measuring range of 0.33-7.09mg/L at the standard 1/10 sample dilution, with sensitivity of 0.33mg/L. High samples are automatically re-measured at the 1/20 sample dilution with a measuring range of 0.67-14.18mg/L with a total assay time of 11 minutes. Within-run precision was assessed by running 5

samples at concentrations across the measuring range twenty times with a single kit lot, on one analyser. The coefficients of variation for these 5 levels were as follows; 2.10% for the sample at 0.57mg/L, 1.21% at 1.22mg/L, 1.13% at 2.52mg/L, 1.08% at 3.69mg/L and 2.36% at 6.14mg/L. Linearity was assessed using a high Cystatin C sample which was serially diluted across the width of the curve at the 1/10 sample dilution (range 0.24-8.4mg/L) and tested in triplicate at each dilution. Results were compared to expected values and demonstrated good linearity when results were analysed by linear regression; y=1.003x-0.0327, R^2=0.9998. No significant interference (within ±6%) was observed when serum pools of known Cystatin C concentrations were spiked with bilirubin (20mg/dL), haemoglobin (5g/L), Intralipid (2000mg/dL) or triglyceride (1000mg/dL) and tested at the standard sample dilution. Comparison was made to the Cystatin C assay for use on the Binding Site SPA PLUS analyser by comparing 115 normal and clinical serum samples (range 0.53-8.54mg/L). Good agreement was observed when the data was analysed by linear regression analysis; y=1.0175x - 0.2179, R^2=0.9917. We conclude that the Cystatin C assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-355

Evaluation of a liposome-based CH50 assay for use on the Binding Site Next Generation Protein Analyser.

J. R. Kerr, K. L. Sharp, A. Kaur, C. Rowe, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

The complement cascade is made up of around 20 serum proteins that form part of the innate immune system. A major function of complement is to lyse bacteria through formation of the membrane attack complex (MAC). The complex interactions of the complement cascade mean that functionality of the MAC cannot necessarily be inferred by apparently normal levels of any single component. Measurement of complement activity is therefore desirable, however existing methods involving antibody-sensitised erythrocytes are limited by the need to create appropriate serum dilutions and by the instability of the erythrocytes. These issues have been overcome through the use of liposomes encapsulating glucose-6-phosphate dehydrogenase (G6PDH) in place of erythrocytes. On addition of sample, antibodies in the reagent combine with dinitrophenyl on the liposomes. The resultant complex activates complement in the sample, which lyses the liposome, releasing G6PDH to react with glucose-6-phosphate and NAD in the reagent. The change in absorbance can be measured turbidimetrically and is proportional to the complement activity in the sample. Complement activity has been correlated with the active stage of systemic lupus erythematosus, rheumatoid arthritis, cryoblogulinemia-vasculitis, some forms of nephritis, and inherited deficiencies of the complement system. Here we describe evaluation of a CH50 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of. The analyser is programmed to construct a six point calibration curve from a single, lyophilized serum-based calibrator. The standard curves are validated by assay of control fluids. The assay range was 12.5 - 100 U/mL using a 1/2 sample dilution. The assay showed a high degree of linearity when serially diluted serum samples were assessed using a weighted linear regression analysis of measured values against expected values (y = 1.01x + 0.26 r^2= 0.998, range 9.987 - 102.230 U/mL). Precision was assessed at five levels across the measuring range. Coefficients of variation for within run and total precision respectively were 20 U/mL - 4.8% and 8.3%, 31 U/mL - 3.7% and 5.8%, 42 U/mL - 1.5% and 4.4%, 52 U/mL - 1.4% and 3.5%, and 80 U/mL - 2.1% and 4.4%. No significant interference (within ±10%) was observed upon addition of haemoglobin (500 mg/dL), bilirubin (20 mg/dL), ascorbic acid (50 mg/dL) or Intralipid (250 mg/dL) to samples with known CH50 values. Good agreement was observed with the Binding Site SPA PLUS CH50 assay when normal and deficient samples (n=28, range 23.091 - 66.143 U/mL) were compared and analysed by Passing-Bablock regression: y=0.96x - 0.24. We conclude that the CH50 assay for the Binding Site next generation protein analyser measures complement activity rapidly, precisely and accurately and shows good agreement with existing assays.

A-356

Evaluation of the urine utility of a multipurpose albumin assay for use on the Binding Site Next Generation Protein Analyser

H. Johnson, F. Murphy, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Albumin measurement in urine is routinely performed for the diagnosis of kidney disease in conjunction with other laboratory and clinical findings. Here we describe the evaluation of a multipurpose albumin assay for use on the Binding Site's next generation protein analyser with respect to the measurement of urine. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to automatically dilute a single calibrator to create a 6-point calibration curve with a measuring range of 11-332mg/L at the standard 1/1 sample dilution, with sensitivity of 11mg/L. High samples are automatically re-measured at the 1/50 sample dilution with a measuring range of 550-16600mg/L and a total assay time of 10.5 minutes. Precision studies (CLSI EP5-A2) were performed testing five urine concentrations in duplicate over 21 working days. Each antigen concentration was assessed for total, inter-lot and inter-instrument precision using three reagent lots on three analysers. The coefficients of variation were 3.9%, 0.97% and 1.24% for the 23.0mg/L sample, 3.2%, 0.26% and 0.50% for the 39.0mg/L sample, 2.1%, 0.87% and 0.31% for the 143.4mg/L sample, 3.2%, 1.21% and 1.23% for the 275.1mg/L sample and 2.6%, 0.94% and 0.84% for the 1490.2mg/L sample. Linearity was assessed using a pool of urine samples spiked with purified human albumin. The fluid was serially diluted and results were compared to expected values. The assay showed good linearity when observed results were compared to expected results and analysed by linear regression; y=0.9933x-0.7688, R^2=0.9995. No significant interference was observed (<±3%) when urine samples of known albumin concentration were spiked with bilirubin (20mg/dL), haemoglobin (25mg/dL), ascorbic acid (20 mg/dL) or total protein (100mg/dL). Comparison was made to the albumin urine assay for use on the Siemens BN™II analyser by comparing clinical urine samples (n=71, range 12.8-1334.8mg/L). Good agreement was observed when the data was analysed by linear regression; y=1.048x + 7.3231, R^2=0.9959. We conclude that the urine albumin assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-357

Patient sensitization profile by ImmunoCAP Solid Phase Allergen Chip (ISAC) in a large Brazilian laboratory.

E. X. Gomes, A. Almeida, F. Prieto, D. Akiyama, E. Taira, N. Maluf, C. Pereira. *Dasa, Barueri, Brazil*

Background: Most atopic patients have positive test results to numerous allergens and the true cause of symptoms can be difficult to identify due to an inconclusive medical history regarding the role of different allergens and reactions. ImmunoCAP ISAC is a microarray technology developed by allergy specialists to allergy specialists for investigation of multisensitized patients. It can also reveal unexpected sensitizations, cross reactive components and risk assessment, providing physicians with relevant information to handle allergen avoidance and individualized patient management.
Objective: The aim of this study was to evaluate the allergy sensitization to some of the important molecular allergens and evaluate the incidence of Latex and Insect Venom. Samples were collected at SP and RJ DASA laboratories from 05 Jun 2012 to 30 Jan 2014. All 66 patients were tested with a panel of 112 different allergens components summing up 7,392 results with ImmunoCAP ISAC (Phadia AB, Thermo Fisher).
Methods: ImmunoCAP ISAC is a semi-quantitative test and results are reported in ISAC Standardized Units (ISU) giving indications of specific IgE antibody levels. The allergen components are spotted in triplets and covalently immobilized to a polymer coated slide. IgE antibodies from the patient sample bind to the immobilized allergen components. The complex is detected by a fluorescence labeled anti-IgE antibody which is measured with a laser scanner. **Results:**
Results for components were evaluated using Phadia Microarray Image Analysis (MIA) software. 27,3% of the reports were found negative (66,7% women/ 33,3% men) and 72,7% of the reports were found positive (56,3% women / 43,7% men). Shrimp Tropomyosin (nPen m1) and Grass Pollen (nCyn d 1) had almost similar positive frequency (16%) and Ovomucoid (nGal d 1) was 50% less compared to

the first two ones (8,33%). Taking patient as calculation basis, the higher incidence was found for insect venom components (15.58%), followed by Latex (8,33%) and Peanuts (2,03%)

Molecular Allergens: % positive

Shrimp Tropomyosin nPen m1 16

Ovomucoid nGal d 1 8,33

Grass Pollen nCyn d 1 16,67

Patients: % positive

Latex 8,33

Insect Venom 15,58

Peanuts 2,03

Conclusion: A high positivity of Tropomyosin allergens, which might indicate cross reactivity between mites, cockroach and shrimp is important to handle shrimp allergy, since these patients might also react to mites. Patients with sensitization to peanuts components rAra h 1 rAra h2 are among the group with high risk to severe allergic reaction to peanut. Ovomucoid is an allergen which indicates that egg symptoms might persists after childhood as well as indicates severity. Positivity to pollen allergens indicates that pollen allergy might be underestimated in our population. The results to Latex and Insect venom show the importance of this toll, since other sensitizations may be found in parallel providing more patient information.

A-358

Autoantibodies directed against moesin N_{1-297} /$C_{471-577}$ are specific serum biomarkers for immune thrombocytopenic purpura (ITP)

Y. Zhang[1], H. Mao[2], A. Zhao[3], F. Du[2], J. Qian[1], J. Yang[1], J. Wei[3], X. Liu[2], Z. Sha[2], Y. Han[1], W. Situ[1], C. Li[1], L. Sheng[1], Y. Xu[2], Z. Shou[2], S. Vardanyan[4], B. Bonavida[5], J. R. Berenson[4], H. Chen[4]. *[1]Shanghai Kexin Biotec Co., Ltd, Shanghai, China, [2]The First People's Hospital of Guiyang, Guiyang, China, [3]Department of Obstetrics and Gynecology , Ren Ji Hospital Affiliated to Shanghai JiaoTong University School of Medicine, Shanghai, China, [4]Institute for myeloma and bone cancer research, West Hollywood, CA, [5]Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA*

Moesin is a member of the ezrin/radixin/moesin (ERM) protein family, and is localized in the cytoplasmic side/end of membrane in filopodia and other micro-extensions on the cell surface. Moesin consists of three domains: N-terminal (N) membrane-binding domain, α-helical (α), and positive charge C-terminal domain. In comparison to other cell types, human platelets demonstrate high expression of moesin but not ezrin or radixin. Phosphorylation of threonine558 in carboxy-terminal actin-binding domain of moesin is associated with the activation of platelets. We have cloned three polypeptides of human moesin: N_{1-297} terminal, $\alpha_{298-470}$ helix domain and $C_{471-577}$ terminal as well as investigated autoantibodies against moesin in patients with ITP. Following informed consent, serum samples from patients with ITP (n=77), patients with non-immune thrombocytopenia or other hematologic diseases (n=47), and gender-matched healthy control subjects (n=50) were evaluated. The titers of moesin autoantibodies were significantly elevated in the sera from patients with ITP compared with healthy subjects (Mean of autoantibodies titers = N_{1-297} 0.515 vs 0.155; P =0.0001). The levels of moesin autoantibody against $C_{471-577}$ in ITP patients were also markedly higher than healthy subjects (Mean of autoantibody titer = $C_{471-577}$ 0.430 vs 0.103, $P < 0.0001$). In contrast, the titer of autoantibody against moesin $\alpha_{298-470}$ helix domains was similar in ITP and healthy subjects. When an autoantibody cut-off value of ±2D in normal control subjects (n=50) was assigned, the serum levels of moesin autoantibodies in ITP patients were found to be elevated in 91% (70/77) for N_{1-297}, 72% (56/77) for $C_{471-577}$ but only 1.3% (1/77) for $\alpha_{298-470}$. Patients with other hematologic diseases, including non-immune thrombocytopenia, anaphylactic purpura, multiple myeloma, pure red cell aplasia, and myelodysplastic syndrome were all negative for moesin autoantibodies. Regardless of the cause of ITP, the autoantibodies against moesin N_{1-297} or $C_{471-577}$ were significantly high than among patients with non-immune-related thrombocytopenia and healthy subjects**.** We used Western blot analysis to confirm the presence of moesin autoantibodies in ITP patients. The results showed that only ITP patients' serum specifically recognized the moesin N_{1-297} and $C_{471-577}$ terminal domains as well as commercial moesin antibodies. In contrast, the antoantibodies from ITP patients' serum did not recognize $\alpha_{298-470}$ helix domains. We also confirmed using Western blot analysis that serum from patients with thrombocytopenia with other hematologic diseases and healthy subjects did not show the presence of autoantibodies against moesin N_{1-297} or $C_{471-577}$ terminal. To further confirm that the autoantibodies against moesin N_{1-297} or $C_{471-577}$ terminal domains were present in ITP patients, antigen competitive inhibition assay was accessed. This N_{1-297} terminal polypeptide (0.5ug/ml or 2.5ug/ml) blocked the detection of autoantibodies against moesin N_{1-297} in the standards whereas an isotype-matched ITP serum did not have any impact on the detection of this autoantibody. Similarly, $C_{471-577}$ terminal polypeptide in the presence of both low (0.5ug/ml) and high (2.5ug/ml) concentrations of the blocking autoantibody against moesin $C_{471-577}$ terminal portion. We propose that autoantibodies against moesin N_{1-297} and $C_{471-577}$ may be specific serum biomarkers for clinical diagnosis and differentiation of ITP from non-immune thrombocytopenia and other hematologic diseases.

A-359

Analytical evaluation of third-generation allergen-specific IgE assay "3gAllergy™" Measurement by Automated Immunoassay System " IMMULITE 2000 XPi "

E. Hamada, M. Maekawa. *Hamamatsu University School of Medicine, Hamamatsu, Japan*

Introduction: With the changes in the living environment including diet and air pollution, allergic diseases have been expanded and diversified. Accordingly, laboratory testing for any kinds of allergy has become important and important. Recently, a new generation of allergy blood testing, 3gAllergy, coupling with IMMULITE has been launched. The full automated random-access multiparameter luminescence immunoassay system, IMMULITE 2000 XPi is based on a solid phase two-site chemiluminescent enzyme immunoassay (CLEIA) and provides quantitative determination of allergen-specific IgE. Here we evaluated analytical performance of the IMMULITE 2000 XPi measurement system of 3gAllergy.

Specimens: We used serum and plasma samples collected from our inpatients/ outpatients and the employees who volunteered, as well as control samples commercially available. This study has been approved by the ethical committee in Hamamatsu University School of Medicine.

Methods: In this study, we evaluated the basic performance and compared IMMULITE 2000 XPi Immunoassay System and its dedicated reagents (Siemens Healthcare Diagnostics, USA) with the ImmunoCAP (Phadia AB, Sweden).

Results: 1. Dermatophagoides pteronyssinus (D1), House dust mites (H1) and Japanese cedar (T17) are the major allergen in Japan. The 3gAllergy reagents showed the following performance.

1) Within-run precision The CVs(%) for control samples measured 20 times with 7 cconcentrations in sequence were 1.7 to 5.98% (D1), 2.7 to 8.7% (H1) and 4.3 to 6.1% (T17).

2) Linearity Linearity observed in high concentration range and in low concentration range indicated favorable results, respectively. However, a downward trend were observed in the range of more than 250 IUA/mL.

3) Minimal detection limit By use of sequential dilution of the specimen with low concentration, the detection limit were shown to be 0.02 IUA/mL.

2. Fourteen tests for Allergen (mite, cedar, alder, cypress, orchard grass, ragweed, mugwort, dog, cat, egg white, ovomucoid, egg yolk, cow's milk and peanuts)

1) Correlation We evaluated correlation with immuno-CAP for the 14 allergens. The class concordance rates and measurement correlation of 14 tests were comfortable. However, slightly higher tendencies in 3gAllergy are observed in the egg white, ovomucoid and egg yolk.

Conclusion: The basic performance of IMMULITE 2000 XPi of "3gAllergy™" and correlation with immunoCAP was satisfactory. The slight higher tendencies in 3gAllergy for egg white, ovomucoid and egg yolk were considered due to the higher sensitivity, and then patients with low titer of allergen-specific IgE could be discovered and followed. 3gAllergy is a third-generation allergen-specific IgE assay, delivering fast and accurate results to help enhance the quality of care and service provided to the patient. In addition, the device has some excellent properties, for example, offering excellent precision, accuracy and lot-to-lot reproducibility, a wide range of test menu, easy handling and maintenance, reducing workloads of manual sample sorting with the innovative sample rack system and time for reporting. The properties could lead to improved patient care.

A-360

Droplet Digital PCR (ddPCR) for Quantitative Analysis of Treg-specific Demethylation Region (TSDR) in peripheral blood compared to Flow Cytometry (FACS)

J. Schmitz[1], S. Götze[1], R. Andag[1], J. Brockmöller[2], O. Kollmar[3], E. Schütz[4], M. Oellerich[1]. *[1]University Medical Center Göttingen, Department of Clinical Chemistry, Göttingen, Germany, [2]University Medical Center Göttingen, Department of Clinical Pharmacology, Göttingen, Germany, [3]University Medical Center Göttingen, Department of General, Visceral and Pediatric Surgery, Göttingen, Germany, [4]Chronix Biomedical, Göttingen, Germany*

Background: Regulatory T cells (Tregs) are crucial for immune homeostasis and regulate several immune responses, especially tolerance induction. In transplanted patients they may be useful for monitoring immune status, whereas the value for tolerance prediction of peripheral Treg determination is still debated. Several methods for Treg detection are in use, either based on analysis of FoxP3 protein expression by FACS analysis or the determination of a specific de-methylated region in the FOXP3 gene (TSDR).

Methods: The TSDR of the FoxP3 gene de-methylation was determined with the QX100™ Droplet Digital™ PCR System (Biorad) using methylation specific assays. DNA was extracted after CD4+ T-cell isolation with Dynabeads® CD4, followed by bisulfite conversion. We analyzed Treg numbers by FACS compared to the TSDR ddPCR in 60 blood samples (31 from male, 29 from female) from 38 patients during the first 13 months after liver transplantation (LTx). Peripheral blood mononuclear cells (PBMC) were isolated with Lymphoprep™ (Axis-Shields) and frozen at -80°C. After thawing the cells were stabilized in RPMI 1640/FCS/Penicillin overnight at 37°C, and subsequently stained. Treg were identified as CD4+CD25+CD127lowFoxP3+/CD4+ cells in a BD FACSCANTO™ II (BD Biosciences). For the CD25 staining an antibody (clone M-A251) recognizing a different epitope than the therapeutically used Basiliximab was chosen to eliminate interference.

Results: Compared to an earlier conventional qPCR method (LightCycler480), the precision was substantially improved with the TSDR ddPCR (6.3% CV) between runs at a level of 1.3% de-methylated TSDR compared to 30% with qPCR using the same control sample. FACS analyses were based on a minimum count of 30,000 CD4+ cells and showed an imprecision of 6.1% (CV) at 2.6% Tregs. In LTx patients TSDR ddPCR showed higher values (5.2%±2.6) compared to the FACS assay (1.4%±1.0). Noteworthy, if FoxP3+/CD4+ cells were considered, resulting values were still lower (1.9%±1.5) compared to TSDR. No significant correlation was found between Treg percentages determined by both methods (r=0.218, p=0.095).

Discussion: The de-methylated FoxP3-TSDR is highly specific for natural Treg cells. It has already been proven that human non-regulatory T cells conserve their methylation status after being activated (imprinting). Hence TSDR ddPCR provides a more robust measure than the analysis of protein expression by the use of antibodies in FACS. The precision of ddPCR is comparable to FACS analyses. Our new ddPCR assay is able to measure all TSDR T-cells, even those, which are undetectable by FACS assay such as "latent" Tregs, which have lost their phenotypic FoxP3 expression. Another reason for the deviation and the lack of agreement might be due to the complexity of the flow cytometric analyses in particular if intracellular staining is needed. In addition in critically ill patients high background signals are common, but hard to compensate for. This new ddPCR assay allows for specific Treg quantification with adequate precision, good reproducibility, fast turn around, low costs and only requires 20% of blood compared to FACS. Further studies will show if the exact ratio of Treg/CD4 cells measured by ddPCR is useful as a biomarker for tolerance assessment in transplanted patients.

A-361

The Association of Serum Free Light Chain Levels with Markers of Renal Function

H. Akbas, B. Karatoy Erdem, F. Davran, V. T. Yilmaz. *Akdeniz University, Faculty of Medicine, Antalya, Turkey*

BACKGROUND: The kidney is often affected in plasma cell dyscrasias, usually due to the effects of nephrotoxic monoclonal free light chains. Renal failure due to a monoclonal gammopathy may be detected by the highly sensitive serum free light chain (sFLC) ratio yet missed by electrophoretic assays. The aim of this study was to assess sFLC levels in relation to markers of renal function.

METHODS: 513 patients were included in this study. sFLC levels were measured by Freelite® (The Binding Site Group Ltd, Birmingham, UK) assay using the BNII nephelometer (Siemens Diagnostics, Germany). κ/λ sFLC ratio was calculated. Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. GFR was estimated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation. Patients were assigned to 2 groups depending on their eGFR values: ≤ 60 mL/min/1.73m2 (group 1, n=103) and >60 mL/min/1.73m2 (group 2, n=410). Data were expressed as median and min.-max. All statistical analyses were done with SPSS version 20.0 and a significance level of 0.05 was considered.

RESULTS: Serum kappa FLC median value was 36.4 (5.62-16000) mg/L, serum lambda FLC was 21.7 (4.91-8770) mg/L, κ/λ sFLC ratio was 1.33 (0.01-3258), serum creatinin was 1.56 (0.63-7.21) mg/dL in group 1. Both of Lambda sFLC and κ/λ sFLC ratio were correlated with eGFR (r=-0.318, r=0.198, p<0.05, respectively). We did not find any significant correlation between κ/λ sFLC ratio and eGFR in group 2.

CONCLUSIONS: We examined the association between polyclonal sFLC concentrations and renal function. Our preliminary findings suggest that serum Lambda FLC might be considered useful marker of predicting renal function. Prospective studies are needed to clarify the usefulness of these parameters for identifying renal failure due to a monoclonal gammopathy.

A-362

Evaluation of a Caeruloplasmin assay for use on the Binding Site Next Generation Protein Analyser

J. K. Robinson, F. Murphy, A. Kaur, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

Caeruloplasmin is synthesised in the liver and has a major role in copper metabolism, carrying approximately 95% of the total copper in serum. Decreased levels of Caeruloplasmin can be caused by hereditary disorders of copper metabolism, for example; inability to transport oxidised copper (Cu2+) from the gastrointestinal epithelium into the circulation (as in Menkes disease), or the inability to insert Cu2+ into the developing Caeruloplasmin molecule (as in Wilson's disease). Dietary copper insufficiency, including malabsorption, also reduces serum Caeruloplasmin concentrations. Here we describe the evaluation of a serum Caeruloplasmin assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is enhanced by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.03-0.82g/L at the standard 1/10 sample dilution, with sensitivity of 0.03g/L. High samples are automatically re-measured at a dilution of 1/20, with an upper measuring range of 0.06-1.64g/L. Precision studies (CLSI EP5-A2) were performed at five levels in duplicate over 21 working days. Five antigen levels were assessed in duplicate, twice daily for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 9.4%, 1.5%, 5.5%, 7.4% and 8.8% for the 0.061g/L level, 7.6%, 1.6%, 3.4%, 6.6% and 6.2% for the 0.156g/L level, 5.6%, 1.7%, 2.3%, 4.8% and 2.9% for the 0.247g/L level, 5.7%, 1.7%, 2.1%, 5.0 and 3.7% for the 0.442g/L level and 6.4%, 2.0%, 1.8%, 5.9% and 3.7% for the 0.867g/L level respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (0.03 - 0.82g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values ($y=0.96x + 0.00$, $R^2 = 0.999$). No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1250 formazine turbidity units) when spiked into a sample with known Caeruloplasmin concentrations and measured using the minimum sample dilution. The assay was compared to the Caeruloplasmin assay for the Binding Site SPA PLUS analyser by running clinical samples (n=36). Good agreement was demonstrated by Passing-Bablok regression; $y = 1.00x + 0.01$. We conclude that the Caeruloplasmin assay for the Binding Site next next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-363

Identification of an IgD biclonal gammopathy

K. Lockington, L. L. Eckelkamp, J. A. Katzmann. *Mayo Clinic, Rochester, MN*

Background: Monoclonal abnormalities are confirmed by immunofixation electrophoresis (IFE) which identifies the immunoglobulin heavy chain and/

or light chain type. Routine IFE includes antisera to gamma, alpha and mu heavy chain and kappa and lambda light chain. When a free light chain is detected without a corresponding heavy chain, IFE is performed with antisera to delta and epsilon heavy chains to rule out a monoclonal IgD or IgE. We do not routinely reflex to anti-delta and epsilon IFE when we detect a free light chain in the presence of an intact immunoglobulin with the same light chain type. We recently initially reported a "monoclonal IgG lambda plus monoclonal free lambda" that eventually was confirmed as a biclonal IgG lambda and IgD lambda. Because the presence of a monoclonal IgD protein is often associated with either multiple myeloma or primary amyloid, this IgD/IgG biclonal gammopathy triggered an evaluation of our anti-delta and epsilon reflex process for free light chains.

Methods: Protein electrophoresis is performed on Helena agarose gels and IFE is performed with Sebia reagent kits. IFE reflex testing uses BioWhittaker antiserum for IgD and Binding Site antiserum for IgE.

Results: During a 1 month period we detected 1245 patients with a serum monoclonal protein. Twenty-eight of the samples (2.2%) were also tested with IgD and IgE antisera. Of these 28 patients, 19 had a monoclonal free light chain and the remaining 9 were eventually reported as having a biclonal gammopathy of 2 intact immunoglobulins with differing light chain type. In addition, there were 26 patients with an intact monoclonal immunoglobulin plus a monoclonal free light chain of the same type as the intact immunoglobulin. As per the laboratory protocol, these samples were not reflexed to the expanded IFE.

Conclusion: Our protocol to reflex monoclonal free light chains to anti-delta and epsilon IFE does not include free light chains that are associated with an intact monoclonal immunoglobulin with the same light chain. This protocol resulted in 2.2% of positive IFE tests being reflexed. If all new monoclonal free light chains are reflexed (regardless of the presence of an intact immunoglobulin with the same light chain), our reflexed IFE testing would approximately double.

A-364

Clinical Evaluation of the New BioPlex Celiac IgA and IgG Kits

M. Snyder[1], J. A. Murray[1], I. Absah[1], S. Paul[2], J. Sama[2], X. Guo[2]. *[1]Mayo Clinic, Rochester, MN, [2]Bio-Rad Laboratories, Hercules, CA*

Background: Serologic testing, specifically for tissue transglutaminase (TTG) and deamidated gliadin peptide (DGP) antibodies, is increasingly being relied upon to establish a diagnosis in patients with suspected celiac disease (CD). Therefore, diagnostic accuracy of CD-specific autoantibodies is critically important. Although monitoring patients with CD who have instituted a gluten-free diet (GFD) is routine practice, how responses compare between various serologic markers is not always clear. This study compares performance of bead-based multiplex immunoassays (MIAs) for TTG and DGP antibodies to that of currently available individual enzyme immunoassays (EIAs) for diagnosis and monitoring.

Methods: Retrospective samples were obtained from healthy controls (n=210), inflammatory disease controls (n=101), and patients with CD and related diseases (total n=105), including adult patients with biopsy-proven CD (n=96; 7 with IgA deficiency), pediatric patients with CD (n=6), and patients with dermatitis herpetiformis (DH) (n=3). An additional retrospective CD cohort was included (n=10), consisting of paired samples pre- and post-GFD (time on GFD 1.6 to 52.6 months). MIA testing was performed on BioPlex® 2200 Celiac IgA and IgG kits (Bio-Rad); EIA testing was performed on QuantaLite R h-tTG and Gliadin IgG II IgA and IgG kits (INOVA Diagnostics). All testing was performed according to manufacturers' instructions.

Results: The specificity of TTG-IgA, TTG-IgG, DGP-IgA, and DGP-IgG by MIA in healthy donors ranged between 96.2% and 99.5%. These were not significantly different from the EIA specificities (97.6% to 99.5%). For the disease control cohort, TTG-IgA and DGP-IgA showed similar specificities between MIA (97% and 100%) and EIAs (100% for both). However, the EIAs had lower specificities for TTG-IgG and DGP-IgG at 92% and 96%, compared to 100% and 99% for the MIA. In the CD/DH cohort, excluding IgA deficient patients, MIAs and EIAs demonstrated identical sensitivities at 92% for TTG-IgA and 86% for DGP-IgA. For TTG-IgG and DGP-IgG, the total CD/DH cohort was analyzed, including IgA deficient patients. The sensitivity of DGP-IgG was 83% and 79% for MIA and EIA; for TTG-IgG, overall sensitivities were 41% and 48% for MIA and EIA. If only IgA deficient patients were analyzed, a sensitivity of 57% was obtained for DGP-IgG by both methods. However, TTG-IgG by MIA had a higher sensitivity than EIA (71% vs 29%). Among patients on a GFD, for individuals positive for DGP-IgA at baseline, 25% remained positive by both MIA and EIA. For those patients positive for TTG-IgA, 11% and 14% remained positive on MIA and EIA, respectively. In contrast, 62.5% and 71.4% of patients positive for DGP-IgG remained positive by MIA and EIA.

Conclusion: The diagnostic accuracy of autoantibody serology for diagnosis of CD appears comparable between MIA and EIA methods. The only significant differences identified between the methods were for TTG-IgG and DGP-IgG. These results also confirm that TTG-IgA displays the best combination of sensitivity and specificity for CD, as demonstrated by previous studies. Further, the IgA isotypes appear to have the most utility for monitoring, based on conversion to a negative serology following implementation of a GFD.

A-365

Diagnostic utility of autoantibodies and HLA-DRB1 Shared Epitope in patients with recent onset Rheumatoid Arthritis

J. L. García de Veas Silva, B. Hernández Cruz, C. González Rodriguez, V. Sánchez Margalet, F. Navarro Sarabia. *Hospital Universitario Virgen Macarena, Sevilla, Spain*

Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis. Anti-cyclic citrullinated peptide (anti-CCP) antibodies have diagnostic value in RA but the role of shared epitope (SE) is unclear. We assessed the diagnostic value of anti-CCP antibodies and SE in patients with symptoms of arthritis in their first visit to the rheumatologist.

Methods: We measured anti-CCP antibodies with QUANTA LiteTM enzyme-linked inmmunosorbent assay (ELISA) kit for the detection of IgG anti-CCP3 (Cyclic Citrullinated Peptide 3) antibodies in patient sera (cut-off value, 40 UI/ml). SE was determined with GenID® Reverse Hybridization kit for detection of SE in HLA-DRB1 alleles kit in patient plasma. They were tested for 211 patients with suspected rheumatoid arthritis. The American College of Rheumatology (ACR) criteria for RA were fulfilled for 106 patients. These patients were diagnosed of RA. The other 105 patients were diagnosed with other rheumatic disease. We also determined rheumatoid factor (RF) with BAYER® FR IgM immunoturbidimetric assay for ADVIA 2400 (cut-off value, 20 UI/ml). We determined the diagnostic value (sensibility, specificity and likelihood ratios) for anti-CCP antibodies, SE and RF. We determined the area under the curve (AUC) for anti-CCP antibodies and RF. Statistical analyses were performed using IBM SPSS Statistics version 19 for Windows (New York, USA).

Results: Sensitivity of anti-CCP antibodies, SE and RF for RA were 66,0%, 72,6% and 81,1%, and specificity were 96,2%, 38,1% and 76,2%, respectively. The AUC for anti-CCP antibodies was 0.875 with 95% CI of 0.828 to 0.922 and the AUC for RF was 0.864 with 95% CI of 0.815 to 0.913. The positive likelihood ratio for anti-CCP antibodies, SE and RF were 17.37, 1.06 and 3.41 respectively. The negative likelihood ratio for anti-CCP antibodies, SE and RF were 0.35, 0.72 and 0.25. Anti-CCP antibodies were positive in 30.0% of RF-negative RA patients. Anti-CCP antibodies and RF were positive in 60.4% of total RA patients and were negative in 13.2% of total RA patients.

Conclusion: the anti-CCP antibodies have a very good diagnostic value in their first visit to the rheumatologist. However, the SE doesn´t have diagnostic value in our population.

A-366

4-Phenyl butyric acid attenuate apoptosis via inhibition of endoplasmic reticulum stress against diabetic cardiomyopathy

J. Xu[1], M. Cui[2], S. Shi[1], Q. Zhou[1]. *[1]First Hospital of Jilin University, Changchun, China, [2]Clinical Medical College of Jilin University, Changchun, China*

Background: Diabetic cardiomyopathy (DCM) is a major cause of death of patients with diabetes. It is known that apoptosis has been considered to play a critical role in DCM. Our recent studies have demonstrated the important role of endoplasmic reticulum stress (ER stress) in diabetes-induced cardiac cell death. The aim of this study was to investigate cardiac protection by 4-Phenyl butyric acid (PBA), a low molecular weight compound that acts as a chemical chaperone to enhance protein folding and ameliorate ER stress, in the development of DCM.

Methods: At 2 weeks, and 2 and 5 months after diabetes onset with Type 1 diabetic mouse model induced with multiple low-dose streptozotocin, cardiac remodeling and dysfunction were determined using echocardiography and hemodynamic evaluation and cardiac fibrosis was detected by Picric acid-Sirius red staining; ER stress signal pathway and apoptosis were detected by western blotting assay and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Mechanism of cardiac protection by PBA was used by cell culture with embryonic rat heart derived cells (H9c2).

Results: Apoptotic cells and CHOP, the activated form of caspase-3 and caspase-12 delineated that diabetes mainly induced cardiac cell death at the early stage of diabetes (2 weeks), but not in the late stages (2 and 5 months). However, there was no apoptotic cell death in the hearts of diabetic mice treated with PBA. In parallel with apoptotic effect, significant up-regulation of the ER chaperones, including phosphorylated eIF2α (p-eIF2α), GRP78, GRP94 and cleaved ATF6 proteins were significantly increased in the heart of diabetic mice at 2 weeks after diabetes onset. However, none of these increased ER chaperones in the hearts of diabetic mice were observed in the heart of diabetic mice treated with PBA. Pre-exposure of H9c2 cells to PBA significantly prevented high glucose or tunicamycin-induced ER stress and apoptosis, while same pretreatments did not have any effect on normal H9c2 cells.

Conclusion: These results suggest that ER stress exists in the diabetic heart, which may cause the cardiac cell death. PBA can attenuate diabetes-induced cardiac cell death via suppression of cardiac ER stress and associated apoptotic effects. This study could provide important theoretical support for prevention and treatment of DCM. The study also might open up a new way for the drugs in research and development for diabetic cardiovascular complications.

Acknowledgments: These studies were supported in part by Grants from National Science Foundation of China (no. 81000310), Jilin Science and Technology Development Program (no. 20100124), Norman Bethune Program of Jilin University (no. 2012223).

A-367

Vitamin D Receptor Polymorphisms and HLA-Class II Genotypes Among Lebanese with Multiple Sclerosis - A Pilot Study

R. T. Daher, R. A. Mahfouz, N. M. Karaky, F. A. Jaber, B. I. Yamout. *American University of Beirut, Beirut, Lebanon*

Background: Multiple sclerosis (MS) is an autoimmune disease with multifactorial etiology. Previous studies showed that HLA-DRB1*15 allele is a major genetic risk factor for MS in other populations possibly through regulation of vitamin D receptor (VDR) complex. In this study, we investigated the HLA class II genotypes and VDR gene polymorphism among a group of Lebanese MS patients and controls.

Methods: Fifty MS patients (remitting/relapsing, aged: 19-74 years, male:female=1:2.1) were selected for this study, based on the Expanded Disability Status Scale. The controls included: 49 healthy subjects (aged: 15-59 years, male:female=1:2) and 51 neurologic patients other than MS (Non-MS, aged 13-70 years, male:female=1:1.12). After a thorough history, blood in EDTA tube was collected. Extracted genomic DNA was used for molecular analysis of VDR genotypes (*ApaI*, *TaqI* and *BsmI*) and HLA class II typing (low resolution HLA-DRB1/3/4/5) (Luminex, San Diego, CA). Differences between groups were evaluated using Mann Whitney-U test. Chi-square test was used for association between various categorical variables. (P<0.05 statistically significant*)

Results: All determined variables were not statistically different between healthy and non-MS patients (p>0.05); therefore both were combined into one control group for analysis. Results are summarized below.

Groups	**Age**(years) (Mean±SD)	**HLA-DRB1*15**(%)	**VDR Gene**								
			ApaI			*TaqI*			*BmsI*		
			AA(%)	Aa(%)	aa(%)	TT(%)	Tt(%)	tt(%)	BB(%)	Bb(%)	bb(%)
MS (n=50)	42.8±13.5	11(22)	15(30)	26(52)	9(18)	20(40)	24(48)	6(12)	7(14)	22(44)	21(42)
Control (n=99)	33.7±12.7	8(8)	37(37)	48(49)	14(14)	31(31)	47(48)	21(21)	20(20)	51(52)	28(28)
P value	<0.001*	0.017*	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Frequency of HLA-DRB1*15 was significantly higher in MS patients compared to controls. None of the VDR genes differed between the two groups. Odds ratio (OR) for MS in the presence of DRB1*15 allele is 3.21 (p= 0.016; 95%CI= 1.20-8.59). Cosegregation of HLA-DRB1*15 and VDR genotypes indicated a slightly increased risk for MS in the presence of A-allele (OR=3.40; p= 0.022; 95%CI= 1.14-10.19). Similarly, combination of DRB1*15 with b-allele resulted in even higher OR of 4.22 although not statistically significant (p= 0.08; 95%CI= 0.75-23.89).

Conclusion: Our results confirm that HLA-DRB1*15 is a strong predisposing factor for MS in Lebanese patients. Furthermore, the interaction between specific VDR alleles and HLA polymorphism may synergistically influence the susceptibility to MS.

A-368

Frequency of antinuclear antibodies (ANA) by indirect immunofluorescence in Brazilian samples

F. B. Flumian, F. O. Paiva, D. C. B. Carvalho, M. A. Rigone, T. N. Carvalho, L. R. Galhardo, C. R. Costa, L. B. Campanholi, E. A. Garcia, O. Fernandes, N. Gaburo Jr.. *DASA, Sao Paulo, Brazil*

Background: Antinuclear antibodies (ANAs) are considered a hallmark of autoimmune rheumatic diseases (ARDs), and the indirect immunofluorescence assay (IFA) on HEp-2 cells is the standard method for ANA detection. During the past decade, as the demand for ANA testing increased, new automated methods have arisen for screening/detection of ANAs.Objective: To describe the frequency of positive ANAs and the most common patterns found in Brazilian samples, following the IV Brazilian Consensus on FanHep2 in 2013.

Methods: During the 2013 year, 192.593 serum samples were screened to ANA detection by the Immunology department of DASA, using IFA technique. In the first semester, a semi-automated technique was used, with slides preparation performed by Quanta-Lyser 240 equipment (Inova Diagnostics, San Diego, CA) and manual reading. In the second semester, the "Integrated Laboratory" (Inova Diagnostics) system was implemented. This system is composed by Nova View equipment and Quanta-link software. It allows the performance of full automated process, from slides preparation to reading. During both phases, the initial dilution of samples was 1/160.

Results: We found that 73% of the analyzed samples showed negative result and 27% showed positive result for ANA. The most frequent pattern found among positive samples was Speckled Nuclear Fine Dense - PSNFD, followed by Speckled Nuclear Fine, as described at Table 1. Some patterns were found in less than 1% of the positive samples as NUMA 1 (Pattern Nuclear Fine Speckled with Mitotic Apparatus), Fine Dense Speckeld Cytoplasmitic, Cytoplasmatic with Isolated Dots, NUMA2 (Mitotic Apparatus Type Mitotic Fuse), Nuclear Type Nuclearmembranous, Polar Speckled Cytoplasmatic, Mitotic Apparatus type Intercellular Bridge, Cytoplasmic Fibrilar, Fine Speckled Cytoplasmatic, Mitotic Apparatus type Centriole and Pleomorphic Nuclear Speckled (PCNA).

Conclusion: Our findings demonstrated that 35.43% of the positive samples presented PSNFD pattern, which according to previous reports are rarely found in ARDs.

Table 1. ANA's patterns detected. Legend: *Other patterns: frequency less than 1%.

Pattern	Number of samples	%
Nuclear Fine Dense Speckled (PSNDF)	18609	35.43
Nuclear Fine Speckled	13832	26.33
Nuclear Quasi-Homogeneous Speckled	5179	9.86
Nuclear Homogeneous	2752	5.24
Nucleolar	2423	4.61
Nuclear Coarse Speckled	2419	4.61
Nuclear Centromere	2121	4.04
Nuclear Coarse Speckled Reticuladet	1401	2.67
Reticular Specled Cytoplasmatic	1364	2.60
Nuclear dots	588	1.12
*Other patterns	1838	3.51
Overall	52526	100

A-369

THE SERUM LEVELS OF DIKKOPF-1 (DKK-1) IN AXIAL SPNDYLOARTHRITIS (AXSPA) ARE RELATED TO DISEASE DURATION

E. Melguizo[1], V. NAVARRO-COMPÁN[2], C. GONZALEZ-RODRIGUEZ[1], F. NAVARRO-SARABIA[1], R. ARIZA-ARIZA[1]. *[1]Virgen Macarena University Hospital, Sevilla, Spain, [2]LA PAZ University Hospital, MADRID, Spain*

Background: Tumor necrosis factor (TNF) alpha is responsible for induction of dkk-1 which down-regulates bone formation. Therefore, it was expected that TNF-blocker therapy would inhibit radiographic progression in patients with axSpA but this effect has not been observed yet. Nevertheless, most of the studies have included patients with long disease duration and it is unknown whether or not this effect would be the same in patients with an early stage of the disease.

Objectives: To investigate if disease duration influences on the serum levels of dkk-1 in patients with axSpA. Methods: Observational study including consecutive patients with axSpA according to ASAS criteria visiting a tertiary hospital between January 2011 and June 2013. All patients were receiving NSAIDs and none of them was under biologic therapy. The following characteristics were recorded at one visit: Demographic (age, gender), symptoms duration, HLA-B27, disease activity indices (BASDAI, CRP, ESR) and function (BASFI). Blood samples to determine dkk-1 serum levels by enzyme immunoassay were collected at the same visit too. Patients were classified as early axSpA (symptoms duration ≤ 5 years) and established axSpA

(>5 years) and the characteristics enumerated above were compared between both groups. Univariate and multivariate linear regression models were employed to identify the characteristics related to dkk-1 serum levels.

Results: Thirty one patients with early axSpA and 21 patients with established disease were included. Patients with early axSpA were younger (32.6 ± 9.3 vs 41.0 ± 10.2 years; p<0.01), had lower degree of disease activity (BASDAI: 4.6 ± 2.7 vs 6.6 ± 1.9; p<0.01 and ESR: 7.7 ± 9.2 vs 18.1 ± 15 mmHg; p<0.05) and worst function (3.2 ± 2.9 vs 5.8 ± 2.5; p<0.01) compared with patients with established disease. Serum levels of dkk-1 were significantly higher in patients with early disease (25.9 ± 11.5 vs 13.9 ± 13.5; p<0.001 ng/dl). No statistically significant differences were found between both groups for the rest of characteristics. In the univariable analysis, symptoms duration and BASDAI were inversely related to dkk-1 levels (std β: -0.435; p<0.01 and Std β: -0.283; p<0.05, respectively). However, only the relationship with symptoms duration remained statistically significant in the multivariable analysis (std β: -0.415; p<0.01).

Conclusions: Serum Dkk-1 levels in patients with axSpA depend on disease duration, being higher in patients with recent onset of the disease. The effect of TNF-blocker therapy on radiographic progression may be different in patients with an early stage of the disease compared with patients with established disease.

A-370

Impact of genetic (HLA-DRB1 Shared Epitope) and environmental (Smoking) factors in the presence of anti-CCP antibodies in Rheumatoid Arthritis

J. L. García de Veas Silva, B. Hernández Cruz, C. González Rodriguez, V. Sánchez Margalet, F. Navarro Sarabia. *Hospital Universitario Virgen Macarena, Sevilla, Spain*

Background: Reumatoid Arthritis (RA) is a multifactorial autoimmune disease where environmental and genetic factors interact in the etiology of the disease and development of anti-cyclic citrullinated peptide antibody. The aim of this study is to investigate whether HLA-DRB1 shared epitope (SE), tobacco exposure (TE) and smoking dose (SD) are associated with the presence of anti-cyclic citrullinated peptide (anti-CCP) antibodies in Spanish patients with RA.

Methods: A cohort of 106 patients with early diagnosed RA was studied. Anti-CCP antibodies and rheumatoid factor (RF) were measured at diagnosis and HLA-DRB1 genotyping was performed for SE. TE was categorized as never or ever. Smoking dose (SD) was categorized in pack-years with a cut-off of 20 pack-years. Contingency tables and models of logistic regression were used to calculate the association between SE and smoking with the presence of anti-CCP.

Results: In univariate analysis, SE (OR=2.68; 95% CI 1.11 to 6.46), TE (OR=2.79; 95% CI 1.12 to 6.97), SD (OR=6.04; 95% IC 1.68 to 21.74) and the presence of RF (OR=8.73; 95% IC 2.84 to 26.80) were associated with the presence of anti-CCP antibodies. In logistic regression analysis, only SE-TE interaction (OR=7.083; 95% IC 1.01 to 49.50) and presence of RF (OR=3.07; 95% IC 1.26 to 7.49) were independently associated with the presence of anti-CCP antibodies.

Conclusion: SE-TE interaction and the presence of RF were significantly and strongly associated with the presence of anti-CCP antibodies.

A-371

Evaluation of the CSF and serum utilities of a multipurpose albumin assay for use on the Binding Site Next Generation Protein Analyser

H. Johnson, F. Murphy, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

The integrity of the blood-brain barrier may be compromised in certain neurological conditions. Measurement of albumin in CSF and serum, and calculation of a ratio, is widely used as a diagnostic tool in these circumstances. Here we describe the evaluation of the serum and CSF utilities of the low level albumin assay for the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay is programmed to create a 6-point calibration curve from a single serum based calibrator. Total, inter-lot and inter-instrument precision was assessed for both sample types by running serum and CSF pools at five concentrations across the respective curves. Each sample was run in duplicate, twice daily for 21 days across three analysers. Linearity was assessed using pools of normal serum and CSF samples spiked with purified human albumin. Both fluids were serially diluted across the width of the standard curve and results were compared to expected values. No significant interference (±3.03%) was observed when CSF and serum samples of known albumin concentration were spiked with bilirubin (20mg/dL), haemoglobin (500mg/dL) or Chyle (1500 FTU's). Comparisons were made to both the serum albumin and CSF albumin assays for use on the Siemens BN™II analyser by comparing clinical samples. The main assay characteristics are summarised in the table below:

Assay		Serum	CSF
Initial sample dilution		1/200	1/1
Initial range		2200-66400mg/L	11-332mg/L
Maximum sample dilution		1/200	1/10
Maximum range		2200-66400mg/L	110-3320mg/L
Sensitivity		11mg/L	11mg/L
Assay time (mins)		10.5	10.5
Total precision (concentration)(C.V)		(3.7g/L) **(2.9%)**	(145.5mg/L) **(5.92%)**
		(13.0g/L) **(3.0%)**	(281.5mg/L) **(5.37%)**
		(28.5g/L) **(2.88%)**	(439.9mg/L) **(3.62%)**
		(37.0g/L) **(2.6%)**	(593.1mg/L) **(3.52%)**
		(54.4g/L) **(3.3%)**	(975.2mg/L) **(8.08%)**
Inter-kit precision (concentration)(C.V)		(3.7g/L) (**0.6%)**	(145.5mg/L) **(1.15%)**
		(13.0g/L) **(0.4%)**	(281.5mg/L) **(2.89%)**
		(28.5g/L) **(1.0%)**	(439.9mg/L) **(1.36%)**
		(37.0g/L) **(0.6%)**	(593.1mg/L) **(1.88%)**
		(54.4g/L) **(0.6%)**	(975.2mg/L) **(1.87%)**
Inter-instrument precision (concentration)(C.V)		(3.7g/L) **(1.1%)**	(145.7mg/L) **(0.77%)**
		(13.0g/L) **(0.5%)**	(282.3mg/L) **(1.59%)**
		(28.5g/L) **(0.5%)**	(441.8mg/L) **(2.30%)**
		(37.0g/L) **(0.5%)**	(594.6mg/L) **(1.30%)**
		(54.4g/L) **(1.4%)**	(977.8mg/L) **(1.41%)**
Comparison	**Sample no.**	106	62
	Range	19,868-55,847 mg/L	33.9-961.9 mg/L
	Passing and Bablock analysis	Y=1.01x + 1010.77	Y= 1.08x +14.88
	R^2	0.9541	0.9782
Linearity	**Linear regression**	y=0.9978x +589.88	y=1.0x-4.72
	R^2	0.9996	0.9995

We conclude that the low level albumin assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing albumin assays.

A-372

Multiplex Assay of Circulating Inflammatory Biomarkers in Patients with Stroke

J. Polivka[1], J. Polivka[2], V. Rohan[2], P. Sadilkova[3], O. Topolcan[4]. *[1]Department of Histology and Embryology and Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [2]Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and Faculty Hospital Plzen, Plzen, Czech Republic, [3]Department of Histology and Embryology, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [4]Central Imunoanalytical Laboratory, Faculty Hospital Plzen, Plzen, Czech Republic*

Background: Neuroinflammation is involved in the pathophysiological mechanisms of stroke. However, the role of inflammatory blood biomarkers in relation to the clinically relevant information remains unclear. This study determines the association of various circulating inflammatory biomarkers with stroke severity, classification into different stroke subtypes and patient 3-month outcome.

Methods: 215 stroke patients (Large artery (LAA), n = 93; Cardioembolic (CE), n = 47; Lacunar (LAC), n= 33; Cryptogenic (CR), n = 7 and Hemorrhagic stroke (HS), n = 35) were prospectively evaluated in the Faculty Hospital Plzen between 2012 and 2013. Stroke severity (National Institutes of Health Stroke Scale; NIHSS) was measured at hospital admission. Functional outcome (modified Rankin Scale; mRS) was assessed after 3 months. A multiplex panel of 14 biomarkers (IL1, IL6, IL10, IL12, MCP-1, OPG, OPN, VEGF, MMP1, MMP2, MMP7, MMP9, 25-OH-Vitamin D, 1,25-OH-Vitamin D) was assessed in plasma samples (collected within 4 hours from symptom onset) by Luminex xMAP™ technology. The associations of circulating inflammatory biomarkers with the stroke severity, classification into different stroke subtypes and patient outcome were evaluated by Spearman's rank correlations and Wilcoxon test.

Results: Positive correlations with stroke severity (NIHSS at baseline) were found for IL6 (r = 0.15, P = 0.02), IL10 (r = 0.16, P = 0.014) and MMP9 (r = 0.14, P = 0.03). The worse 3-month outcome (mRS) was correlated with IL6 (r = 0.14, P = 0.036) and blood leukocytes (r = 0.14, P = 0.038). Higher plasma levels of IL6 (P = 0.02), IL10 (P = 0.006) and MMP9 (P = 0.029) and startlingly lower cholesterol (P = 0.029) were found in patients with more severe stroke at baseline (NIHSS > 10). Patients with

worse 3-month outcome (mRS ≥ 3) had higher plasma level of MMP9 (P = 0.04), glucose (P = 0.025), higher NIHSS at baseline (P < 0.0001), lower cholesterol (P = 0.0082) and trend to higher IL6 (P = 0.084). The biomarkers that varied by the stroke classification were OPG (osteoprotegerin, P = 0.018), IL10 (P = 0.015), MMP2 (P = 0.0004), cholesterol (P = 0.0066), NIHSS at baseline (P < 0.0001) and 3-month mRS (P < 0.0001). Patients with ischemic (LAA + CE + LAC) in comparison to hemorrhagic stroke had lower NIHSS at baseline (P = 0.0192), mRS at 3 months (P = 0.003), OPN (osteopontin, P = 0.016), OPG (P = 0.0087) and MMP2 (P = 0.0004).

Conclusion: In our study, various inflammatory circulating biomarkers correlated with stroke severity, such as IL6, IL10 and MMP9. IL6 and blood leukocytes correlated with 3-month outcome. Lower level of OPN, OPG and MMP2 were found in ischemic in comparison with hemorrhagic stroke patients and should be further studied as diagnostic biomarkers of stroke classification. Patients with hemorrhagic stroke had more severe neurological deficit at baseline and worse 3-month outcome. Circulating inflammatory biomarkers should be further examined in patients with stroke.

Supported by MH CZ - DRO (Faculty Hospital Plzen - FNPl, 00669806) and by the project ED2.1.00/03.0076 from the European Regional Development Fund.

A-373

Evaluation of an IgM assay for use on the Binding Site Next Generation Protein Analyser

S. Amin, F. Murphy, S. J. Harding, P. J. Showell. *The Binding Site Ltd, Birmingham, United Kingdom*

IgM is the first immunoglobulin produced in a primary immune response. It constitutes around 10% of the total serum immunoglobulin, and is largely in a pentameric form. Pentameric IgM is able to flex into a "crab" or "staple" formation when binding to antigen and activates the classical pathway of complement. Here we describe the evaluation of a serum IgM assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The instrument automatically dilutes a single calibrator to produce a calibration curve with a measuring range of 0.2 - 7.5g/L at the standard 1/20 sample dilution, with sensitivity of 0.1g/L. High samples are remeasured at a dilution of 1/400 with an upper measuring range of 4.0 - 150.0g/L. The assay time is 10.5minutes and is read at end point. Precision studies (CLSI EP5-A2) were performed at eight levels in duplicate over 21 working days. Antigen levels of 0.19g/L, 0.28g/L, 0.38g/L, 1.44g/L, 1.65g/L, 2.68g/L, 5.11g/L and 10.21g/L were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. The coefficients of variation were 6.2%,1.4%, 2.3% and 5.5% for the 0.19g/L sample, and 5.6%, 1.5%, 3.8% and 3.8% for the 0.28g/L sample, 5.6%, 1.2%, 3.7%, 4.1% for the 0.38g/L sample, 4.0%, 2.0%, 3.4% and 0.0% the 1.44g/L sample, 3.4%, 1.6%, 2.6% and 1.4% for the 1.65g/L sample, 3.6%, 1.2%, 3.0%, 1.6% for the 2.68g/L sample, 4.0%, 2.2%, 3.0% and 1.3% for the 5.11g/L sample and 5.6%, 2.5%, 3.6% and 3.4% for the 10.21g/L sample respectively. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (0.198 - 7.662 g/L) and comparing expected versus observed results. The assay showed a high degree of linearity when expected values were regressed against measured values; y=0.9953x + 0.0235, R^2 = 0.9993. No significant interference (within 10%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units) when spiked into a sample with known IgM concentrations and measured at the minimum sample dilution. Correlation of this assay with the equivalent assay for the Binding Site SPA PLUS was performed using both normal and clinical samples (n=115, range 0.135 - 60.424 g/L). Good agreement was demonstrated by Passing-Bablock regression; y=0.98x - 0.02 g/L. We conclude that the IgM assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

A-374

Antibodies-to-Infliximab: Assay Development and Correlation with Infliximab Concentrations in Serum Samples of Treated Patients

M. A. V. Willrich, J. G. Balsanek, P. M. Ladwig, D. R. Barnidge, D. L. Murray, M. R. Snyder. *Mayo Clinic, Rochester, MN*

Background: Infliximab (IFX) is a chimeric therapeutic monoclonal IgG1 kappa antibody targeting tumor necrosis factor-α and is FDA-approved for treatment of several inflammatory disorders. Patients undergoing therapy may form antibodies-to-Infliximab (ATIs), which can reduce circulating drug concentrations. Measurement of IFX concentration and ATIs is useful for guiding therapy in patients who have lost clinical response. Here we present the development of an electrochemiluminescent immunoassay (ECLIA) for detection of ATIs. Methods: A "bridging" ECLIA was developed in which the ATI forms a link between biotin- and sulfo-tag-labeled IFX (MesoScale Discovery LLC). An 8-point standard curve was established using a high-affinity human IgG1 ATI (AbD Serotec), and an acid-dissociation step was performed to disrupt immune-complexes. Residual sera with physician-ordered IFX and ATI were evaluated (n=37) and compared to results from a reference laboratory (Esoterix). Serial sera from patients on IFX were collected at trough levels (n=36), 48-72 hours post-infusion (n=33) and 28-32 days post-infusion (n=33). IFX was measured using clonotypic heavy chain peptides by LC-MS/MS. Results: The analytical measurable range for the ECLIA ATI assay was established as 19.5-2,500ng/mL (R^2=0.9911). Limit of quantitation was defined as 19.5ng/mL; results >19.5ng/mL were classified as positive. Using residual serum samples, the ECLIA showed an overall qualitative concordance of 86.4% to a commercial method. In the cohort of serial samples, 22% (8/36) of trough samples, with IFX concentrations of 8.5±8.8µg/mL, were positive for ATI; in 7/8 samples the ATIs persisted 28-32 days after IFX infusion (IFX concentration 15±11µg/mL). Samples obtained 48-72h after IFX infusions were negative, suggesting that high concentrations of IFX (77±40µg/mL) interfere with ATI measurement. Presence of ATIs was associated with lower concentrations of IFX at trough (8.8±8.9µg/mL in ATI negatives *vs.* 3.3±4.8µg/mL in positives, p=0.0038), 48-72h post-infusion (78±41µg/mL *vs.* 47±12µg/mL, p=0.0357) and at 28-32 days after (15±7µg/mL *vs.* 7±10µg/mL, p=0.0048). Out of the 8 positives measured at trough, 7 had IFX<5µg/mL. In the residual sera cohort used for method comparison samples with IFX<5µg/mL (n=18) showed 100% concordance for ATI by the two methods. In contrast, samples with IFX≥5µg/mL (n=19), had a concordance of 73.7%. In the presence of detectable trough levels of IFX, significance of ATIs is unclear. Based on these data, we propose an algorithm for assessment of patients showing a loss of response to IFX. Initial testing would be performed for quantitation of trough IFX by LC-MS/MS with a reflex to ATIs in cases when IFX concentrations are <5µg/mL. Conclusions: Using a newly-developed ECLIA bridging method, we have demonstrated that the presence of ATIs correlated with lower IFX concentrations and that the majority of ATIs were found in patients with trough IFX<5µg/mL. In addition, interference by endogenous IFX may be an issue, specifically when assessed at peak levels. An algorithmic approach starting with the quantitation of IFX measured at trough with a reflex to ATI could provide guidance to clinicians in identifying patients who might respond to increased doses of IFX compared to patients for whom another biologic agent might be more appropriate.

Tuesday, July 29, 2014

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

Mass Spectrometry Applications

A-375

Tandem mass method for disaccharide units of urinary glycosaminoglycans from MPS patients

C. Chuang, H. Lin, C. Tsai, S. Lin. *Mackay Memorial Hospital, Taipei, Taiwan*

Background: Identification of acid mucopolysaccharide by liquid chromatography/ tandem mass spectrometry method (LC-MS/MS) of predominant disaccharide units of glycosaminoglycans (GAGs) (chondroitin sulfate, CS; dermatan sulfate, DS; heparan sulfate, HS) after methanolysis is validated and applicable for mucopolysaccharidosis (MPS) phenotype determinations.

Methods: A total of 76 urine samples were collected and analyzed, including 9 MPS I patients, 13 MPS II, 7 MPS III, 8 MPS VI, and 39 normal controls. Urinary GAG was first precipitated by Alcian blue method followed by a treatment of 3N HCl methanol. The protonated species of the methylated disaccharide products were detected by using a multiple reaction monitoring experiment. Internal standards, [$^2H_6$] CS, [$^2H_6$] DS and [$^2H_6$] HS, were in-house prepared by deuteriomethanolysis of CS, DS and HS.

Results: The within-run and between-run precisions were good, and the recoveries were 94.3% for DS and 95.1% for HS. Linearity of DS and HS was calculated individually and the correlation coefficients (r) were 0.9914 for DS and 0.9935 for HS, respectively. One particular disaccharide for each GAG was selected, in which the parent ion and its daughter ion after collision were *m/z* 426.1→236.2 for DS (*m/z* 432→239 for dimmers derived from [$^2H_6$] DS) and *m/z* 384.2→161.9 for HS (*m/z* 390.4→162.5 for the [$^2H_6$] HS dimmer). The results were correspondent well when comparing with the two-dimensional electrophoresis method. The quantities of DS and HS were determined, which were varied from one MPS phenotype to the others, and the results can be used to evaluate the severity of MPS subgroups, as well as the amelioration of follow-up after enzyme replacement therapy (ERT).

Conclusion: The modified LC-MS/MS method for MPS phenotype determination is specific, sensitive, validated, accurate and applicable for simultaneous quantifications of urinary DS and HS. This method can help to make correct diagnosis of MPS patients and evaluate the effectiveness of ERT.

A-376

Improving detection limits of prohibited substances and therapeutics by Solid Phase Microextraction (SPME) coupled to LC-MS/MS

F. Ruparelia[1], J. Pawliszyn[2], N. Reyes-Garcés[2], G. Augusto Gómez-Ríos[2], B. Bojko[2]. [1]*IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada,* [2]*University of Waterloo, Waterloo, ON, Canada*

This work presents the enhancement achieved in limits of detection for a set of prohibited substances and therapeutics by coupling a completely automated thin-film SPME analytical protocol to a powerful LC-MS/MS system developed by IONICS Mass Spectrometry.

LC-MS/MS analyses were performed using a triple quadrupole MS 3Q-320 (IONICS, Bolton, Ontario, Canada). Complete separation was achieved using a pentaflourophenyl column (Phenomenex, Torrance, CA, USA) and a ternary gradient of water, methanol and acetonitrile (0.1% formic acid in all mobile phases). Sample preparation was performed using a Thin Film Microextraction (TFME)-Concept autosampler. The automated procedure consisted of the following steps: TFME pre-conditioning (samples were simultaneously incubated at 30 ºC) (30 min), extraction (75 or 90 min from pooled urine samples spiked at different concentration levels), washing step (10 s), and desorption in a proper solvent for 20 or 60 min. HLB and C18 coatings in thin-film SPME configuration were prepared in-house following a protocol developed in our laboratory.

In order to preserve sporting ideal and ensure fair play game, the World Anti Doping Agency (WADA) has banned performance-enhancing substances in competitive sports. In most cases, despite the use of extensive and cumbersome sample preparation protocols, the extraction and detection of these compounds in complex matrices such plasma, urine, and blood, can be a challenge. Solid phase microextraction (SPME), a green chemistry technique that combines sampling/sample preparation in a single step, has shown to be a powerful tool for the determination of multiple prohibited drugs in complex matrices. However, in some circumstances the quantification capabilities of SPME are constrained by the instrumental limits of detection and quantification of the LC-MS/MS systems.

This work presents a SPME method coupled to an effective MS/MS system towards the quantification of compounds with low minimum required performance level (MRPL) values set by WADA. Analytes selected in this study (boldenone, cannabidiol, cannabinol, dihydrotestosterone, fentanyl, fluoxymestrone, methyl-testosterone, nandrolone, testosterone and 19-norandrosterone) were of particular interests here as they did not meet the MRPL levels with the SPME-LC-MS protocol previously developed in our group. Analysis of neat standards (prepared in methanol at a concentration range of 0.05 to 100 pg/µL) performed in a triple quadrupole MS IONICS 3Q-320 demonstrated allowed meeting the MRPL values required by WADA, and selected compounds displayed outstanding limits of quantification. For instance, instrumental LOQs of 33 and 200 ag/µL were achieved for fentanyl and testosterone, respectively, with a correlation coefficient equal or better than 0.999. In addition, therapeutics such as pancuronium and rocuronium exhibited similar instrumental LOQs using the same MS analyzer. These findings provide an opportunity to expand the applicability of SPME to quantify low concentrations not only for ex-vivo analysis but also for in-vivo applications in which the temporal resolution, sensitivity and accuracy provided by SPME is highly desired.

A-377

Enhanced Resolution and Matrix Interference Reduction for the Analysis of Vitamin D Metabolites

C. Aurand, D. Bell, T. Ascah-Ross. *Supelco, Bellefonte, PA*

Analysis of Vitamin D metabolites has continued to be a topic of interest in recent publications, primarily as biomarkers for possible disease states and vitamin sufficiency. While Vitamin D is present in two forms, current ELISA methods cannot distinguish D2 and D3 forms of the vitamin metabolites resulting in a reporting of total 25-hydroxyvitamin D. In this study, an LC/MS method for the analysis of Vitamin D metabolites is expanded to include dihydroxy metabolites along with the epi-homologs. Chromatographic resolution is utilized for the quantitation of hydroxy and dihydroxy Vitamin D2 and D3 metabolites including the isobaric epimers. In addition, sample preparation techniques are evaluated for the impact of biological matrix ionization effects.

Chromatographic method development consisted of screening C18, Cyano, Phenyl Hexyl and pentyl fluorophenyl (F5) stationary phase. Method development experminets resulted in conditions for the direct quantitation of isobaric metabolites 25 hydroxyvitamin D3, 3-epi-25 hydroxyvitamin D3 1-α hydroxyvitamin D3 along with 25 hydroxyvitamin D2, 3-epi-25 hydroxyvitamin D2. In addition, human serum samples were processed using standard protein precipitation techniques along with novel phospholipid depletion plates for the comparison of matrix interference impact. . The unique combination of the selectivity of the F5 separation along with the novel sample preparation technique allow of a robust and accurate LC/MS method for quantitation of all the associated Vitamin D metabolites

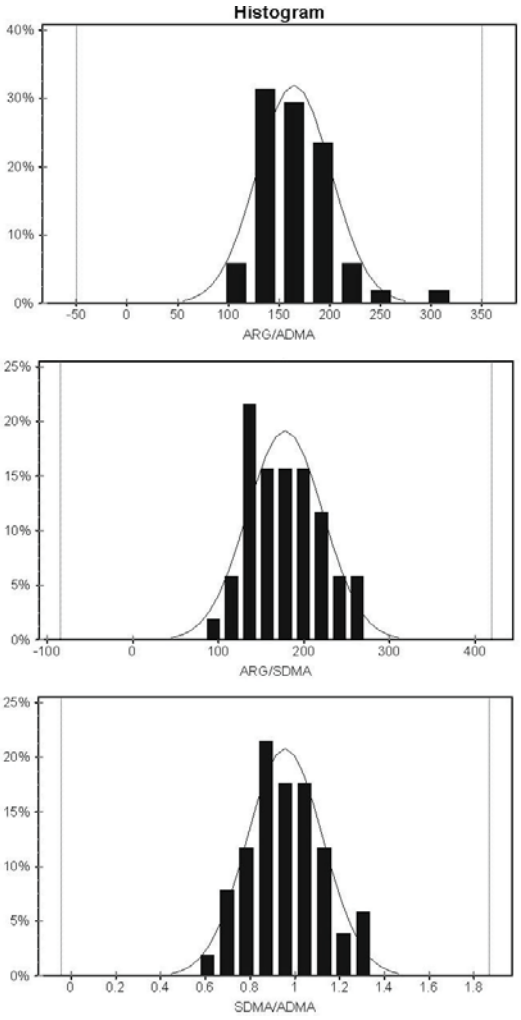
A-378

Reference interval determination for the ratios of L-arginine (ARG), symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA)

J. M. El-Khoury, D. R. Bunch, B. Hu, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA), metabolic products of methylated L-arginine (ARG) containing proteins, play an important role in regulating nitric oxide production. Recently, SDMA and ADMA have been extensively evaluated as biomarkers of renal and/or cardiovascular diseases with indication of potential use of the ratios of ARG/ADMA and SDMA/ADMA. More specifically, the ratio ARG/ADMA has been shown to be an independent predictor of mortality in patients with dilated cardiomyopathy, while the ratio SDMA/ADMA has been investigated as a biomarker of hypertension in rats and was found to be a better predictor for disease activity and progression than the individual parameters alone. However, reference intervals (RIs) for the ratios ARG/ADMA, ARG/SDMA and SDMA/ADMA have not been reported in the literature. Our objective in this study was to determine RIs for these ratios in a healthy adult population using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. **Methods:** Collection of blood samples for RI determination was approved

by the Institutional Review Board. In brief, EDTA whole blood samples (n = 51) were collected from healthy adults (39 females) identified via a questionnaire, aged 19-64 y (38.8 ± 12.6), after a minimum of 8 hour fasting. A published LC-MS/MS assay was used for the analysis of ARG, SDMA and ADMA. EP Evaluator release 10 was used for statistical analysis. **Results:** The central 95% RI for ARG/ADMA, ARG/SDMA and SDMA/ADMA were 108 to 247, 95 to 261, and 0.64 to 1.27, respectively. Based on EP evaluator's determination a transformed parametric method for ARG/ADMA and a parametric method for ARG/SDMA and SDMA/ADMA were used. Histograms are shown in the figure. **Conclusion:** RIs for ARG/ADMA, ARG/SDMA and SDMA/ADMA were determined using a well-defined healthy population by an LC-MS/MS method. These values are important in defining the clinical utility of these parameters.



A-379

A unique brain lipidome and metabolome biosignature in Alzheimer's Disease

G. Astarita[1], G. Paglia[2], J. Langridge[3], S. Lai[3]. *[1]Georgetown University/ Waters Corporation, Milford, MA, [2]Center for Systems Biology, University of Iceland, Reykjavik, Iceland, [3]Waters Corporation, Milford, MA*

Alzheimer's disease (AD) is the most common cause of adult dementia, but the cause of this inexorable neurodegenerative disease remains still elusive. Alterations in both lipid and polar metabolites biochemical pathways have been associated with AD. Here we conducted an unbiased investigation of the underlying biochemical alterations in AD human tissues. We used an integrated lipidomics and metabolomics approach to survey frozen brain tissue samples from clinically characterized AD patients and age-matched controls. Lipids and polar metabolites were extracted using a biphasic, liquid-liquid extraction procedure. Polar metabolites were separated using a hydrophobic interaction liquid chromatography (HILIC), whereas lipids using an integrated microfluidic device packed with reversed phase C18. Travelling-Wave ion mobility mass spectrometry was used to improve peak capacity and CID fragmentation specificity. Moreover, ion mobility-derived collision cross sections provided orthogonal physicochemical data that were used with retention time, accurate mass and MS/MS data to increase confidence of metabolite identification. Data was collected using both negative and positive ionization mode in the data-independent acquisition mode with an alternate low and elevated collision energy method to acquire both precursor and product ion information in a single analytical run. Lipidome and metabolome data were fused and mined using multivariate statistical and pattern-recognition tools. Initial observations were confirmed using more targeted approaches for quantification. Pathway analysis was then used to incorporate the novel molecular information into the known biochemical pathways. The results obtained were further integrated with clinical data to generate testable hypotheses on the functional significance of the abnormalities observed in AD. Our preliminary results reveal novel molecular alterations in AD and a unique lipidome and metabolome biosignature that differentiates the brains from individuals with AD compared from those from control subjects.

A-380

Pain Management Drug Monitoring in Urine using HPLC-MS/MS

J. K. Wolken, T. Doran, P. Smith, A. Iwanski, D. Wiebe. *University of Wisconsin Hospital and Clinics, Madison, WI*

Background: With prescription drug abuse reaching epidmic proportions, clinicians are seeking solutions to monitor aptients on long-term pain management prescriptions. These monitoring programs need to be sensitive to low level amounts of pain management therapeutics in a patient population correctly adhering to physicians orders while screening for non-prescribed pain medication and drugs of abuse. The University of Wisconsin Hospital and Clinics (UWHC) Toxicology group has developed a clinical test which will monitor 35 drug substances with minimal sample preparation in urine in a 10 minute HPLC-MS/MS run. **Methods:** The following compounds are monitored in the following catagories: **Drugs of abuse**: 6-MAM (heroin metabolite), amphetamine, benzoylecgonine (cocaine metabolite), MDA, MDMA (ecstasy), and methamphetamine. Benzodiazepines: alprazolam, hydroxyalprazolam, 7-aminoclonazepam, diazepam, nordiazepam, lorazepam, midazolam, oxazepam, and temazepam. Opioids: buprenorphine, norbuprenorphine, codeine, fentanyl, norfentanyl, hydrocodone, hydromorphone, meperidine, normeperidine, methadone, EDDP (methadone metabolite), morphine, naloxone, naltrexone, oxycodone, oxymorphone, tapentadol, N-desmethyltapentadol, tramadol, and N-desmethyltramadol. Patient urine is mixed 1:1 with an internal standard which consists of 8 deuterium labeled compounds (a subset of the 35). This is injected onto a BiPh Restek column, and using a Agilent 1200 HPLC the compounds are eluted into a AB Sciex 4000 triple quadruploe mass spectrometer. Each compound has 2 ion transitions for identification and quantitation. The AMR for most of the compounds are 25-1000 ng/ml with most of the benzodiapizines having an AMR of 10-1000 ng/ml. Results: The within run CV range for the compounds at the low end cutoff (25ng/ml for most compounds) was between 1.5-10.1% where the day to day precision at the same low cutoff was 4.0-15.5%. Carryover has not been shown to be an issue for any drug substance despite some extremely high patient samples being analyzed. Comparison of methods to an in-house GC-MS method showed increased sensitivity for most compounds, most notably 6-MAM, morphine, and oxycodone. Assayed College of American Pathologist (CAP) Toxicology surveys from 2011 and 2012 showed 100% qualitative agreement for all 30 survey samples for the compounds in the Pain Management Profile. Conclusion: The new pain panel method shows very little limitation in 'real world' patient samples. Even with minimal sample preparation steps very few issues have arisen with interferences, ion suppression, and retention time shifts. We advise our clinicians that the panel results are only a snapshot in time. The dose, time of dose, state of hydration, and individual metabolism all play a part in the concentration of drug substances in the urine. Intermittant testing over time is recommended to help compile a clearer picture of the patients' compliance.

A-382

Determination of Tacrolimus and Sirolimus in whole blood by liquid chromatography electrospray ionization tandem mass spectrometry

M. E. R. Diniz, L. S. Vilhena, T. M. C. C. Barbosa, B. P. Paulo, E. C. C. Mateo, A. C. S. Ferreira. *Instituto Hermes Pardini, Vespasiano, Brazil*

Tacrolimus and Sirolimus are immunesuppressive drugs used in organ transplantation, exhibit narrow therapeutic ranges and adverse effects are common. Methods based on Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) are considered

the gold standard in therapeutic monitoring of these drugs. So a simple, rapid and sensitive LC/MS/MS method has been developed and validated for the determination of Tacrolimus and Sirolimus in whole blood using Ascomycin as the internal standard (IS). In this process, 100 µL of whole blood samples containing the internal standard were treated by liquid-liquid extraction using ethyl acetate and subjected to LC-MS/MS analysis using positive electrospray ionization (ESI+). Chromatographic separation was performed on a Symmetry C18 column (3.5 µm 4.6 x 75 mm) and mobile phase acetonitrile:methanol:water (80:10:10, v/v/v) with 0,1% of formic acid and 0,02% of Ammonium hydroxide 25% at 400 µL/min. The MS/MS detection was conducted by monitoring the fragmentation ions of 821.7→768.5 (m/z) for Tacrolimus, 931.5→864.5 (m/z) for Sirolimus and 809.7→756.7 (m/z) for Ascomycin. Ammoniated adducts of protonated molecules were used as precursor ions for all analytes. The method had a chromatographic running time of approximately 4 min. The linear analytical range of the procedure was between 1.0 and 51.0 ng/mL for Tacrolimus and 2.0 and 52.0 ng/mL for Sirolimus. The medium range of recovery for the Tacrolimus was 98.1-103.2% over a interval of 2.0-37.5 ng/mL and for the Sirolimus 97.1-106.1% over a range of 2.0-39.0 ng/mL. The intra and inter-day precision was less than 6.8% for Tacrolimus and 10.8% for Sirolimus. In conclusion, the LC-MS/MS method has been developed successfully for the quantitative analysis and therapeutic monitoring of these immunosuppressive drugs.

A-383

Evaluation of high performance liquid chromatography and liquid chromatography-tandem mass spectrometry methods for 25 (OH) D_3 assay

B. Omer, F. A. Aydin, P. Mikailova, S. Genc. *Istanbul University Istanbul Medical Faculty, Istanbul, Turkey*

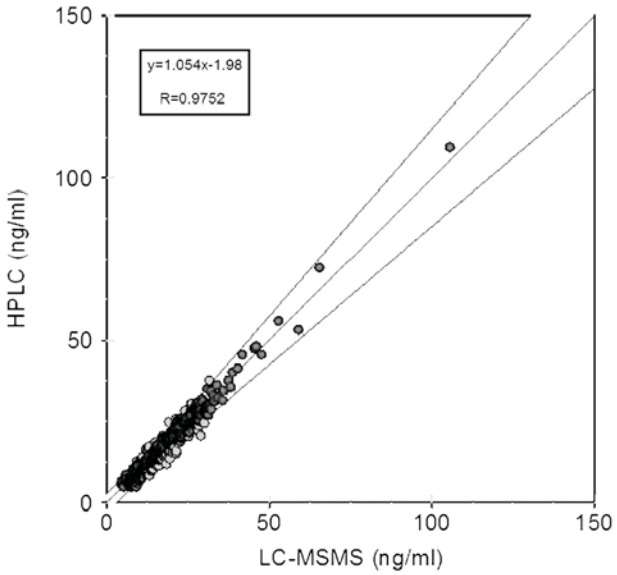
Background: Growing evidence about the role of vitamin D on health in different fields of medicine emerged the necessity of establishing more reliable and accurate 25 (OH) vitamin D_3 assessment methods. This study was designed to compare performance characteristics of two different 25 (OH) vitamin D_3 assessment methods.

Methods: 25 (OH) D_3 vitamin levels were quantified using two methods as follows: high-performance liquid chromatography (HPLC) with Thermo Finnigan TSP (Florida-USA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) with Zivak Technologies ZinMass-200 LC-MS/MS System with APCI ionization source (Istanbul-TURKEY) as the reference method. Assay performance characteristics were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) .The comparison studies were done using randomly chosen 306 plasma samples from routine clinical samples submitted for 25 (OH) D_3 vitamin measurement.

Results: The LC-MS/MS assay had within-run coefficient of variation (CV) 7.3% and between-day CV 6.0% for low control (22.4±3.3 ng/mL), for high control (82.3 ±11.8 ng/mL) within-run CV 7.2% and between-run CV 9.6%. HPLC method had within-run CV 6.9% and between-day CV 12.5% for low control, and for high control, within-run and between-run CVs were 5.6 and 8.7 respectively. The linearity studies showed good correlations between expected and obtained values for both methods. When the relationship between the results obtained from HPLC and LC-MS/MS assays was investigated in 306 subjects, the HPLC assay showed an acceptable correlation with the LC-MS/MS (y=1.054x-1.98, R=0.9752).

Conclusion: With good precision and accuracy, HPLC system revealed an acceptable correlation with LC-MS/MS for 25 (OH) vitamin D_3 assay.

Figure 1: Comparison of plasma 25 (OH) vitamin D_3 levels measured by HPLC vs LC-MS/MS.



A-384

Elimination of false positives and false negatives for the screening of amphetamines, PCP, and benzoylecgonine by Agilent RapidFire

D. Ames. *IQ Laboratories, Fort Wayne, IN*

This research shows a more accurate alternative to using an immunoassay for the screening of amphetamines, PCP and benzoylecgonine. Immunoassays are the typical method used to screen for amphetamines, PCP, and benzoylecgonine. However, immunoassays are known to produce false positives, false negatives and require the use of expensive reagents. By utilizing an Agilent RapidFire 300 High-throughput System we eliminated false positives and false negatives. False positives and false negatives were eliminated because RapidFire uses an Agilent QQQ triple quad mass spectrophotometer as the detection method rather than enzymes or antibodies. The use of a triple quad mass spectrophotometer as the method of detection also allows for selectivity that is not possible with immunoassays. In this study on RapidFire PCP, benzoylecgonine, amphetamine, methamphetamine, MDMA, MDA, MDEA, methylphenidate and ritalinic acid were screened for and on the DxC 600i the reagents from Beckman Coulter for amphetamines, PCP, MDMA and benzoylecgonine were used. Patients were screened on a Beckman Coulter DxC 600i and then on an Agilent RapidFire 300 High-throughput System connected to an Agilent 6460 QQQ triple quad mass spectrophotometer. Those patients found to be positive on either system were then analyzed by LC-MS/MS for further confirmation. The use of RapidFire allowed us to eliminate false positives and false negatives and increase selectivity.

A-386

Determination of urinary ethyl glucuronide and ethyl sulfate by LC/MS/MS for clinical research

L. Cote. *Agilent Technologies, St-Laurent, QC, Canada*

Background: Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of urinary ethyl glucuronide (EtG) and ethyl sulfate (EtS). A dilution procedure and a solid phase extraction (SPE) procedure are evaluated and compared based on ease of use, analyte recovery and post-extraction cleanliness.

Methods: A dilution procedure and a solid phase extraction (SPE) sample preparation procedure were developed and compared for the simultaneous extraction of ethyl glucuronide and ethyl sulfate in urine. Calibrators were created by spiking synthetic urine (Surine-Cerilliant) with various concentrations of EtG and EtS standards (Cerilliant). The chromatographic system consists of a Polaris 3 C18-Ether column coupled with a guard column and a mobile phase comprised of acetonitrile and water containing 0.1% formic acid. Quantifier and qualifier transitions were monitored. EtG-D5 and EtS-D5 internal standards (Cerilliant) were included to ensure accurate and reproducible quantitation. Urine controls (UTAK Laboratories) were used and samples were kindly supplied by collaborators.

Results: The separation of EtG and EtS from isobaric interferences is especially critical; without proper separation by retention time, impurities present in both compounds can cause interferences with one another and lead to inaccurate quantitation. The described method achieves the required functional sensitivity and is capable of quantitating analytes over a sufficiently wide dynamic range with a single injection. All analytes displayed excellent linearity from 25 to 10000 ng/ml. All calibration curves displayed an R2 > 0.9993. Back calculated accuracies for all calibrators ranged from 93% to 120% for the dilution procedure and from 96 to 113% for the SPE procedure. Commercially available quality control material was used to test the reproducibility of this method. Measurements were repeated on three separate days to assess interday reproducibility and CVs were found to be below 6%.

Conclusion: A method has been developed for quantifying ethyl glucuronide (EtG) and ethyl sulfate (EtS) in urine for clinical research. Two sample preparation procedures consisting of a simple dilution from urine and SPE are shown. Chromatographic separation of all analytes and interferences with conditions compatible with LC/MS/MS have been developed. Typical analytical method performance results are well within acceptable criteria.

A-387

A Quantitative Determination of Methadone and its Metabolite (EDDP)in Dry Blood Spot by LC-MS/MS

J. Ye, H. Qiao, E. Majdi, L. Cousins. *IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada*

Background: Critically ill children routinely receive opioids for analgesia and sedation to reduce pain and stress, facilitate ventilation, and avoid secondary complications. The typical course of treatment often induces tolerance, and withdrawal symptoms may be precipitated if the drugs are discontinued abruptly. Withdrawal symptoms are not only unpleasant but can be life-threatening and may prolong the need for mechanical ventilation and potentially extend hospitalization. Methadone is a synthetic opioid receptor agonist widely used in the treatment of severe pain and in maintenance treatment of opioid addicts. It is approved by the U.S. Food and Drug Administration for detoxification treatment of opioid addiction in adults, but does not have a label for pediatric use. Therefore it is necessary to monitor the dosage and to avoid the abuse. The present study provides a much simplified approach whereby a novel, highly sensitive LC-MS/MS triple quadrupole mass spectrometer is used to measure directly the methadone and EDDP concentrations through the dried blood spot (DBS) samples.

Methods: Fresh human whole blood was spiked with different concentrations of methadone and EDDP. 30 ul of the blood were spotted onto Whatman DMPK-C cards. Cards were air dried for about 2 hours. 6 mm spot were punched placed into a vial containing 100ul IS (0.01ng/mL) working solution. Samples were vortexed for 3 minutes and centrifuged for 5 minutes at 4000 rpm. The supernatants were transferred into HPLC vials for analysis. An IONICS 3Q 220 mass spectrometer was used. This instrument is equipped with heated coaxial flow ion source and "Hot Source-Induced Desolvation" interface, with a multi-orthogonal channel and laminar flow sampling. The samples were injected using Shimadzu UFLC XR system. Sample was loaded to a Chromolith-RP18E column (100X3 mm, 3 um) with a gradient method at 0.5 mL/min. Mobile Phases were A (0.1% formic acid 100% H2O) and B (0.1% formic acid in 100% ACN). The total LC run time is 3.5 minutes.

Results: Calibration curve of the neat methadone and EDDP solution showed good linearity over a range of 0.0025-10 ng/mL with correlation values of R^2=0.9997 and 0,9998, respectively. In DBS extraction the calibration curves for methadone and EDDP over a range of 0.1 to 100 ng/mL were created. The curves also showed good linearity with weighting factor of 1/x for both analytes. The correlation values were R^2=0.997 and 0.998 for methadone and EDDP, respectively. At LLOQ of 0.1 ng/mL, a good accuracy of 108% and 99% and CVs of 9% and 8.3% were obtained for methadone and EDDP, respectively.

Conclusion: The results in this study show a fast, accurate, and precise LC-MS/MS method with IONICS 3Q 220 mass spectrometer for quantifying methadone and EDDP in DBS samples. The LLOQs for both samples are 0.1 ng/mL with good precision and accuracy. The sample preparation procedure is simple and rapid without SPE and LLE extraction. Therefore, the LC-MS/MS method in this study has confirmed its clinical applicability and can be used in routine bioanalysis, especially for methadone level monitoring.

A-388

Increased Throughput for the Analysis of delta-9-THC in Oral Fluids using Triple Quadrupole Mass Spectrometry coupled to Automated Dual-Channel HPLC

K. McCann, A. Szczesniewski. *Agilent, Santa Clara, CA*

Background: Many forensic labs are interested in improved sample throughput to get better utilization of the testing instrumentation. Through this work we demonstrated the ability to increase mass spectrometer productivity through the automated use of a dual channel high performance liquid chromatography (HPLC) system. A newly developed software interface intelligently determines the timing of all HPLC components and coordinating the analytical utilization of the mass spectrometer.

Methods: The integrated LC/MS/MS system is comprised of a triple quadrupole mass spectrometer coupled to a configurable HPLC system, all controlled by a single software application. For the purposes of this work, the HPLC system consists of a high-capacity autosampler, two binary pumps, two HPLC columns, two temperature-controlled column compartments and one switching valve. To operate the system, a standard data file collected by LC/MS/MS is loaded into the software. The data analysis method is extracted from the data file and a window of interest is specified using the data file's chromatogram. Based on that information, the software automatically coordinates all timing related to running the HPLC system.

Results: The analysis of delta-9-THC is performed in many forensic toxicology labs analyzed by LC/MS/MS where sample throughput is a major concern. An established LC/MS/MS method for the analysis of this analyte from oral fluids was used for testing the capabilities of this new instrument. The standard method uses an autosampler, binary pumps, HPLC column and temperature-controlled column compartment. With a runtime of 5 minutes, the analytes of interest reach the mass spectrometer between approximately 2 minutes to 4 minutes. Hence, more than 50% of the data collected by the mass spectrometer is of no interest.

The standard method utilizes what is considered a single HPLC stream. The new HPLC system mirrors certain components of this single stream system to provide a second stream, operating in parallel to the first stream. By loading the standard method and window of interest into the automation software, the software is able to determine the most efficient method of injecting and analyzing a list of delta-9-THC samples without any user configuration necessary. By staggering injections on parallel streams and switching between the two streams at the appropriate time, throughput of the integrated expanded system can double the throughput achieved with the standard method, without any sacrifice to the quality of quantitation.

Conclusion: Fully automated software controlling a completely integrated LC/MS system consisting of two parallel LC streams has been developed and implemented in the analysis of Δ9-THC. No special method development is required; the user supplies a standard method and defines a window of interest, allowing the software to determine all necessary timing and coordination of the analysis. Throughput for this method has been doubled through the use of an Automated Dual-Channel HPLC.

A-389

Quantitative Analysis of Acetaminophen and Salicylic Acid in Urine by Rapidfire Coupled with Triple Quadrupole Mass Spectrometry

F. Mbeunkui, S. Sullivan, R. B. Dixon. *Physicians Choice Laboratory Services, Rock Hill, SC*

Background: A rapid and quantitative method for the direct quantitation of acetaminophen and salicylic acid in biological matrices is warranted in clinical and toxicological chemistry. Compliance monitoring and therapeutic drug monitoring of these compounds can inform clinicians when assessing potential side-effects of the drugs including hepatocellular damage or tinnitus. The predominate method for measuring acetaminophen and/or salicylate in biological matrixes is by enzyme immunoassay (EIA). EIA techniques and reagents are labeled and limited to use for serum testing. The purpose of this assay is to provide specific drug and metabolite quantitative data in alternative biological matrices.

Methods: Rapidfire is an automated sampling instrument that injects the sample onto a small cartridge packed with stationary phase. The instrument is programmed to load the sample onto a cartridge then rinse with aqueous mobile phase. After rinsing the sample, the Rapidfire diverter valve switches the flow path to the mass spectrometer. A highly organic mobile phase then elutes the purified compounds which are ionized by electrospray ionization prior to detection by the triple quadrupole mass spectrometer. The panel detects acetaminophen, acetaminophen-glucuronide, and salicylic acid. Deuterium labeled internal standards for acetaminophen and salicylic acid are utilized for isotope dilution. Quantitation is enabled by calibration of the system with 7 levels

of matrix spiked standards constructing a standard curve prior to sample analysis.

Results: We have developed a quantitative Rapidfire mass spectrometry (RFMS) method for the analysis of acetaminophen and salicylic acid in urine. The sample preparation method is simple. Samples are hydrolyzed, then diluted with an internal standard solution prior to loading on the RFMS. The analytical procedure is approximately 10 seconds from sample to sample. Due to the high variability of matrix affects in patients undergoing chronic pain or mental health treatment, it is necessary to have a robust analytical method. To that end, we have investigated a wide range of samples with variable creatinine concentrations. The calibration range of the method is 0.5-200 µg/mL for acetaminophen and 1-400 µg/mL for salicylic acid providing a wide analytical measurement range. External QC materials were also tested with acceptable imprecision and accuracy (<15%). Real patient samples have been analyzed and this procedure demonstrates a robust and reproducible method for this assay.

Conclusion: This technique provides rapid and quantitative analysis of acetaminophen and salicylic acid from biological specimens with minimal sample preparation.

A-390

Determination of Insulin-Like Growth Factor-1 in serum by HRAM LC-MS for clinical research

L. Cote[1], K. McCann[2], C. Chu[2]. *[1]Agilent Technologies, St-Laurent, QC, Canada, [2]Agilent Technologies, Santa Clara, CA*

Background: High Resolution Accurate Mass (HRAM) Liquid Chromatography Mass Spectrometry (LC-MS) is ideally suited for the rapid analysis of biomolecules. A highly sensitive and specific HRAM LC-MS method has been developed for the quantitation of Insulin-Like Growth Factor-1 (IGF-1) in serum. This method uses a simple sample preparation combined with an online sample cleanup procedure coupled to a high resolution accurate mass quadrupole time-of-flight mass spectrometer.

Methods: An efficient sample preparation procedure was developed for the extraction of IGF-1 in serum. Calibrators were created by spiking clean serum with various concentrations of IGF-1. The chromatographic system consists of a C8 extraction column coupled with a high resolution, 300 angstrom pore size analytical column and a mobile phase comprised of acetonitrile and water containing 0.2% formic acid. Quantifier and qualifier transitions were monitored and Rat IGF-1 internal standard was included to ensure accurate and reproducible quantitation.

Results: Online sample cleanup and chromatographic separation of a sample is achieved in less than three minutes. The separation of Rat IGF-1 and Human IGF-1 is especially critical since these compounds share common interferences. Without proper separation by retention time, impurities present in both compounds can cause interferences with one another and lead to inaccurate quantitation. The described method achieves the required functional sensitivity and is capable of quantitating IGF-1 over a sufficiently wide dynamic range. This method displayed excellent linearity from 5.63-990 ng/mL. All calibration curves displayed an R2 > 0.999. Back calculated accuracies for all calibrators ranged from 84% to 105% and showed intra-day CVs below 8%. Separately prepared incurred samples were used to test the accuracy and reproducibility of this method. Measurements were repeated in triplicates and on three separate extractions to assess intra- and inter- day reproducibility and CVs were found to be below 10%.

Conclusion: A robust method for quantifying Insulin-Like Growth Factor-1 in serum with excellent reproducibility and accuracy has been developed.

A-391

Fast Determination of Serum Methylated Arginines By Liquid Chromatography Tandem Mass Spectrometry

A. Unlu, S. Abusoglu, F. Akyurek. *Selcuk University, Konya, Turkey*

Background: NG,NG-dimethyl-l-arginine, or asymmetric dimethylarginine is a naturally occurring amino acid that circulates in plasma. Free ADMA, and related amino acids, NG-monomethyl-l-arginine, as well NG,N_G-dimethyl-l-arginine are produced normally by all cells from hydrolysis of proteins containing methylated l-arginine residues. SDMA shares the pathway for cell entry with arginine. Endogenous methylarginines are important potentially modifiable molecules that may be associated with impaired synthesis of nitric oxide. The aim of this study was to implement a fast, accurate and simple mass spectrometric method for serum methylated arginine.

Methods: 100 µL of internal standard in methanol were added to 200 µL of serum and centrifuged at 13.000 rpm for 10 minutes. Supernatant was evaporised with N2 flow at 65 ◦C. Derivatisation step was performed dissolving the dried extract in 200 µL of a freshly prepared butanol solution containing 5% (v v^{-1}) acetyl chloride and kept at 65 ◦C for 30 min. The solvent was removed by evaporation under nitrogen flow at 65 ◦C. The samples were dissolved in 200 µL of water–methanol (90:10, v v^{-1}) containing 0.1% (v v^{-1}) formic acid and 40 µL injected into system. Multiple reaction monitoring was performed with a continuous infusion of a 50 µM solution of each analyte. Recovery test was calculated as average of "measured value/expected value" ratio (%). Limit of detection and quantification were determined by a signal to noise ratio of 3:1 and 10:1, respectively. Inter- and intra-assay precision were evaluated by analysis of ten replicates of C1, C2 and C3, daily for 3 days and expressed as mean, SD and CV%.

Results: This method's intra-assay CV and % bias values were 15.6,10.2; 9.72,7.88 and 6.45,6.02 for 0.4, 0.8 and 1.6 µmol/L ADMA, respectively. Calibration curves in serum were obtained using concentrations of ADMA, SDMA, NMMA at 0.2, 0.4, 0.8, 1.6, 3.2, 32 µM and of Arg and Cit at 1, 25, 50, 100, 250 µM. The linearity of calibration curves in plasma was estimated by the coefficients of correlation (r^2), which ranged from 0.987 to 0.999. The standard curves for serum asymmetric dimethylarginine was linear within the range of 0.2-32 µmol/L. The equation for calibration was y=0.943x + 7.469 and R^2=0.992. Total run time was 5 minutes. Recovery was found to be between 90-105%. Limit of detection and limit of quantification were 0.1 and 0.25 µmol/L for ADMA, respectively. The intra- and inter-assay CV values were below 20% for SDMA; LNMMA, arginine and citrulline.

Conclusion: Satisfactory characterization, stability of the label during chromatography as well as mass spectrometry, standardization of commercially available as well as of self-synthesized stable-isotope labeled analogs of analytes, and final added concentration of the internal standard in the matrices being analyzed is essential and crucial for reliable

quantitative analysis. Data from calibration curves and method validation reveal that the method is accurate and precise. The short and fast run time, the feasibility of high sample throughput and the small amount of sample required make this method very suitable for routine analysis in the clinical setting.

A-392

Comparison of Q-TOF Acquisition Modes for Quantitative Analysis of Tetrahydrocannabinol Metabolite

H. Park[1], N. Chindarkar[2], R. Fitzgerald[2]. *[1]Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [2]University of California-San Diego, San Diego, CA*

Background & Objectives:

In clinical chemistry laboratories, unit resolution tandem mass spectrometry (MS) platform is considered the gold standard for quantitative analysis of small molecules. High resolution mass spectrometry (HRMS) is also a viable option with higher specificity and retrospective full scan analysis capability. The previously limited quantitative capability of HRMS has improved, such that both high performance screening and acceptable quantitation may now be feasible. We evaluated the quantitative performance of a quadrupole-time of flight (Q-TOF) analyzer for urine 11-nor-9-carboxy tetrahydrocannabinol (THC-COOH). Three Q-TOF acquisition modes MS^E, Q-TOF, and Q-TOF with enhanced product ion sensitivity (EPIS) were optimized and compared to unit resolution tandem MS.

In the Q-TOF mode, the quadrupole selects a precursor mass followed by collision cell fragmentation and TOF full scan analysis of product ions. Q-TOF-EPIS mode is the same except product ion sensitivity is increased by limiting the TOF scan range around a set m/z value. MS^E works in dual scan mode to collect TOF full scan data with low collision energy (precursor ion scan) and high collision energy/ramp (fragment ion scan). The quadrupole is RF only in MS^E mode.

Experimental: Waters Acquity UPLC with 1)Xevo G2 Q-TOF and 2)Xevo TQ-S were used with a Phenomenex (Kinetex, C8, 2.6µm, 50x2.1mm) column. Mobile phases A/B were 5mM ammonium formate (pH 3)/0.1% formic acid in water/acetonitrile respectively (flow 0.4mL/min). The gradient was 30-90% B in 4min. During solid phase extraction 2mL of urine, internal standard (THC-COOH-d3) and diluent were applied on Phenomenex Strata-X-Drug B cartridges. Eluates were dried and reconstituted in 100uL of mobile phase of which 10uL was injected on UPLC. Calibration was carried out by using 5 calibrators over a range of 12.5-200ng/mL spiked in drug free urine. All MS were used in negative mode, with optimized settings of cone voltage (40V), capillary voltage (2.5V), desolvation temperature (500°C) and desolvation gas (1000L/h).

Results and Conclusion:

Each Q-TOF mode was first optimized for ionization of THC-COOH. We compared the signal intensity of THC-COOH in each mode by acquiring data by infusing pure

THC-COOH in methanol and by analyzing patient urine specimens that screened positive for THC metabolites by immunoassay. These patient specimens and a CAP PT sample were analyzed for this study. The concentration range of 13.2-175.8ng/mL was observed among patient specimens. We found excellent correlation of THC-COOH concentrations among all TOF modes and the unit resolution tandem MS. Q-TOF-EPIS was 2.4 fold more sensitive than Q-TOF mode. Of the TOF modes, MS^E provided the maximum sensitivity for quantitation (m/z 343.2 for MS^E and 343.2/299.2 for Q-TOF-EPIS). MS^E mode was approximately 2.5 fold more sensitive than the Q-TOF-EPIS mode but was less sensitive than unit resolution tandem-MS. Our data indicate that quantitative analysis on the Xevo G2 Q-TOF compares favorably with traditional unit resolution MS but is less sensitive. Future experiments will determine if the high mass resolution achievable with the TOF can be used to shorten chromatographic run times without sacrificing specificity.

A-394

Ultrafast, high-throughput quantitative analysis of creatinine in serum by laser diode thermal desorption coupled to tandem mass spectrometry

J. Lacoursiere, P. Picard, A. Birsan. *Phytronix, Quebec, QC, Canada*

Background: Serum cretatinine is used as an important indicator of renal health. Elevated level of creatinine in serum is a key clinical biomarker of impaired kidney function in humans. In some clinical or pre-clinical studies, only small amounts (µL) of serum are available for creatinine analysis. Several analytical methods are available for creatinine detection and quantification, but require large serum volume or take more than 3 minutes per sample analysis. An alternative approach to the analysis of creatinine enabling high- throughput analysis and needing low serum volumes was developed, using the laser diode thermal desorption (LDTD) coupled to a tandem mass spectrometer (MS/MS).

The objective of this work is to analyze multiple real patient samples using the LDTD-MS/MS and cross validate the results against known traditional methods for the detection and quantification of creatinine in serum.

Methods: Sample preparation consisted of a protein precipitation extraction by adding 190 µL of IS solution (2000 ng/mL d3-Creatinine) in acetonitrile to 10 µl of serum. After vortex-mixing and centrifugation (2 min at 14000 rpm), a 2 µL aliquot was deposited in a LazWell plate, and allowed to dry at room temperature. Creatinine is an endogenous compound and calibration curve cannot be made into the serum matrix. Standards were prepared into water and treated similarly to real sample. A 2µl aliquot of the final extracts was deposited into Lazwell plate and dried completely before analysis. The LDTD laser power was ramped from 0 to 65% of maximum power in 6 seconds and maintained 1 second at 65%. The mass spectrometer is operated in negative ionization MRM mode, monitoring transitions 112 -> 41 for creatinine.

Results: A simple high-throughput protein precipitation method for creatinine analysis in serum was developed and validated. The optimization of instrumental parameters and a method application will be presented. The method demonstrated a linear dynamic range over two orders of magnitude, between 0.04 and 4 mg/dL. Standard curves of creatinine spiked serum extracted using this method shows good linearity (R2 between 0.9995 to 0.9992 over the quantification range. Three levels of QC samples were prepared by spiking known creatinine levels in serum for the validation tests. The endogenous concentration of creatinine was determined before the QC spiking addition. Sum of endogenous concentration and spiked concentration were used as nominal concentration. The accuracy measured of QC samples ranged from 92.9 to 99.9%. The quality controls had a precision error (% CV) of less than 15 % for inter- and intra-day assay. The use of negative ionization mode gave better signal intensities and eliminated interferences from the serum extract giving no false positives .The cross validation study with a traditional method for the analysis of creatinine confirmed the effectiveness of this new analytical approach using the LDTD-MS/MS. Analysis time (8 sec per sample) as well as sample throughput are significantly improved.

Conclusion: The LDTD-MS/MS method is an effective tool for the quantitation of creatinine at a rate of 8 seconds per sample to support preclinical and clinical studies.

A-396

Analysis of Pain Panel Medications in Urine on Raptor™ Biphenyl by LC-MS/MS

F. Carroll. *Restek Corporation, Bellefonte, PA*

The Restek™ Biphenyl has been the column of choice for clinical diagnostic and Pain Management drug screening testing because of its ability to provide highly retentive, selective, and rugged reversed-phase separations of drugs and metabolites. By bringing the speed of Superficially Porous Particles to the Biphenyl family, Restek's Raptor™ Biphenyl provides clinical labs with an even faster option for a wide variety of clinical assays. Drug screening applications can be difficult to optimize and reproduce due to the limited selectivity and ruggedness of the analytical column. The Raptor™ Biphenyl has been engineered to be rugged and selective with a pain management analyses that can be performed with a 5-minute cycle time and complete isobaric resolution. The Raptor™ Biphenyl beats popular competitor methods in both selectivity and performance.

Comparison analyses were performed on the Raptor™ Biphenyl 2.7µm, 50 x 3.0mm and competitor phenyl hexyl and C18 columns. Each manufacturer's own optimized method conditions were used in this evaluation. A pain panel drug standard was prepared in diluted urine and injected for assessment of retention and resolution. The ruggedness of the Raptor™ Biphenyl column was tested by performing a minimum of 2500 injections of a minimally diluted urine standard on a single column with the guard cartridge changed every 1000 injections. Retention time and response were monitored throughout the experiment. Analysis for both experiments were performed on a Shimadzu UFLC-XR HPLC equipped with an ABSCIEX 4000 LC-MS/MS using electrospray ionization in positive ion mode.

The Raptor™ Biphenyl displayed increased retention over the competitor phenyl hexyl and C18 columns. By improving the separation of the early-eluting compounds such as morphine, oxymorphone, and hydromorphone from hydrophilic matrix interferences, ion-suppression was decreased resulting in an increase in sensitivity. In addition, isobaric compounds such as morphine and hydromorphone display increased resolution and response. The Raptor™ Biphenyl has also proven to be rugged under high through-put conditions. In the first lifetime experiment a pain panel drug standard was diluted in urine 6x and filtered through 0.2 µm PVDF Thomson filter vials. The filtered matrix standards were injected on a single column with the replacement of the guard cartridge every 1000 injections. Under these conditions, the column lasted through 3000 injections when the study was ended. A second lifetime study was executed for 2500 injections using a new column and the same interval for guard cartridge replacement however the matrix standards did not receive any filtration. All 2500 injections were completed without a drastic change in response or peak shape. The Raptor™ Biphenyl 2.7µm, 50 x 3.0mm column has proven to withstand over 2000 injections of matrix samples regardless of filtration. It is the recommendation of Restek that with guard cartridge changes every 500 injections the column can last up to 3000 injections or beyond.

A-397

A reduced workflow SPE- LC-ESI-MS/MS method to distinguish healthy from elevated concentrations of metanephrine and normetanephrine in patient plasma samples

F. A. Kero[1], J. Ye[2], T. Enzweiler[1], V. Vandell[1], E. Gairloch[1], E. Majdi[2], H. Qiao[2], L. Cousins[2]. *[1]Biotage, Charlotte, NC, [2]Ionics, Bolton, ON, Canada*

Background: Adrenal neuroendocrine tumors known as pheochromocytomas induce excessive production of catecholamines in mammalian blood and urine. The salient metabolites, metanephrine and normetanephrine, are routinely screened as biomarkers for this condition in both matrices. The bottleneck of these analytical methods has traditionally been laborious sample preparation methods that mitigate the variability in matrix inherent with patient samples. Additional issues include the complexity of the measurement that challenges detectors that lack sensitivity and robustness. This report details a "load, wash, elute" weak cation exchange solid phase extraction procedure amenable to both plasma and urine samples. The extracts are subsequently injected into an LC-MS/MS system. The preliminary sample preparation method was developed at the Biotage US Applications lab (Charlotte, NC).The method was then transferred to Ionics (Bolton, ON, Canada) to facilitate the nmole/L measurements of the selected biomarkers by laminar flow tandem mass spectrometry. The SPE-LC-ESI-MS/MS method parameters were first optimized using pooled mixed gender plasma. A set of patient samples (n=32) was later supplied by the Mayo Clinic (Rochester, MN) that had previously been analyzed by a validated referee method. The population represented measured values across a range of clinical relevance.

Methods: Plasma samples were processed using a Biotage PPM96 positive pressure manifold. Plasma samples (100 microliters) were diluted with 50 mM ammonium acetate (300 microliters). The plasma samples were then loaded onto a Biotage EVOLUTE EXPRESS 30mg WCX 96 well plate. The plates did not require conditioning or equilibration steps. The samples were sequentially washed with 50/50% MeCN/MeOH and H2O. The analytes were eluted with 47.5/47.5/5% MeCN/MeOH/formic acid. Samples were then dried down using a Biotage SPE Dry nitrogen evaporator. The reconstituted extracts were analyzed using a Shimadzu LC system in tandem with an Ionics 3Q 220 triple quadrupole mass spectrometer.

Results: Pooled plasma samples yielded >80% recovery for both analytes. Analyte

suppression was determined to be <10%. The patient data obtained by this reduced workflow method compared well to historical data obtained by the validated referee method. The method LOQ was 0.1 nmole/L for normetanephrine and 0.05 nmole/L for metanephrine.

Conclusion: It is anticipated that this time saving and sensitive SPE-LC-ESI-MS/MS method will have significant impact in population screening strategies for these metabolites.

A-399

Sensitive LC-MS/MS assay for detecting testosterone in female, pediatric and male serum

C. Grote, S. Erfurth. *PeaceHealth Laboratories, Springfield, OR*

Introduction: Testosterone (Te) measurements are widely used to assess steroid hormone status in children and adults of both sexes. Immunoassays are often not sensitive enough to determine the lower Te concentrations found in children and females. An LC-MS/MS method was developed that provides a single assay with the necessary accuracy ($R^2 \geq 0.97$), precision (CV<10%) and sensitivity (LOQ<5 ng/dL) to routinely measure low concentrations of Te in children and females and higher Te concentrations in males.

Method: Serum (200 µL) extraction involves protein precipitation with 0.1 M zinc sulfate, SPE with Bond Elut Plexa columns and derivatization with 25% hydroxylamine. The chromatographic system consists of a Zorbax Eclipse Plus-C18 guard column, Zorbax Eclipse XDB-C18, 2.1x30 mm, 1.8 micron analytical column and a mobile phase comprised of A: 0.1% formic acid and 1 mM ammonium formate in water and B: 0.1% formic acid in acetonitrile. MRM transitions for qualifier and quantifier ions were monitored and a deuterated Te internal standard was added to each calibrator and specimen. Calibrators (1.0 to 2000 ng/dl Te) were prepared in 2% BSA. LC-MS/MS instrument included an Agilent 1260 HPLC Series SL binary pump, vial plate auto sampler with thermostat, temperature controlled column compartment and an Agilent 6460 QQQ with JetStream Technology and ESI source.

Validation: Ion suppression was evaluated by injecting extracted serum from two low (13 and 53 ng/dL) serum pools while monitoring sixteen phospholipid/lysophospholipids and Te MRM transitions. No phospholipid interference was observed at the Te retention time. Structurally related compounds (androstenedione, nandrolone, cortisol, corticosterone, 11-deoxycortisol, DHEA, progesterone, 5-alpha-dihydrotestosterone, 17-alpha-hydroxyprogesterone, 17-alpha-methyltestosterone and aldosterone) were evaluated for interference. An isotopic molecular ion of androstenedione monooxime, an isobar of Te, increased the concentration of Te by ≈ 0.8%. DHEA, an isomer of Te, produced a product ion transition of Te at a retention time of 0.81 minutes and does not interfere at physiologic concentrations. The average absolute and extraction recovery determined with pooled serum at 13 and 53 ng/dL was 100.4% and 78.2% respectively. Intraday and interday imprecision (CV) using 5 serum pools between 14 and 1031 ng/dL were < 4% and < 6% respectively. The LOQ determined by analyzing Te calibrators was 1 ng/dL. Extracted Te calibration curves from 1.0 to 100 ng/dL using a linear curve fit and 1.0 to 2000 ng/dL using a quadratic curve fit showed correlation coefficients of R^2=0.9996 and R^2=0.9998 respectively. Correlation of Te serum levels for females and children (n=88) with a national laboratory showed an R^2=0.974 and y=0.975x - 1.342 and combined serum levels for children and adults of both sexes (n=171) showed R^2=0.984 and y=1.069x - 1.495.

Conclusions: The method described is a highly sensitive (LOQ=1 ng/dL) and specific LC-MS/MS method suitable for analysis of serum Te in females, children and males. The formation of a Te oxime derivative allows use of a short 30 mm C18 column with a total run time of 3.0 minutes. The analytical protocol is free of cross reactivity, interference from structurally related steroids and phospholipids.

A-400

Application of an Immunocapture-LC-MS/MS Insulin Analogue Method to Clinical and Postmortem Insulin Investigations

S. L. Wong[1], G. Van Der Gugten[2], D. T. Holmes[2]. *[1]University of British Columbia, Vancouver, BC, Canada, [2]St. Paul's Hospital, Vancouver, BC, Canada*

Background: Synthetic insulins, or insulin analogues, are routinely prescribed in type 1 and advanced type 2 diabetes mellitus. Detection and quantitation of synthetic insulins are useful in confirming accidental or intentional overdoses of insulin analogues, as well as in the workup of aberrant insulin results.

In most laboratories, insulin concentration is determined by immunoassay. Insulin immunoassays exhibit variable cross-reactivities to synthetic insulins, are prone to interferences from insulin precursors/degradation products/autoantibodies, and fail to provide information on the type(s) of analogue(s) present. Given these limitations, we have developed an LC-MS/MS method for the identification of 5 commonly prescribed pharmaceutical insulins.

Methods: Twenty-five microliters of 500 µU/mL of bovine insulin (internal standard), and 5 µL of 5 g/L dextran sulfate + 0.5 M $MgCl_2$, were added to 1 mL of patient serum or calibrator (insulin analogues in 20% acetonitrile) in Eppendorf® LoBind tubes. The mixtures were incubated with 500 µL of paramagnetic beads coated with monoclonal mouse anti-insulin antibodies at room temperature for 1 hour. The beads were washed 3 times with 1 mL 0.01M PBS, and the insulin analogues extracted with 2 x 100 µL 1% acetic acid into a BSA-treated 96-well plate. Chromatographic separation was achieved with an ACE 300 C18 column (5 x 2.1 mm, 5 µm ID) with a run-time of 8.5 minutes. The samples were analyzed on an AB SCIEX QTRAP® 5500 system in positive ESI mode, with MRM transitions monitored for regular insulin (qualifiers m/z 1162/226, 1162/652; quantifier m/z 1162/345), lispro (qualifier 969/217; quantifier 1162/217), aspart (qualifiers 972/226, 1166/219; quantifier 972/136), detemir (qualifiers 987/454, 1184/357; quantifier 1184/454), and glargine (qualifiers 1011/1164, 1011/1179; quantifier 867/136). Calibration curves were constructed with linear regression using 1/x weighting.

Results: The method demonstrated good linearity over a concentration range of 5-200 µU/mL, with R >0.997 for all insulin analogues. Analytical recoveries were between 90.3% and 113.4%. Approximate LLOD and LLOQ were 3.5 µU/mL and 5 µU/mL, respectively. Within-run CVs ranged from 3.2% to 14.8%. The utility of the method was shown in a series of case studies: (a) postmortem investigations of deaths secondary to suspected insulin overdose (b) clinical workup of a type 1 diabetic patient who presented with hypoglycemia, and questions of whether excess synthetic insulin was administered deliberately, and (c) confirmation of insulin concentration in an insulinoma patient with discrepant insulin results from 4 commercial immunoassays.

Conclusion: We have developed a robust LC-MS/MS assay for the quantitation of 5 popular insulin analogues. It is valuable in the workup of insulin-related clinical and forensic cases, and has overcome some of the limitations exhibited by current commercial insulin assays.

A-401

Ultra-sensitive simultaneous LC-MS/MS quantification of human insulin, glargine, lispro, aspart, detemir and glulisine in human plasma using 2D-LC and a novel high efficiency column: method development and application in an overdose case

E. E. Chambers[1], K. Fountain[1], T. Rosano[2], C. Kim[2], J. Desemone[2]. *[1]Waters Corporation, Milford, MA, [2]Albany Medical College, Albany, NY*

Background: Recombinant human insulin and its analogs represent the primary treatment for insulin dependent Type I and Type II diabetes patients. Many these insulin formulations have or will be coming off patent shortly, generating a tremendous interest in quantitative methods for pharmacokinetic and bioequivalence assessments. In addition, there is interest in insulin quantification from an anti-doping and forensic perspective. Historically, insulins have been analyzed using radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). LC-MS analysis of insulins is needed due to the many shortcomings of these ligand-binding assays, chiefly: lack of standardization, cross-reactivity, limited linear dynamic range, and sample preparation time. Hybrid assays (affinity capture + LC-MS) have been the most effective though they lack the simplicity and throughput required for routine testing and bioanalysis. This work provides single method for quantification of intact human insulin and 5 insulin analogs in human plasma. This investigation solves both the selectivity and sensitivity problems encountered for accurate quantification of insulins in plasma since the former is not possible with conventional assays and the latter with conventional LC-MS/MS. We then retrospectively apply the method to a unique case of insulin glargine and aspart overdose which required prolonged dextrose infusion to prevent hypoglycaemia.
Methods: Blood samples were collected when the patient was admitted and approximately every 5 hours thereafter until symptoms no longer presented.Plasma samples are prepared using protein precipitation to remove high abundance proteins, followed by mixed-mode strong-anion exchange SPE to selectively eliminate closely related interferences and provide orthogonal ity. The multidimensional LC system includes at-column-dilution and trap and back elute components which improve sensitivity (through increased loading) and selectivity (clean-up achieved during the trapping phase.) Insulins are separated with formic acid in water (A) and ACN (B) on a superficially-porous, charged surface column packed with sub-2 µm particles using a linear gradient from 15 to 40% B.
Results: Method LOD's of 50-200 pg/mL were achieved for each insulin. Average

accuracy for standard curve points was 99-100%. Average inter-and intra-day accuracies for QC's samples were 98 and 94 %, respectively. Average inter- and intra-day precisions were 7.5 and 5.3 %, respectively. Matrix factors were calculated in 6 sources of human plasma and CV's of matrix factors for all analogs were <15 %. In addition, the presence of artificially high human insulin did not affect quantification of any of the analogs. Samples from two over-dose incidents were quantified. Dextrose infusion was required for 110 and 96 hours in the two cases.M1 metabolite and aspart were detected up to 90 and 22-29 hours, respectively, after admission. Higher levels of glargine M1 metabolite correlated to higher rates of dextrose infusion. **Conclusion:** This method represents a single, simple method for the simultaneous, direct quantification of intact human insulin and analogs in human plasma which achieves detection limits in the 50 pg/mL (8.6 fmol/mL) range. This assay was successfully applied to quantify glargine, its metabolite, and aspart in samples from two overdose cases. Disclaimer: This method is intended for clinical research use only, not for use in diagnostic procedures

A-402

Analytical and Clinical Validation of an LC-MS/MS Method for Urine Leukotriene E_4: a Marker of Systemic Mastocytosis

J. W. Meeusen, A. Lueke, A. Gray, L. J. Donato, J. H. Butterfield, A. K. Saenger. *Mayo Clinic, Rochester, MN*

Background: Systemic mastocytosis (SM) is a disorder that results in excessive accumulation of clonally derived mast cells in various tissues. When triggered, these mast cells release large amounts of histamine, prostaglandins and leukotrienes. This release of signal molecules causes intermittent "spells" with symptoms of itching, flushing, lightheadedness, tachycardia, gastrointestinal distress, or even loss of consciousness. Diagnostic criteria include the presence of mast cells in tryptase-stained biopsy sections (typically bone-marrow) plus one of the following: abnormal mast cell morphology; KIT Asp816Val mutation, CD25 positive mast cells, or serum tryptase > 20 ng/ml. Urine concentrations of N-methyl histamine (NMH) and 11-beta prostaglandin $F_{2\alpha}$ (BPG), the primary metabolites of mast cell derived histamine and prostaglandin, can aid in screening, reduce unnecessary biopsies and guide therapy. However, NMH and BPG lack sensitivity. Leukotriene E_4 (LTE_4) is the primary stable metabolite of total cysteinyl leukotrienes. We hypothesized that secretion of LTE_4 would be increased in SM and could be used alone or in combination with NMH and BPG to optimize screening for SM. Here we describe a novel liquid chromatography-tandem mass spectrometry assay to accurately and precisely quantitate LTE_4 in urine and outline its clinical utility in SM screening.

Methods: D_5-labeled internal standard was added to urine specimens and followed by an acetonitrile precipitation before injection into a Turboflow MAX mixed-mode anion exchange column with subsequent chromatographic separation using a C8 2.5-µm analytical column. LTE_4 was measured in negative ion mode using an AB Sciex API 5000. All LTE_4 concentrations were normalized to urine creatinine (enzymatic method, Roche).

Results: Intra-assay precision (%CV) determined in pooled urine specimens ranged from 2.6% to 5.0% at mean LTE_4 concentrations of 41, 631, and 1452 pg/mL (n=20). Inter-assay %CV determined over 20 days ranged from 6.9% to 8.2% at mean LTE_4 concentrations of 44, 445, and 1380 pg/mL. Linearity was determined between 0-2000 pg/mL and the mean recovery from mixing studies performed in triplicate was 100% ($y=1.01x-2.28$, $r^2=0.9985$). Limits of detection and quantitation were determined at 2 and 8 pg/mL, respectively. The normal reference range of <104pg/mg creatinine was determined based on the 95th percentile in a cohort of 128 healthy individuals.

Clinical performance was determined in 409 patients referred for clinical evaluation. SM was diagnosed in 66 (16%) patients according to World Health Organization criteria. Clinical sensitivity was 53% for BPG (>1000ng/24h) and 71% for NMH (>200µg/g creatinine) in our cohort of 409 symptomatic patients. Sensitivity improved to 86% with a specificity of 68% when BPG or NMH were both considered. Including LTE_4 (>104pg/mg creatinine) improved the SM diagnostic sensitivity to 97%, with minimal change in specificity (61%).

Conclusion: We have developed a sensitive and precise LC-MS/MS assay for quantitation of LTE_4 in urine. This assay has significant potential utility as a useful screening marker of SM, greatly improving screening sensitivity when used in combination with other biomarkers of mast cell activation. Incorporating LTE_4 into a panel including BPG and NMH provides a much needed screening tool for a complicated disease with non-specific symptoms and invasive confirmatory testing.

A-403

Addition of solid phase extraction to opiate sample preparation for UPLC-MS/MS

H. F. Mohammad[1], P. J. Bates[1], C. A. Hammett-Stabler[2]. *[1]University of North Carolina Hospitals, Chapel Hill, NC, [2]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC*

Background: Opiate testing has increased in parallel with the rise in patients seeking pain management care. These often prescribed drugs must be monitored due to their potential toxicity. Since qualitative immunoassay screening methods fail to distinguish specific opiates/opioids, clinical laboratories have turned to liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Sample preparation is a critical step in LC/MS/MS analysis. Currently our samples are prepared using "dilute and shoot (D/S)" after digestion with β-glucuronidase. The goal of this study was to assess the inclusion of an additional step involving solid phase extraction (SPE) in the sample preparation.

Methods: Drug free urine spiked with buprenorphine, norpubrenorphine, codeine, hydroxycodone, oxycodone, morphine, hydromorphone, oxymorphone, and 6-monoacetylmorphine (Cerilliant Corp) at known concentrations and previously tested patient samples were used in this study. In brief: 100 µl of urine was incubated with β-glucuronidase at 50° C for 1 h, and centrifuged. For the D/S method, 100 µl of the enzyme-digested sample was diluted 1:1 with water prior to analysis. For the SPE method, Oasis MCX µElution plates (Waters Corp, Milford, USA) were pre-conditioned with 200 µl methanol and 200 µl 5% formic acid. 100 µl of the enzyme-digested sample was applied and washed with 5% methanol in 5% formic acid solution. Samples were then eluted with 100 µl of 5:90 methanol:acetonitrile containing 5% of NH4OH solution. The elutes were dried under N2 and reconstituted with 100 µl water and analyzed. Opiate measurements were obtained using UPLC/MS/MS (ACQUITY UPLC/MS, Waters Corp) as described previously (1). Data were analyzed using EP Evaluator software (Data Innovations, LLC).

Results: The addition of the SPE step increased total analysis time by 10-15 minutes. We did not achieve a significant change in assay performance in terms of the analytical measuring range and linearity. The response curves for each of the nine drugs and metabolites were linear from 50-1000 ng/ml. However, recovery improved for all analytes; but particularly for 6-monacetylmorphine, hydroxymorphone, and norbuprenorphine at the lower concentrations where we saw improvement from 71-87% to >94% with the addition of SPE. Method comparison data using linear regression demonstrated good agreement with all correlation coefficients (R2) > 0.95.

Conclusion: The addition of SPE to our sample preparation increases cost by ~$3/sample and turnaround time by 10-15 minutes. However, we saw improved recovery across all analytes which may be attributed to a reduction in interferents from the sample matrices. This in itself is desirable as cleaner samples extend column life and reduce maintenance. Thus we believe the benefits of this additional step outweigh the costs and time.

1. Bates PJ, et al. Simultaneous detection of nine opiates, including buprenorphine and norbuprenorphine in urine using UPLC-MSMS. www.msacl.org

A-404

Using intact immunoglobulin light chains to quantitate rituximab by mass spectrometry

J. R. Mills, D. R. Barnidge, A. B. Girtman, P. M. Ladwig, D. L. Murray, M. R. Snyder. *Mayo Clinic, Rochester, MN*

Background: The therapeutic monoclonal immunoglobulin rituximab is used to deplete $CD20^+$ B-cells in the treatment of lymphoproliferative and autoimmune disorders. In autoimmune diseases, the exact mechanism by which B-cell depletion modulates disease activity is unknown and instances of clinical relapses can occur in the absence of detectable B-cells. The lack of methods to measure rituximab has restricted clinical studies aiming to better understand how drug levels relate to disease relapse.

Objective: Recently we demonstrated that microLC-ESI-Q-TOF mass spectrometry is capable of detecting residual levels of monoclonal light chains in human serum. We hypothesize this technology would be able to quantitate rituximab light chains in patient sera as a diagnostic tool, eliminating the need for tryptic digestion. Here we compare intact light chain quantification using microLC-ESI-Q-TOF MS to our current method using proteotypic SRM quantification on a triple quadrupole MS.

Methods: Rituximab intact light chain (iLC) quantification was performed on immunoglobulin-enriched serum reduced with DTT, separated on an Eksigent Ekspert liquid chromatography system, and analyzed on an ABSciex 5600 Triple TOF® mass

spectrometer. The peak area for the rituximab iLC (molecular mass - 23,034 Da) was found by integrating the molecular mass peak observed after deconvolution of the summed mass spectra from the rituximab elution time. The therapeutic mAb infliximab was added to each sample prior to Ig-enrichment as an internal standard. For the proteotypic quantitation, peptides unique to rituximab heavy chain (HC) and light chain (LC) variable regions were quantified by SRM from ammonium sulfate-crashed serum that was reduced, alkylated and digested with trypsin at 37°C for 12h. A proteotypic peptide from horse IgG was used as an internal standard and stable isotope labeled peptides were added to monitor retention times. Tryptic peptides were separated using a Thermo TLX-2 system then analyzed on an ABSciex API 5000 triple quadrupole mass spectrometer. Linearity, LOD, LOQ, intra-assay precision were assessed for both assays using rituximab spiked into human serum. For both methods, 6-point standard curves were generated [0-100 ug/mL] by spiking known amounts of rituximab into pooled human serum.

Results: Linearity was established by performing serial dilutions in human serum (100-1.0ug/mL; R^2=0.99). The rituximab iLC molecular mass peak area was detectable above the polyclonal immunoglobulin background with an LOD of 1.2 ug/mL and an LOQ of 2 ug/mL. Intra-assay precision was 6.7% at 100 ug/mL and 16.7% at 2ug/mL. We have established rituximab HC and LC proteotypic peptides have an LOQ ~2-fold lower. A method comparison using weighted linear regression between Intact and light or heavy chain peptides were comparable ([slope =0.99, y-intercept =-0.04 and R^2>0.99] and [slope =0.99, y-intercept =-0.03 and R^2>0.99], respectively).

Conclusion: Measurement of rituximab iLCs based on accurate mass assessment is a viable analytical approach. Quantitation of iLCs compares well to a proteotypic peptide approach, although differences in the analytical sensitivity of the methods may exist. Further studies using this methodology are warranted to understand how rituximab levels correlate with disease relapse.

A-405

Optimization and evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for the determination of total cholesterol in human serum and a comparison with field methods

H. Lee[1], S. Park[1], E. Ahn[1], S. Park[1], G. Lee[2]. *[1]Korea Research Institute of Standards and Science, Daejeon, Korea, Republic of, [2]Chungnam National University, Daejeon, Korea, Republic of*

BACKGROUND: Abnormal cholesterol levels are strongly associated with cardiovascular disease because these promote atheroma development in arteries. Lowering total cholesterol reduces the risk of coronary heart disease. For the reference intervals of cholesterol levels in blood are narrow, accurate methods of analysis for cholesterol in serum are essential, and the establishment of a reference and a definitive method has been needed to evaluate the field method. Isotope dilution gas chromatography-mass spectrometry has been accepted as a definitive method. Although ID GC-MS is considered to be highly accurate, it contains complex procedures such as derivatization. We describe simple ID LC/MS/MS as another method for the determination of total cholesterol in serum and its optimization conditions here. In order to compare with field method, proficiency testing programs were performed on the base of this method.

METHODS: Human serum samples were obtained from pooling of healthy human serum free from HIV, HCV, and HBV. Cholesterol-d_4 was used as an internal standard. 0.1 mL of serum sample was taken into an amber vial. An appropriate amount of isotope standard solution was spiked into the sample vial to make a 1:1 weight ratio. After adding 0.6 mL of an aqueous 8.6 mol/L KOH solution and 4 mL ethanol, we heated the solution in thermomixer for 3 h at 70 ℃ to hydrolyze the cholesterol esters. After the solution cooled, we added 5 mL of water and 10 mL of hexane, shook the tube for 5 min, separated the hexane phase, evaporated the solvent under vacuum, and dissolved the residue with 1 mL of ethanol. Aliquot of the ethanol solution was filtered and then analyzed by LC/MS/MS. The LC column was C_{18}, and kept at 50 ℃ during the chromatographic run. The mobile phase was methanol containing 0.1% acetic acid, and the flow rate was 0.3 mL/min. Serum samples were distributed to about 180 clinical laboratories in korea for the comparison with field methods.

RESULTS: The optimum volume of KOH solution for hydrolysis of cholesterol ester was about 5% of total sample mixture, and reaction time was 3 h. The optimized ID LC/MS/MS method was verified through the measurement of NIST SRM 909b and the participation in key comparison, and showed good agreement with the SRM values. The pooled serum samples were certified by this method, and used as materials for the proficiency testing programs. Expanded uncertainty of certification was about 2% within the 95% confidence interval. Proficiency testing programs of field laboratories have shown some discrepancies of 6.2% CV and 5.3% CV in total cholesterol results among the laboratories.

CONCLUSION: An optimized ID LC/MS/MS method was proposed as another method for the determination of total cholesterol in serum. This method was verified through comparison with the NIST SRM. We developed the two levels of total cholesterol CRM on the basis of this method and used them as materials for proficiency testing programs. Through the proficiency testings, it was possible to view the state-of-the-art of total cholesterol measurement by field laboratories.

A-406

LC-MS/MS Method For The Detection Of Free Thyroxine And Free Tri-Iodothyronine Using The Ionics 3Q 320 Triple Quadrupole Tandem Mass Spectrometer

V. Gounden, Z. Yuan, S. J. Soldin. *National Institutes of Health, Bethesda, MD*

Background: The majority of routine clinical laboratories perform free thyroxine (FT4) and free tri-iodothyronine (FT3) measurements on immunoassay (IA) platforms. These IA's are affected by changes in binding protein concentrations, have a weak inverse linear log relationship to TSH in hypo- and hyperthyroid individuals and have poor performance at the upper & lower values of the reference intervals. The gold standard for free thyroid hormone analysis involves preparation of sample using equilibrium dialysis. This is a time consuming and technically difficult technique. However liquid chromatography-tandem mass spectrometry (LC-MS/MS) following ultrafiltration of the sample at 37° C (method as previously described by Gu et al. Clin Biochem. 2007;40;1386-1391) has been shown to perform better than IA in the above described circumstances and involves a simpler more convenient sample preparation than equilibrium dialysis. Our objective was to improve on the sensitivity of this initial method. Here we describe our 3rd generation LC-MS/MS method with improved sensitivity over the initial mass spectrometry method.

Method: Sample preparation was performed by ultrafiltration of 500ul of serum using a 30-kDa centrifugal filter (Centrifree YM-30, Millipore). Following addition of sample to filtering device samples were centrifuged in a temperature controlled centrifuge at 1113g for 30 minutes at 37 °C. 150 ul of the ultrafiltrate was added to 450ul of methanol containing deuterium-labeled internal standards (IS) for FT4 and FT3 and centrifuged. 350 uL of the supernatant was further diluted with water, vortexed and 200 uL injected into LC-MS/MS. FT4, FT3 were detected by electrospray ionization in negative mode with the following transitions: FT4 775.6>126.7 and FT3 649.9>126.7. LC-MS/MS setup consisted of a Shimadzu UFLCXR HPLC system interfaced to a Ionics 3Q 320 triple quadrupole tandem MS. Chromatographic separation was performed using a Poroshell 1.7µm C18 column (100mmx2.1mm) with a gradient mobile phase (A: 2% Methanol in water containing 0.01% acetic acid; B: 98% Methanol, at a flow rate of 0.5mL/min. Run time per injection was 13 minutes.

Results: The method described displayed good linearity over a concentration range of 0-25 pg/ml (FT3) and 0-5 ng/dL (FT4) with r2 >0.995. Between day precision CVs for across the concentration range were: FT3 4.8-8.8 %; FT4 7.5-7.8%. Lower limit of Quanititation (LLOQ) at signal to noise ratio(S/N) =10 was 0.2 pg/ml for FT3 and 0.05 ng/dL(S/N=20) for FT4.

MS MS comparison r values with our first generation method were 0.87 and 0.82 for FT4 and FT3 respectively.

Conclusion: The sensitivity of the 3rd generation FT4/FT3 method described above is greatly enhanced due to improvements in mass spectrometer and column technology. LLOQ is now 10 fold lower than that found for previous FT4/FT3 methods.

A-407

A Simple and Robust Targeted Quantitative Method for Insulin and its Therapeutic Analogs

E. E. Niederkofler[1], S. Peterman[2], A. Leber[1], K. Antwi[1], T. Schroeder[3], U. A. Kiernan[1], K. A. Tubbs[1], B. Krastins[2], A. Prakash[2], J. Frahm[1], M. Lopez[2]. *[1]Thermo Fisher Scientific, Tempe, AZ, [2]Thermo Fisher Scientific BRIMS, Cambridge, MA, [3]Thermo Fisher Scientific, Somerset, NJ*

Background – The measurement of Insulin has been identified as a paramount metric in clinical research, drug development, forensic toxicology, and sports doping applications. Conventional insulin assays are plagued by the inability to differentiate endogenous insulin from exogenous insulin analogs. The use of LC-MS can overcome this shortcoming; however, the LC-MS methods to-date lack the analytical sensitivity demanded by the field. Therefore, a highly-analytically selective sample interrogation workflow is required to address the complexity of plasma samples and, ultimately, for accurate and analytically sensitive LC-MS detection and quantification. To meet these

requirements, a Mass Spectrometric Immunoassay (MSIA) method was developed for the high-throughput, analytically-sensitive quantification of insulin and its analogs from human plasma.

Methods – Both neat and plasma samples containing a mix of insulin and its analogs at various concentrations were analyzed. A heavy version of insulin was used as an internal reference standard and spiked into each sample prior to target selection. In a 60-minute protocol (per 96 samples) the Thermo Scientific™ MSIA D.A.R.T.'S™ pipette tips derivatized with a pan-anti insulin antibody were used for insulin target selection. After affinity enrichment, MSIA detection and quantification was achieved in a 10 minute LC-MS method on a Thermo Scientific™ Ultimate™ 3000 LC system coupled to a Thermo Scientific™ Q Exactive™ mass spectrometer. Full MS scans were acquired, thus enabling the full characterization and quantification of multiple insulin analogs from a single sample.

Results - One of the primary limitations to current insulin assays is the inability to distinguish between endogenous and exogenous insulin analogs. The immobilized insulin pan-antibody in the MSIA D.A.R.T.'S pipette tips recognizes a common epitope region in the beta chain that is conserved across all of the analyzed variants, which allows the capture and detection of all variants from the sample. Further, utilizing full scan MS mode in the analysis stage of the MSIA workflow enables simultaneous detection of multiple insulin analogs and the ability to screen for unsuspected insulin analogs post-acquisition. Accurate intact mass and fragmentation of the insulin analogs confirmed the identity of each variant.

An additional limitation to high-throughput targeted quantification of insulin and its analogs are inefficient sample preparation protocols that result in their lack of analytical sensitivity and robustness. Using the MSIA Insulin workflow described above, we achieved an LLOQ of 15 pM (87 pg/mL) and an LOD of < 7.5pM (~47 pg/mL) for the intact variants in plasma. Further, reproducibility studies demonstrated inter- and intra-day CV's of < 3% and spike and recovery resulted in recoveries of 96-100%. In addition to the improved analytical sensitivity, the MSIA workflow significantly reduces the background matrix. The reduced complexity affords shorter LC gradients, and, therefore, shorter LC-MS analysis times. Altogether these results demonstrate the high analytical sensitivity, reproducibility, and robustness of the MSIA Insulin workflow in clinical research methodology.

Conclusion – A robust clinical research methodology incorporating antibody-directed target selection from a complex matrix with highly-analytically sensitive LC-MS detection was developed for the qualitative and quantitative simultaneous analyses of multiple insulin analogs.

A-408

Development and Validation of a High Performance Liquid Chromatography Tandem Mass Spectrometry 9 Steroid Panel using Minimal Sample Volume

B. Stolze[1], V. Gounden[1], J. Gu[1], S. J. Soldin[2]. *[1]National Institutes of Health (Clinical Center), Bethesda, MD, [2]National Institutes of Health (Clinical Center) and Department of Medicine, Georgetown University, Washington DC, Bethesda, MD*

Background and Objectives: Steroid profiles play a critical role in the evaluation of endocrine disorders. High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), with superior sensitivity, specificity, and simultaneous multi-analyte quantitation capabilities, is the preferred method for steroid analysis. Our first generation steroid profile by HPLC-MS/MS simultaneously measured 9 steroids in 18 minutes using 760µL of serum. Our second generation reduced the sample volume to 200µL with a 1 mL injection. Our objective was to improve on our initial methods whilst reducing sample volume and run time. Here we describe our third generation steroid profile assays, quantifying 9 steroids with a run time of 11.5 minutes using a 50uL sample volume and 100uL injection volume, while providing better sensitivity, specificity, and a ten-fold lower limit of quantitation due to improvements in mass spectrometric and column technology.

Method: An Agilent 6490 triple-quadrupole MS coupled with an Atmospheric Pressure Photoionization (APPI) source and Agilent 1200 Infinity series HPLC were used employing isotope dilution with deuterium labeled internal standard for each analyte. 50uL of serum were deproteinized by adding 75µL of acetonitrile containing internal standards. After centrifugation, 75µL of supernatant was diluted with 250uL of water and a 100µL aliquot was injected onto a Poroshell 120 EC-C18 column. After column washing the steroids were eluted using a methanol gradient as follows: 80% A (methanol: water 2:98, v/v) for 3 minutes, 50% B (methanol: water 98:2, v/v) to 58% B over 3 minutes, 58% B to 90% B over 1 minute, holding at 90% B for 1.5 minutes, and finally 90% B to 20% B in 0.01 minutes. Quantitation for all 9 analytes was performed in positive MRM mode. Instrument parameters were as follows: gas temperature 325 oC, vaporizer 400 oC, gas flow of 11 L/min, nebulizer 60psi, and capillary 4000V.

The MRM for each analyte and compound dependent parameters are listed below:

Cortisol 363.3/121.1 Collision energy (CE) 26; Cortisone 361.2/163 CE 22; 11-deoxycortisol 347.3/97.1 CE 30; Corticosterone 347.2/121.2 CE 22; 17α-hydroxyprogesterone 331.2/109.1 CE 30; Progesterone 315.3/109.1 CE 26; Testosterone 289.1/109 CE 22, Androstenedione 287.1/97.1 CE 18, 21-deoxycortisol 347.4/311.3 CE 13,

Results: Within-day CVs ranged from 2.4-9.5% and between-day CVs from 3.0-9.9%. Method comparison analysis was performed using split sample analysis of 20- 75 serum samples. MS to MS comparison studies yielded r-values between 0.943 and 0.997 with recoveries from 90-105%. Regression analysis slope and intercept values for all steroids in the panel were as follows: slope range 0.89-1.1; intercept range -0.3 to 6.4

Conclusions: Our method measures 9 steroids in 11.5 minutes with minimal sample volume and preparation. This method is advantageous in a clinical environment because of simple sample processing, increased sensitivity, and high-throughput.. The low sample volume used permits assessment of steroid status in neonates and infants thereby optimizing early diagnosis of endocrinopathies. The low limits of quantitation make this method ideal for measurement of androgens and estrogens in women and prepubertal children.

A-409

Quantitation of 1α,25-dihydroxyvitamin D using solid-phase extraction and fixed-charge derivitization in comparison to immunoextraction

N. Chan, E. Kaleta. *Houston Methodist Hospital, Houston, TX*

Background: Quantitation of 1α-25 dihydroxyvitamin D (DHVD) has been a difficult task due to the relative concentration of this metabolite with respect to 25-hydroxyvitamin D, and well known cross-reactivity of immunoassays. Immunoextraction (IE) techniques have been well characterized, but can be costly due to antibodies required for extraction. As an alternative to IE, solid-phase extraction (SPE) coupled to enhanced ionization with fixed-charge derivitization has been made commercially available. This work describes the validation of the SPE method directly comparing against IE with traditional triazole-dione derivitization.

Methods: DHVD was extracted by SPE and IE preparations prior to LC-MS/MS. SPE was performed using Amplifex™ C1000 and S500 cartridges with diisopropyl ether and hexane/isopropanol extraction, and Amplifex™ Diene reagent derivitization (AB Sciex, Framingham, MA). Immunoextraction was performed using ImmunoTube® 1,25(OH)2 Vitamin D extraction kits (ALPCO), with derivitization using 9mmol/L 4-phenyl-1,2,4-triazole-3,5-dione (PTAD, Sigma). LC was performed for quantitation using an Acuity UPLC BEH C18 column (Waters), A: H_2O, 0.1% formic acid and B:acetonitrile, 0.1% formic acid, from 37%B to 51%B over a 3.5 min linear gradient. MS/MS was performed using an AB Sciex 5500 Q-Trap with multiple reaction monitoring (MRM) for DHVD2 and DHVD3. Clinical validation was performed for each laboratory developed test, including accuracy, intra- and inter-assay precision, reportable range, reference range, sensitivity and specificity.

Results: Extraction of DHVD by SPE and Amplifex™ derivitization demonstrated similar assay performance to the traditional IE followed by traditional PTAD derivitization.

	SPE-Amplifex		IE-PTAD	
	DHVD2	DHVD3	DHVD2	DHVD3
Linear Range	4-200 pg/mL	4-200 pg/mL	4-200 pg/mL	4-200 pg/mL
Intra-assay Precision				
12 pg/mL	7.1	5.5	11.4	8
60 pg/mL	11.4	9.2	8.9	6.1
Inter-assay Precision				
12 pg/mL	13.30%	7.20%	8.80%	12.80%
60 pg/mL	4.00%	5.50%	6.10%	8.20%
Limit of Detection	1.9 pg/mL	2.7 pg/mL	2.7 pg/mL	1.7 pg/mL
Limit of Quantitation	4 pg/mL	4 pg/mL	4 pg/mL	4 pg/mL

The SPE method requires more technologist time (1hr vs 30 min), but requires equal derivitization and LC time, making overall assay time comparable. Cost analysis shows the SPE method to be lower cost than IE by avoiding expense associated with antibody extraction.

Conclusion: Work demonstrated that SPE shows comparable analytical performance to IE, showing promise for utility as a clinical method for sensitive measurement of DHVD.

A-410

Development of an Ultra Pressure Liquid Chromatography -Tandem Mass Spectrometry Method for Pain Management Drugs in Urine

A. A. Ejilemele[1], S. Sharma[1], D. Patel[1], J. Petersen[2], R. Patel[1]. *[1]Universal Clinical & Research Laboratories, Webster, TX, [2]University of Texas Medical Branch, Galveston, TX*

Background: Provision of adequate pain relief is an important standard of care in the management of chronic pain and the use of opioids is one of the mainstays of the management plan for this category of patients. These medications provide partial analgesia and maintain or improve function. However, their use must be monitored by regular urine screening in order to monitor compliance, identify diversion as well as the concomitant use of drugs of abuse.

Methods: The UPLC-MS/MS quantitative method detected 17 drugs and 5 metabolites. Calibrators were prepared in drug free urine using certified reference materials from Ceriliant at five levels ranging from 50 -1000 ng/mL for 20 high concentration analytes and 5 - 100 ng/mL for 2 low concentration analytes. Quality control samples were also prepared in drug free urine at three target levels (125 ng/mL, 375 ng/mL and 750 ng/mL for the 20 high analytes and 12.5 ng/mL, 37.5 ng/mL and 75 ng/ml for the two low concentration analytes). Preparation of sample/standards/quality control for analysis required the dilution of 100 µL with 850 µL of diluent (0.1% formic acid in 100% methanol) and 50 µL internal standard. The internal standard contained 22 analytes at a concentration of 1000 ng/mL. The 22 analytes were separated on a Waters Acuity TQD instrument using a BEH C18 1.7µm 2.1 X 50 mm column and binary mobile phase (A: 0.1% formic acid in 5 mM ammonium acetate; B: 0.1% formic acid in 100% Methanol) within 7.5 minutes and a flow rate of 0.3 mL/min. Analytes were detected on the tandem mass spectrometer in a positive ion mode. Chromatographic peaks of each analyte were acquired using quantitating and confirmatory ion transitions at cone voltages and collision energies specific to each compound. Accuracy was tested using recovery experiments.

Results: All analytes had linear calibration curves (r2 >0.950 for the 20 high concentration analytes; r2 > 0.999 for the 2 low concentration analytes). The within run coefficient of variation (CV) for the low, medium and high QC ranged from 1.9 - 12.2% for the high concentration analytes and 1.6 - 12.4% for the low concentration analytes at three levels. The between run CV for the low, medium and high QC ranged from 0.05 - 10.8% for the high concentration analytes and 0.1 - 8.6% for the low concentration analytes. Matrix effect, carryover and interference were minimal. Samples could be diluted a minimum of eight fold and still remain linear. Analysis of 24 positive and 50 negative CAP samples as well as 10 spiked samples gave excellent correlation with expected concentrations (r2 > 0.97 for all analytes).

Conclusion: We developed a rapid, linear, accurate and sensitive UPLC-MS/MS method for the measurement of 17 pain management medications and 5 metabolites which is suitable as a screening and confirmatory method.

A-412

Evaluation of Q-Exactive coupled with liquid chromatography for Total Testosterone and Dehydroepiandrosterone Quantification in Serum

G. Altawallbeh[1], D. R. Bunch[2], J. Gabler[3], S. Wang[2]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH, [3]ThermoFisher Scientific, San Jose, CA*

Background: Clinically, total testosterone (TT) and dehydroepiandrosterone (DHEA) are measured in men and women for androgen abnormalities, and in pediatrics for cases of delayed or precocious puberty. Historically, immunoassays were most often used to measure TT. However, studies have shown that immunoassays overestimate the serum TT at the lower concentrations typically found in females and pediatrics. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been identified as the "gold standard" technology for steroid determination. Most of the published LC-MS/MS methods for TT and DHEA analysis are performed on triple quadrupole MS. Recently, a high resolution quadrupole-Orbitrap (Q-Exactive) MS is available and may offer improved specificity. **Purpose:** To evaluate the bench-top Q-Exactive MS coupled with LC for the quantification of low levels of TT (2.5 ng/dL) and DHEA (20 ng/dL) in serum and to investigate possible interferences from blood collection tubes. **Methods:** Charcoal stripped serum was spiked with testosterone and DHEA then serially diluted at 10 concentrations to demonstrate linearity. Female serum specimens were obtained in BD vacutainer tubes with serum separator (SST) and without serum separator (Non-SST) and compared to check for interference. All samples (200 µL) were spiked with testosterone-d3 internal standard (25 µL; 225 ng/mL) and extracted with methyl tert-butyl ether. The supernatant was evaporated at 40°C under a stream of nitrogen then derivatized with hydroxylamine (50 µL; 100 mg/mL). Methanol (50 µL) was added then it was incubated for 30 min at room temperature before injection of 50 µL. The analysis was carried out on an LC-Q-Exactive system using an Accucore C18 column (50 x 2.1 mm, 2.6 µm). **Results:** The coefficient of variation for the linearity study was <15% for both DHEA and testosterone. DHEA was linear from 4-4000 ng/dL using mass transition 304.2>253 with a correlation of R^2=0.9877. Testosterone was linear from 0.12–120 ng/dL and had an R^2=0.9706 for 304.2>111.6 and R^2=0.9727 for 304.2>123.6. The SST to Non-SST comparison demonstrated interference with testosterone for mass transition 304.23>111.57 in SST which confirms published findings. Accurate mass was unable to eliminate the interference, however there was no interference for mass transition 304.2>123.6. Testosterone and DHEA were separated both chromatographically and with unique mass transitions post-derivatization. **Conclusions:** The Q-Exactive MS coupled with LC can be used to quantify TT and DHEA at very low concentrations.

A-413

A Sensitive and Rapid Liquid Chromatography-Tandem Mass Spectrometry Method for Quantification of Arginine Derivatives

D. A. Payto, C. Heideloff, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Arginine (Arg) is the substrate of nitric oxide synthase for the production of nitric oxide. Arginine can be methylated to form asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) through the activity of methyltransferases. ADMA is an endogenous inhibitor of nitric oxide synthase and a biomarker for endothelial function. SDMA is a biomarker for renal function that has been shown to outperform creatinine based equations for determining estimated glomerular filtration rate (GFR) in predicting kidney function when compared to measured GFR (mGFR). **Objective:** To develop a sensitive and rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measurement of Arg, ADMA, and SDMA in plasma. **Methods:** EDTA plasma (50µl) and 50µL of internal standard (IS) solution (1.8µM Arg-IS as L-arginine:HCL [U-^{13}C6, 97-99%], 0.5µM of ADMA-IS as ADMA:HCl:H_2O [2,3,3,4,4,5,5-d_7, 98%] and SDMA-IS as [N^G,N^G-Dimethyl-L-arginine-d_6]) were vortex mixed. 1% ammonium acetate in methanol (300µL) was added to the mixture, vortex mixed and centrifuged. Supernatant (50µL) was mixed with 150µL of 1% formic acid in acetonitrile and 10µL was analyzed on an LC-MS/MS system using a Polaris Si-A analytical column. Total chromatographic run time was 3.5 minutes. Multiple Reaction Monitoring (MRM) transitions were 175.00-70.40, 203.05-46.50, 203.00-172.10 for Arg, ADMA, and SDMA respectively. **Results:** Matrix effects were shown to be compensated by the deuterated internal standards through a mixing study. No carryover was observed up to 822.1µM, 8.9µM, and 9.3µM for Arg, ADMA, and SDMA respectively. Analytical Measurement Range (serial dilution of a spiked patient pool), analytical recovery, and CV (based on CLSI EP10-A3 guidelines) are shown in table 1. **Conclusion:** This validated LC-MS/MS method offers sensitive and rapid quantification of Arg, ADMA, and SDMA in EDTA plasma.

Table 1: Method Validation Data

	Arginine	ADMA	SDMA
Analytical Measurable Range	7.40-1022.3 µM	0.09-9.54 µM	0.09-11.50 µM
Analytical Recovery (%)	87.6-114.9	89.3-114.0	100.2-106.6
Total CV (%)	8.2-10.4	6.8-9.1	6.4-8.8
Intra-Assay CV (%)	5.7-10.3	4.6-8.2	4.4-6.1

A-414

Flow injection-tandem mass spectrometry for inborn error metabolism research using a meta calculation software

J. Lai[1], B. Hart[1], C. De Nardi[2], M. Kozak[1], K. Van Natta[1], B. Duretz[3], D. C. Kasper[4]. *[1]Thermo Fisher Scientific, San Jose, CA, [2]Thermo Fisher Scientific GMBH, Dreieich, Germany, [3]Thermo Fisher Scientific, Villebon, France, [4]Medical University of Vienna, Vienna, Austria*

Background: Since bacterial inhibition method was first developed for research of inborn error metabolism in 1960, the technology has changed drastically from EIA, RIA, FIA, ELISA to LC and Tandem MS over the past 50 years.

Tandem MS allows for higher quality results compared to the old approaches. However, manual data processing in the post-analytical phase still remains a common cause of errors in the total testing processing.

This research describes a method of flow injection-tandem MS in analyzing donor samples for the quantitation of amino acids and acylcarnitines with a meta calculation software.

Methods: Samples were extracted from dried blood spot cards; the internal standards

were added during the extraction procedure and extracted samples were derivatized prior to injection onto an LC-Tandem MS system. QC samples were added to the batch.

The flow injection was conducted using a LC with open-tube providing an automated sample introduction to a Tandem MS (Thermo Scientific) without chromatographic separation. The Tandem MS used Selected Reaction Monitoring scanning for the detection of amino acids and acylcarnitines. This beta version software is developed for an automatic calculation of mass ion ratio and user defined formulas uaing data files generated from Tandem MS.

Results: A total of 41 samples and 779 analytes were processed.

The comparison result of this sample set shows that over 99% of concentration calculations (Analytes and Formulas) are within 10% of bias. Over 87% of Formulas Ratios are within 10% of bias. Table below shows comparison between software calculations (One-Step) and manual calculations (Multiple-Steps).

Conclusion: This offline automated data processing tool shows a good agreement with manual calculation process, and it can process both concentration and user defined formulas. This meta calculation software improves time effectiveness by eliminating manual calculation process and removing transcription errors in post-analytical phase.

Comparison Between Software Calculation and Manual Calculation

Type of Calculations	Analyte/ Formula	Number (N)	Bias%	R2	Linearity Equation	Value Range
Analyte Concentration	C0, C8, C14, C14:1, C16, Cit, Met, Orn, Phe, Tyr	410	< 20%	0.9986	Y = 0.0593 + 0.9993 X	0.81 to 199.15(ng/mL)
		407	< 10%			
		381	< 5%			
Formula Concentration	F1=C0 + C14:1	41	< 5%	0.9992	Y = -0.7703 + 0.9996 X	43.98 to 167.04 (ng/mL)
Formulas Ratios	F2=(Orn - Phe)/ Tyr	82	< 40%	0.9966	Y = 0.0039 + 0.9983 X	-0.98 to 2.25
		80	< 20%			
	F3=(C8 + C14:1 - C16)/ (Orn + Tyr)	72	< 10%			
		61	< 5%			

A-415

Analysis of serum testosterone and androstenedione for clinical research using either manual or automated extraction

D. Foley[1], B. Keevil[2], L. Calton[1]. *[1]Waters Corporation, Wilmslow, United Kingdom, [2]University Hospital of South Manchester, Wythenshawe, United Kingdom*

Background: Here we evaluate a UPLC/MS/MS method used to measure serum testosterone and androstenedione enabling investigation of metabolic dysfunction for clinical research purposes. An analytically selective method was developed using a mixed-mode Solid Phase Extraction (SPE) sorbent in 96-well plate format. Either manual or automated extraction was employed, providing flexibility in sample preparation options depending on the laboratory environment.

Methods: Certified testosterone and androstenedione reference material purchased from Cerilliant (Round Rock, TX) were used to create calibrators and QC material in stripped pooled serum purchased from Golden West Biologicals (Temecula, CA). Hormone Standardization (HoSt) testosterone certification program samples from the CDC (Atlanta, GA) were used to provide an initial assessment of analytical method bias. A set of serum samples (University Hospital of South Manchester, UK) were analyzed using the newly developed method and an independent LC/MS/MS method for testosterone and androstenedione and results were compared. This same set of serum samples was used to show equivalence of the manual and automated extraction techniques. All samples were pre-treated with ammonia, zinc sulphate and methanol. SPE was carried out with a Waters® Oasis® MAX µElution 96 well plate to reduce ion suppression and concentrate the samples without the need for evaporation. Automated extraction was performed using the Waters Offline Automated Sample Preparation Station (OASPS). Using an ACQUITY UPLC® I-Class system, samples were injected onto a 2.1 x 50 mm Waters ACQUITY UPLC HSS C18 SB column using a water/ methanol/ammonium acetate gradient and quantified with a Waters Xevo® TQD Mass Spectrometer.

Results: The method was shown to be linear from 0.05 - 15 ng/mL for testosterone and androstenedione. Coefficients of variation (CV) for total precision and repeatability on 5 separate days for low (0.15 ng/mL), mid (1.0 ng/mL) and high (10 ng/mL) QC samples were all < 6% (n = 30) for both testosterone and androstenedione using manual or automated extraction. Comparison with the values assigned to HoSt testosterone certification program samples analyzed with this method was described by the Deming equation y = 1.07x - 0.03 and Bland Altman mean bias was shown to be < 5% for testosterone. Comparison with samples previously analyzed by the independent LC/MS/MS method were described by the Deming equations y = 1.06x + 0.03 and y = 1.00x - 0.09 for testosterone and androstenedione, respectively. Comparison of the manual and automated extraction techniques within our laboratory was described by the Deming Equations y = 1.01x + 0.01 and y = 0.97x + 0.17 for testosterone and androstenedione, respectively. Bland Altman mean bias between the manual and automated methods was shown to be < 2.5% for both testosterone and androstenedione.

Conclusion: We have successfully quantified serum testosterone and androstenedione using both manual and automated SPE with UPLC/MS/MS for clinical research purposes. The method demonstrates excellent linearity, precision and accuracy.

For Research Use Only, Not for Use in Diagnostic Procedures.

A-416

Alternative Calibration Strategies for LC-MS Based Analysis of Broad Reportable Range Analytes

C. Crutchfield, M. T. Olson, W. Clarke. *The Johns Hopkins University School of Medicine, Baltimore, MD*

Background: Traditional LC-MS calibration strategies are associated with increased cost and time to result relative to response factor (Rf) based calibration strategies. Most reports using Rf based calibration have been applications where the AMR is less than two orders of magnitude. Testosterone requires an AMR of ~3 full orders of magnitude to encompass both male and female reference ranges (Females: 2-45 ng/ dL; Males: 250-1,100 ng/dL). Accurate determination of the analyte:internal standard ratio is problematic as the ratio diverges from unity particularly when A/IS = <0.1, >10. We wanted to examine the response factor performance of using ***two*** non-isobaric isotopically labeled internal standards, Testosterone-$^{13}C_3$ and Testosterone-$^{2}H_5$, placed at different concentrations in the same solution.

Methods: Testosterone calibrators at 2, 5, 10, 20, 50, 100, 200, 500, 1000 & 2000 ng/dL were prepared with internal standard concentrations of 20 ng/dL Testosterone-$^{13}C_3$ and 200 ng/dL Testosterone-$^{2}H_5$. QC material was prepared with testosterone at 2.2 ng/dL, 75 ng/dL, & 1,800 ng/dL. Measurements were performed using a Prelude SPLC coupled to Thermo TSQ Vantage mass spectrometer equipped with a HESI-II probe using reverse phase chromatography. Response factors were generated as n=12 replicate measurements (Testosterone-$^{2}H_5$ Rf = 1.03 $\pm$ 0.09; T-$^{13}C_3$ Rf = 0.71 $\pm$0.17, 95% CI). Complete linear regression included all calibrators in the set with 1/ x^2 weighting; constrained linear regression excluded the bottom 3 and top 3 calibrators for the High IS and Low IS, respectively.

Results: The concentration of the internal standards influenced performance with respect to the recovery and CV of the QC materials. Interestingly, the higher concentration internal standard performed as well as the low concentration internal standard. The table includes a complete comparison.

Conclusion: These data demonstrate the feasibility of applying RF based calibration to testosterone analysis; however, optimization of internal standard placement and resolution of the disparity of IS response factors warrant further investigation.

		Calibration Strategy					
		Complete Linear Regression		Constrained Linear Regression		Response Factor	
		Low IS	High IS	Low IS	High IS	Low IS	High IS
High QC	Bias	18%	19%	28%	8%	39%	20%
	CV	7.0%	4%	7%	4%	7%	4%
Mid QC	Bias	5%	12%	14%	8%	24%	12%
	CV	7%	4%	7%	3.8%	7%	4%
Low QC	Bias	11%	7%	9%	235%	13%	-7%
	CV	10%	6%	11%	2%	7%	7%

A-417

Comparison of Voriconazole Levels Using LC-MS/MS and HPLC.

M. Hinsdale[1], A. Veenis[2], S. Wong[1], E. Palavecino[1]. *[1]Wake Forest Baptist Medical Center, Winston Salem, NC, [2]Bridgewater College, Bridgewater, VA*

Background: Voriconazole is a triazole antifungal agent used for treatment of invasive fungal infections. Large variations in voriconazole pharmacokinetics may be associated with decreased efficacy or with toxicity and therefore monitoring of voriconazole levels is highly recommended. The objective of this study was to investigate the performance of a newly developed method for measuring voriconazole levels using LCMS/MS compared to standard HPLC methodology.

Material and Methods: Serum samples from patients receiving voriconazole therapy were collected according to our institution standards protocols. Aliquots from each sample were tested by HPLC at a reference laboratory and by the LCMSMS method developed on site and validated according to standard recommendations using for

separation an Aria two channel HPLC with a Cyclone (50 x 0.5mm) column from ThermoFisher for online cleanup and a Hypersil Gold (50 x2.5 mm 3um particle size) column from ThermoFisher for the separation. The detection was accomplished by a ThermoFisher Vantage tandem quadrapole mass spectrometer The LCMS/MS protocol was the following: A 100 µl aliquot of each serum sample, control, and calibrator was added to 300ul of extraction mixture (50ng/ml voriconazole D3 in MEOH). The mixture was vortexed for 20 seconds and then centrifuged at 12000 rpm for 5 minutes. The supernatant of this mixture was diluted 1:1 with water and 100 µl of this supernatant was injected into the column. Once the sample was introduced into the LCMS/MS system, automated turbo-flow analysis was followed by LCMS/MS. The mass spectrometer was run in HESI mode with positive polarity. The spray voltage was 4500v and the vaporizer temperature was 400°C and the sheath gas pressure was 20 psi and the N2 gas pressure was 100 psi. We detected fragments in atomic mass units of 281.2 and 224.1 from voriconazole 350.1.The internal standard voriconazole D-3 amu 353.15 yielded fragments 284.2 and 130. Runtime was (6.3 min) with a detection window of 3min/sample. Performance of the LCMS/MS method for detecting voriconazole levels in 21 clinical serum samples was compared with that of the HPLC method.

Results: The LCMS/MS method for voriconazole was linear over the analytical range of 0.25 to 6 mcg/mL and r^2= 0.9958. This study found that the LCMS/MS is precise with an intra- and inter-assays coefficients of variation of <6% and <4% respectively. The correlation between the LCMS/MS method and the standard HPLC was very good with r^2= 0.9711 (y= 0.833x + 0.4724).

Conclusions: The LCMS/MS method is a rapid and accurate method for measuring voriconazole levels and compared well with the values obtained by standard HPLC procedure. This method is an efficient tool for monitoring voriconazole levels in serum samples from patients receiving voriconazole therapy.

A-418

Unified LC-MS/MS Assays for Therapeutic Drug Monitoring and Clinical Trials

D. A. Svinarov, L. Kassabova. *Medical University of Sofia, Sofia, Bulgaria*

Background: Triple quadrupole LC-MS/MS with its unique selectivity and sensitivity for quantification has become unbeatable tool to resolve analytical challenges in the field of therapeutic drug monitoring (TDM), clinical toxicology, steroid analysis, vitamin D, thyroid hormones and many newer biomarkers. Pharmacokinetic assays for clinical trials (CT) are another major application of this technique. This study presents an unified analytical system for TDM and CT based on LC-MS/MS. **Methods:** Twelve assays were combined in a common analytical system via unified sample preparation and chromatography: analytes and internal standards were extracted from 50-100 µl of human whole blood (WB) or plasma (PL) with respective organic solvent, isocratic separation was performed on a C18 analytical column with a mobile phase consisting of 85% aqueous methanol with 0.005 mM ammonium acetate and 0.1% formic acid. Electrospray positive ionization and selected reaction monitoring were used to follow the respective predominant transitions. Mass chromatograms were collected and processed by specialized software, and linear regression was performed to determine analyte concentrations. Validation strategy was strictly adhered to industrial guidance. **Results:** For each analyte selectivity was assessed with 6 individual sources of WB or PL with matrix effect in the range 90÷112%; extraction recoveries of 70÷93%; stability: freeze-thaw was determined for three cycles of 24 h; post-preparative was documented for 36÷72 h at 8°C, short-term - at ambient temperature was proven for 6÷24 h in the dark and for 2÷6 h at daylight; stock solution and long term in WB or PL - for 1÷4 months at -20°C. List of analytes, application profile, and rest of validation characteristics are as follows:

Matrix	Compound	Accuracy	Precision	Linearity Range	Application
PL	Alprazolam	± 11%	6 %	0.1 ÷ 24 µg/L	CT
PL	Amlodipine	± 10 %	9 %	20÷14 400 ng/L	CT
PL	Clarithromycin	± 4 %	5 %	0.4 ÷ 1725 µg/L	CT
PL	Clopidogrel	± 7 %	10 %	5 ÷ 2160 ng/L	CT
WB	Cyclosporine A	± 11 %	7 %	10 ÷ 2000 µg/L	TDM, CT
WB	Everolimus	± 11 %	10 %	1 ÷ 45 µg/L	TDM, CT
PL	Fexofenadine	± 6 %	8 %	0.8 ÷ 322 µg/L	CT
PL	Galantamine	± 10 %	11 %	0.2 ÷ 8 µg/L	CT
WB	Indapamide	± 8 %	8 %	0,2 ÷ 79,0 µg/L	CT
PL	Midazolam	± 12 %	5 %	0,1÷ 100,0 µg/L	CT
PL	Sildenafil	± 4 %	7 %	0.4 ÷ 740 µg/L	CT
WB	Sirolimus	± 11 %	10 %	1 ÷ 40 µg/L	TDM, CT
WB	Tacrolimus	± 11 %	10 %	1 ÷ 42 µg/L	TDM, CT
PL	25-Hydroxyvitamin D	± 11 %	7 %	1 ÷ 150 µg/L	TDM

Conclusion: With validation according to current industrial requirements, a throughput of 100÷200 samples per working day and immediate method switching, this unified system provides convenience and optimal versatility for a single LC-MS/MS instrument.

A-419

Simultaneous Analysis of Multiple Azole Antifungal Drugs in Plasma for Clinical Research using a simple Protein Precipitation Extraction Protocol

B. J. Molloy[1], B. Keevil[2], L. J. Calton[1]. *[1]Waters Corperation, Wilmslow, United Kingdom, [2]University Hospital of South Manchester, Manchester, United Kingdom*

Background: Here we evaluate a UPLC/MS/MS method to simultaneously measure the azole antifungal drugs voriconazole, fluconazole, itraconazole, ketoconazole and posaconazole in plasma for clinical research purposes to determine pharmacodynamic and pharmacokinetic properties to understand possible drug-drug interactions. A simple, sensitive, precise and robust analytical method was developed using a simple protein precipitation protocol and state of the art UPLC/MS/MS technology.

Methods: Voriconazole, fluconazole, itraconazole, ketoconazole and posaconazole were purchased from Sigma-Aldrich Company Ltd (Dorset, UK) and were used to create calibrators and QC material in pooled plasma obtained from Sera Laboratories International (West Sussex, UK). Stable labelled forms of all analytes were used as internal standards and were purchased from Toronto Research Chemicals (Toronto, Canada).

Linearity, precision, analytical sensitivity, carry-over and matrix effects were all assessed. The method was also compared to an independent LC/MS method for the measurement of voriconazole, and the effect of potential interferences on the method was assessed. All samples were prepared by precipitation with a solution of the internal standards in methanol. Using an ACQUITY UPLC® I-Class system, diluted samples were injected onto a 2.1 x 30 mm Waters CORTECS UPLC C18 column employing a water/methanol/ammonium acetate/formic acid gradient for separation and quantified with a Waters Xevo® TQD Mass Spectrometer.

Results: The method was shown to be linear from 0.060 - 9.5 mg/L, 0.062 - 10.2 mg/L, 0.055 - 10.4 mg/L, 0.048 - 8.8 mg/L and 0.067 - 10.2 mg/L for voriconazole, fluconazole, itraconazole, ketoconazole and posaconazole respectively. Total precision and repeatability was assessed over five days with five replicates per day and is expressed as coefficient of variation (CV). For all analytes the CV for the low QC (0.25 mg/L) was ≤ 5.6% and the CV for the mid (3.5 mg/L) and high (7.5 mg/L) QC was ≤ 3%. Comparison of the results with those for the same samples previously analyzed by an independent LC/MS/MS method for voriconazole was described by the Deming equation y = 0.95x + 0.05.

Conclusions: We have successfully quantified voriconazole, fluconazole, itraconazole, ketoconazole and posaconazole in plasma using a simple protein precipitation extraction protocol with UPLC/MS/MS for clinical research purposes. The method demonstrates good linearity, precision, analytical sensitivity and lack of significant matrix effects.

For Research Use Only. Not for use in Diagnostic Procedures

A-420

Development and Validation of a Dried Blood Spot Method for 25-Hydroxyvitamin D

Z. Wu, D. Bass, K. Urek, J. A. Maggiore. *Doctor's Data, Inc., Saint Charles, IL*

Background: With sustained consumer and professional demand for Vitamin D testing, we sought to develop a convenient, precise, and accurate method for 25-hydroxyvitamin D analysis of a dried blood spot (DBS) sample. Single use and self-retracting bloodletting devices enable the self-collection of capillary blood from lay users. Filter paper collection and transport media have become highly standardized and are increasingly used for the analysis of several analytes in the clinical laboratory. The employment of Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS) serves to expand the DBS offerings in clinical laboratories for the reliable analysis of micronutrients. Combining these components provides the basis for the Doctor's Data, Inc. (DDI) DBS method for 25-Hydroxyvitamin D.

Methods: Capillary blood samples are self-collected using SurgiLance™ sterile lancets, and spotted onto PerkinElmer 226 Spot Saver Cards and permitted to dry. Cards are desiccant packaged and shipped via US or International postage to DDI Laboratory.Two 6-millimeter spots are punched from homogeneous blood spots, and extracted using a methanol-rich solvent solution which also contains deuterated internal standards for 25-hydroxyvitamin D2 and D3. Extracts are further processed and purified using solid phase extraction, eluted, and prepared for injection through a C-18 analytical column on an Agilent 6460 LC-MS/MS System. Results are read from a 5-point calibration curve, derived from certified standards for 25-hydroxyvitamin

D2 and D3. Analytical precision, linearity, recovery, accuracy, interference, and stability were assessed.

Results: For 25-hyroxyvitamin D3, intra-assay precision coefficients of variation (CV) (n=24) at 35.3 and 74.8 ng/mL were 2.7% and 1.7%, respectively. Inter-assay CV (n=24) at the same levels were 3.2% and 3.5%, respectively. For 25-hydroxyvitamin D2, the intra-assay CV (n=24) at 15.9 and 76.8 ng/mL were 3.7% and 2.6%, respectively. Inter-assay CV (n=24) at the same levels were 5.0% and 3.2%, respectively. For assay linearity (n=5), 25-hydroxyvitamin D3 was confirmed linear between 2.0 and 224.0 ng/mL, with recovery between 98.9% and 105.1%; 25-hydroxyvitamin D2 was confirmed linear between 0.5 and 76.5 ng/mL, with recovery between 98.9% and 104.2%. Volunteers provided DBS and paired serum samples allowing sample matrix comparison. Least-squares regression analysis comparing Total 25-hydroxyvitamin D values in serum to DBS (n=46, range 7.5 - 92.6 ng/mL) yielded a correlation coefficient (R^2) of 0.972, y = 0.957x + 8.48; standard error of estimate = 3.58. Both forms of vitamin D demonstrated one-year stability when collection cards are stored desiccated in sealed Ziploc™ bags at ambient (25°C) temperatures or lower. No detectable analytical interference from hemoglobin was apparent. Of the first 2000 DBS samples submitted to DDI for Vitamin D testing, 99.1% of DBS cards received contained blood spots of sufficient quantity and quality to permit processing and analysis.

Conclusion: The analytical method developed and validated by DDI for DBS 25-hydroxyvitamin D testing provides a precise and accurate means of determining Vitamin D status. The collection system for this method has proven to be well-accepted by lay users, while the transportation system provides extended stability to preserve sample integrity to facilitate shipping from remote locations to a central laboratory for analysis.

A-421

Development of an Assay for Methotrexate and its Metabolites 7-hydroxymethotrexate and DAMPA in Serum by LC-MS/MS

R. Schofield[1], K. Murata[1], L. V. Ramanathan[1], M. E. Grace[2], M. Fleisher[1], M. S. Pessin[1], D. Carlow[1]. *[1]Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, [2]Department of Pathology, Mount Sinai School of Medicine, New York, NY*

Background: Methotrexate (MTX) is a folic acid antagonist that is widely used as an immunosuppressant and chemotherapeutic agent. After high dose administration of MTX serum levels must be monitored to determine when to administer leucovorin, a folic acid analog that bypasses the enzyme inhibition caused by MTX and reverses its toxicity. Patients in renal failure who are given high-dose MTX are often given carboxypeptidase-G2 ($CPDG_2$) to reverse the effects of MTX. $CPDG_2$ is an enzyme that converts MTX into glutamate and 4-amino-4-deoxy-N-methylpteroic acid (DAMPA) that are much less toxic. DAMPA cross-reacts in immunoassays rendering them unsuitable for monitoring patients given $CPDG_2$ therapy.

Objective: The objective was to develop a very sensitive and specific assay for MTX by LC-MS/MS that had no cross-reactivity with DAMPA or other metabolites, including the major metabolite 7-hydroxymethotrexate (7-OH MTX). The assay needed to be relatively simple to allow its use in a clinical laboratory. In addition, the assay needed to be able to accurately measure the levels of 7-OH MTX and DAMPA to support clinical trials utilizing $CPDG_2$ and related compounds.

Methods: Serum samples were prepared by protein precipitation using methanol containing deuterated MTX as internal standard. LC-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 and a Hypersil Gold C8 analytical column. The HPLC gradient elution was 20-80% of 10 mM ammonium formate/0.1% formic acid in methanol over 1.8 minutes. Calibrators (7) were prepared in blank human serum.

Results: The LOQs of MTX and DAMPA were 10 nmol/L and for 7-OH MTX it was 20 nmol/L. The analytical measurement ranges for MTX, 7-OH MTX and DAMPA were 0-1000 nmol/L; the calibration curves were linear over the AMR with correlation coefficients $R^2 \geq 0.995$. Dilutions of 10, 100 and 1000-fold were validated giving a clinically reportable range of 0-10^6 nmol/L. The accuracy of MTX was evaluated by comparison to a dihydrofolate reductase (DHFR) enzymatic inhibition assay, Abbott TDx immunoassay (Abbott Laboratories, Abbott Park, IL), and an alternate LC-MS/MS assay. The slopes of the linear regression curves comparing the 4 assays were all +/- 1% with excellent correlation coefficients. MTX recoveries at concentrations spanning the AMR were between 98 and 103%. The accuracy of 7-OH MTX and DAMPA was evaluated using recovery experiments; recoveries of 7-OH MTX and DAMPA at five different concentrations spanning the entire AMR were between 98.8% and 105.1%. Within-day and between-day (N=10) CVs at concentrations spanning the AMR were less than 10% for all three analytes.

Conclusion: We have developed a simple, accurate and sensitive assay to measure MTX levels in serum by LC-MS/MS. Unlike immunoassays this assay shows no cross-reactivity with either DAMPA or 7-OH MTX and can be used in the setting of $CPDG_2$ therapy. In addition, the assay accurately measures the levels of 7-OH MTX and DAMPA to support clinical trials utilizing $CPDG_2$ and related compounds.

A-422

Use of complementary scanning methods by LC-MS/MS in the detection of urinary synthetic glucocorticoids in patients being investigated for Cushing's syndrome

N. K. Djedovic, S. J. Rainbow. *North West London NHS Trust, Middlesex, United Kingdom*

Introduction: Liquid chromatography tandem mass spectrometry methods (LC-MS/MS) are now routinely being used for the analysis of steroids in clinical biochemistry laboratories. The majority of methods use multiple reaction monitoring mode (MRM), which confers the highest specificity. The purpose of this study was to assess the usefulness of complementary LC-MS/MS scan methods such as precursor ion (PI) and neutral loss (NL) used in doping analysis studies for the open detection of synthetic glucocorticoids and their metabolites in patients under investigation for Cushing's syndrome. PI scanning may be used to detect steroids which share a product ion, while NL scanning detects analytes with a common loss irrespective of the parent ion mass.

Method: The ionization and fragmentation behaviour of eight synthetic glucocorticoids (prednisolone, methylprednisolone, betamethasone, dexamethasone, triamcinolone acetonide, fluocinolone acetonide, beclomethasone dipropionate and fluticasone propionate) and two endogenous glucocorticoids (cortisol and cortisone) was assessed in positive and negative electrospray on an API 3000 tandem mass spectrometer equipped with a TurboIonSpray source. Common fragments and neutral losses were identified, and MRM, NL and PI scan methods were developed. In MRM mode, two m/z transitions were monitored for each analyte in positive electrospray: 363.3 > 97.3/121.2 for cortisol, 361.3 > 121.3/162.9 for cortisone, 361.2 > 147.1/279.1 for prednisolone, 375.2 > 161.0/279.0 for methylprednisolone, 393.0 > 147.1/237.1 for betamethasone and dexamethasone, 435.4 > 212.9/339.2 for triamcinolone acetonide, 453.0 > 121.2/337.1 for fluocinolone acetonide, 521.4 > 279.1/337.1 for beclomethasone dipropionate, and 501.2 > 121.3/293.0 for fluticasone propionate. In PI mode, four fragment ions were monitored in positive electrospray: m/z 121.0, 147.0, 275.0 and 279.0. In NL mode, two neutral losses were monitored in negative electrospray: m/z 76.0 and 104.0. Samples were analysed after liquid-liquid extraction with dichloromethane of 500 µL urine spiked with internal standard (d4-cortisol). Chromatographic separation was achieved using an Agilent 1100 system HPLC system and BDS Hypersil C8 column (50 x 2.1 mm, 3 µm).

Results: In order to assess the precision and sensitivity of each method, a urine sample was spiked with a mixture containing the selected corticosteroids at two different concentrations: 5.00 and 50.0 nmol/L. In MRM mode, the limit of detection (LOD) was 5.00 nmol/L, while, in PI and NL modes, the LOD was 50.0 nmol/L. Inter- and intra-assay precision (n = 10) was less than 15% at the LOD. Interference from isobaric compounds was detected using the branching ratios established for each compound. Dexamethasone and betamethasone were resolved mathematically. Patient samples containing synthetic glucocorticoids (methylprednisolone, prednisolone, betamethasone and dexamethasone) were identified by MRM, and metabolites of these compounds were detected using the PI and NL modes. Negative samples from patients were analysed using the established methods to identify endogenous metabolites.

Conclusion: We have developed an MRM method specific for certain synthetic glucocorticoids and scanning methods for the potential detection of other exogenous glucocorticoids based on structural similarities. This additional information should improve patient management.

A-423

Analysis of plasma catecholamines and metanephrines by mixed-mode SPE and HILIC LC/MS/MS

J. Danaceau, E. Chambers, K. Fountain. *Waters Corporation, Milford, MA*

Background: In clinical research, elevated concentrations of urinary catecholamines can be used in conjunction with their O-methylated metabolites (metanephrines) to indicate the presence of conditions such as pheochromocytomas, neuroblastomas,

ganglioblastomas and ganglioneuromas. However, these compounds (in particular, norepinephrine, epinephrine, and dopamine) can be a challenge to analyze via reversed-phase LC/MS/MS due to their high polarity. As a result, many research laboratories still analyze this panel using ion-pairing reagents and ECD detection. While reversed-phase LC/MS/MS has been used successfully, challenges still exist due to ion-suppression from matrix components, insufficient retention, and inadequate separation of normetanephrine and epinephrine. This work describes a single extraction and analysis method for monoamine neurotransmitters and metanephrines from human plasma.

Methods: 250 µL plasma samples were pretreated with 50 mM NH_4CH_3COO, and loaded onto pretreated wells of mixed-mode µElution SPE plates. SPE wells were then washed with 20 mM NH_4CH_3COOH and 50:50 ACN:IPA and eluted with 2 x 25 µL aliquots of 85:15 ACN:H_2O with 2% formic acid. HILIC-based chromatographic separation was achieved using an UHPLC silica-hybrid amide column. MPA and MPB consisted of 30 mM NH_4COO dissolved in 95:5 H_2O: ACN and 15:85 H_2O: ACN, respectively. Compounds were detected by MRM in ESI positive ionization mode.

Results: All compounds eluted within 2.0 minutes, with baseline separation between normetanephrine and epinephrine enabling their unambiguous identification and quantification. Recoveries ranged from 45-90% and averaged 76%. Matrix effects were less that 25% for dopamine and norepinephrine and under 10% for the remaining analytes. Calibration curves were linear from to 10-2000 pg/mL for dopamine, 3-MT, metanephrine and normetanephrine, and from 50-10,000 pg/mL for epinephrine and norepinephrine. Calibration curves for all compounds had R^2 values of 0.999 or greater. %CV and bias values for quality control samples were less than 10% for all analytes at even the lowest QC concentration (40 pg/mL).

Conclusion: This combination of mixed-mode sample preparation and HILIC chromatography results in a rapid, robust method with excellent linearity, accuracy, and precision that is suitable for measuring even the lowest endogenous concentrations of these compounds.

For Research Use Only, Not for Use in Diagnostic Procedures.

A-424

Simultaneous Detection of 60 Pain Management Drugs and Metabolites in Urine with A High Performance Liquid Chromatography - Tandem Mass Spectrometry (HPLC-MS/MS) Method

Z. Cao, E. Kaleta, P. Wang. *Houston Methodist Hospital, Houston, TX*

Background: For chronic pain management, there is a growing need to closely monitor patients taking pain medications for compliance and illicit drug use. In recent years, LC-MS/MS based methods that are highly sensitive, specific and cost-effective have been reported. However, most of these existing methods are limited to common drugs, such as opiates, benzodiazepines, amphetamines or designer drugs. Therefore, a method monitoring multiple drugs and drug classes is necessary, especially for patients with chronic pain that are frequently prescribed multiple medications. The objective of this work was to develop an HPLC-MS/MS method simultaneously detecting 60 drugs and metabolites from the following groups: opiates, synthetic opioids, benzodiazepines, stimulants, anticonvulsants and opioid antagonists.

Methods: Sixty drug standards and 37 deuterated internal standards were monitored using scheduled multiple reaction monitoring (sMRM) on an AB Sciex QTRAP® 5500 mass spectrometer with electrospray ionization in a positive ion mode. Reversed-phase HPLC separation was performed using a Kinetex™ Phenyl-Hexyl column (50x2.6 mm, 2.6 µm particle size) (Phenomenex, CA) with a binary mobile phase (A: 10 mM ammonium formate in water; B: 0.1% formic acid in methanol) by gradient (5-90% mobile phase B) with a 0.6 mL/min flow rate. Four-level calibrators were prepared in drug-free urine (Bio-Rad, CA) in a range of 5-1000 ng/mL (with exceptions of fentanyl: 0.2-25 ng/mL; gabapentin and pregabalin: 10-2000 ng/mL). All internal standards were prepared at 50 ng/mL in mobile phase A. For sample preparation, 50 µL patient urine or calibrator and 50 µl internal standard mixture were diluted with 400 µl mobile phase A before LC injection.

Results: In this lab developed HPLC-MS/MS assay, all analytes were chromatograpically resolved. Without additional sample clean-up, none of the analytes was affected by ion-suppression with this dilute-and-shoot method. All calibration produced linear calibration curves (R^2 >0.940), with the within-run coefficient variations of 2-33%. We tested 21 patient urine samples previously screened positive by the Alere Triage ® TOX Drug Screen assay. Using LC-MS/MS cut-offs consistent with other reference laboratories, our HPLC-MS/MS assay was 35/40 (88%) in agreement with the positive Triage results in the following drug classes: opiates (11/11), amphetamine (7/7), methamphetamine (5/5), cocaine (8/8) and benzodiazepines (4/9). The 5 samples missed by our LC-MS/MS assay were positive for benzodiazepine in the Triage assay: one was prescribed alprazolam; four were prescribed lorazepam. In urine, lorazepam is mostly present as its metabolite lorazepam-glucuronide, which was detected by Triage but not included in the LC-MS/MS assay. The HPLC-MS/MS assay also identified some blinded-spiked drugs not detected by the Triage assay in 21/22 (95%) urine samples. The identities of these drugs were confirmed by comparing to standards.

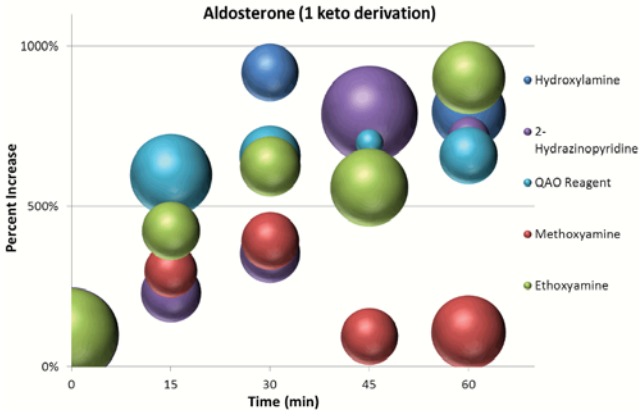
Conclusion: We presented an HPLC-MS/MS method that can simultaneously detect and quantify 60 pain management drugs and metabolites with complete separation. Investigation of the cause of false negative in alprazolam, and further optimization and validation of the assay is ongoing. This work provides a solid foundation for further development of this method into a robust quantitative assay for clinical workflows.

A-425

Steroid Ionization Efficiency as a Function of Derivation using Multiple Derivation Reagents

D. R. Bunch, S. Cahalan, S. Wang. *Cleveland Clinic, Cleveland, OH*

BACKGROUND Ionization plays a crucial role for mass spectrometry measurements. Blood steroid measurements are important for the diagnosis of various endocrinological disorders. Conditions such as congenital adrenal hyperplasia would benefit from simultaneous measurement of multiple steroids with high sensitivity. However, some of these compounds have inefficient LC-MS/MS ionization, which is required to achieve the sensitivity for clinical use. The aim of this study was to explore the impact of derivatization reagents and reaction time on ionization efficiency for multiple steroid compounds. METHODS Steroids (pregnenolone, cortisone, cortisol, aldosterone, testosterone, 17-hydroxylprogesterone, progesterone, 11-deoxycortisol,) at 3.6 µM in methanol were derivatized using 50 L of hydroxylamine, methoxylamine, ethoxyamine, and 2-hydrazinopyridine each at 100 mg/mL at room temperature and QAO reagent (AB Sciex, Framingham, MA) per the manufacture instructions. Aliquots were collected at 0, 15, 30, 45, and 60 min and analyzed in real time. Each time course was performed in triplicate and each sample was spiked with 10 L of reserpine (521 mg/mL; 609 m/z), which was used as an ionization internal standard. The precursor masses were collected for 150 scans (0.1 s each). Ionization was normalized to the reserpine peak. RESULTS Due the large amount of data a representative figure is presented (Figure 1) for aldosterone. The impact of derivation differed significantly using different reagents. The areas of the circles represent the coefficients of variation for the triplicate measurements. The y-axis is the ionization normalized to reserpine and time zero. The x-axis is the time course for the experiments. CONCLUSION Ionization was greatly increased through derivation with these reagents. Hydroxylamine and QAO derivations produced the highest and most consistent responses.



A-426

Identifying four serum peptides as biomarkers for T2DM early diagnosis by MALDI-TOF MS

Q. Meng[1], W. Yan[1], Q. Ma[2], W. Xu[3], X. Liu[4], F. Wang[4], W. Wang[5]. *[1]The PLA 301 Hospital, Beijing, China, [2]Peking University, Beijing, China, [3]Shenyang Pharmaceutical University, Shenyang, China, [4]Bioyong Technology Co. Ltd., Beijing, China, [5]Capital Medical University, Beijing, China*

Background: Currently, there is no ideal serum biomarker for the early diagnosis of type 2 diabetes mellitus (T2DM). Established diagnostics for T2DM include oral glucose tolerance (OGT), fasting blood glucose (FBG) level, and hemoglobin A1c

level, all of which are markers for the late stages of the disease. The aim of this study was to apply magnetic bead fractionation coupled with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) to screen serum samples from patients with T2DM and healthy controls to screen and identify T2DM specific peptides.

Methods: We (1) performed a discovery screen for peptide differences in serum proteomic profiles using magnetic-bead enrichment; (2) used a model based on a genetic algorithm (GA) to distinguish between the serum peptide profiles of patients with T2DM from healthy controls, to establish a training set, and to validate an independent test set; and (3) identified the most promising protein/peptide biomarkers of T2DM using linear ion trap (LTQ)-Orbitrap-MS. Patients selected in the study were pathologically diagnosed with T2DM, with FBG levels > 7.0 mmol/L and 2-h OGT (75 g) > 11.1 mmol/L. A total of 306 patients (141M/165F) and 330 healthy volunteers (83M/247F) were recruited and divided into training sets (206/230) and test set (100/100). Serum samples were collected before meals, prepared, and fractionated using weak cation exchange magnetic beads (MB-WCX) according to the manufacturer's instructions (Bioyong Tech, Beijing, China). The resultant samples were diluted, spotted onto a ClinTOF® target and performed the MALDI-TOF-MS measurements by calibrated ClinTOF® instruments (Bioyong Tech, Beijing, China). All spectra in this research were analyzed using BioExplorer® (Bioyong Tech, Beijing, China) to subtract baseline, normalize spectra, and determine peak m/z values and intensities in the range of 1,000 to 10,000 Da.

Results: Using LTQ-Orbitrap-MS detection, the sequences of seven diagnostic peptides with m/z values of 1691.7, 1778.7, 1865.5, 2022.1, 2210.3, 2929.3, and 4093.2, which were used to establish the GA model, were found to represent four different proteins. Four peaks (1691.7 m/z, 1778.7 m/z, 1865.5 m/z, and 2022.1 m/z) were identified as complement C3f, which is cleaved from C3b by factor I and enters the alternative complement pathway to promote the generation of iC3b. One peak (2210.3 m/z) was identified as the kininogen-1 isoform 1 precursor. Two peaks (2929.3 m/z and 4093.2 m/z) were identified as the fibrinogen alpha chain precursor. An 1473.3 m/z peak was not recognized by this assay, but we identified this peak as transthyretin according to previous results.

Conclusion: A diagnostic model was generated using a genetic algorithm which may discriminate T2DM patients from healthy subjects. Four peptides were derived from complement Cf3 (1691.7 m/z, 1778.7 m/z, 1865.5 m/z, and 2022.1 m/z), kininogen-1 isoform-1 precursor (2210.3 m/z), fibrinogen alpha chain precursor (2929.3 m/z and 4093.2 m/z), and transthyretin (1473.3 m/z). The presence of these peptides at elevated levels and our laboratory findings may provide new biomarkers for the early detection of T2DM.

A-427

A Sensitive and Specific Ultra-High Pressure Liquid Chromatography - Tandem Mass Spectrometry Method for the Quantitation of Hepcidin in Human Serum

D. Chu[1], C. Hedin[1], D. Chollet[2], D. Bertelson[1], E. Ellis[1]. *[1]Covance, Indianapolis, IN, [2]Covance, Geneva, Switzerland*

Background: Hepcidin is a 25-amino acid peptide hormone produced in the liver and is considered to be the central regulator of iron metabolism. It is a promising biomarker for the diagnosis and monitoring of iron metabolism disorders such as anemia, hypoxia and inflammation. Until recently, the assays for measuring hepcidin have lacked precision, accuracy, and specificity. The objective of this study was to develop and validate a sensitive and specific ultra-pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for the quantitative determination of hepcidin in human serum samples.

Methods: Calibrators were created by spiking charcoal stripped human serum with hepcidin concentrations ranging from 0.2 to 100 ng/mL. Human serum samples (0.2 mL) were combined with labeled internal standard (13C18,15N3-hepcidin), and extracted using a 96-well format solid phase extraction (SPE) plate (Waters Oasis HLB). Hepcidin and its internal standard were analysed using a Waters UPLC system coupled to an AB Sciex QTRAP 5500 mass spectrometer in MRM mode. Chromatographic separation was performed using a Waters Acquity reverse phase column (1.7µM, 2.1x100mm). The mobile phases consisted of 0.5% acetic acid in water (mobile phase A), and 0.05% acetic acid in methanol:acetonitrile (50:50, v:v) (mobile phase B). The linear gradient started at 20% B and ramped up to 100% B over 6 minutes, followed by 1 minute of re-equilibration. Hepcidin and its internal standard were detected by positive electrospray ionization with the following transitions: hepcidin m/z 558.7→693.7 and internal standard m/z 562.8→697.1.

Results: The method described displayed good linearity over a concentration range of 0.2-100 ng/ml with r2 >0.99. Intra-day and inter-day precision for all 3 QC levels showed CVs =<3.5% and =<5.9%, respectively. Accuracy was evaluated using a spike and recovery experiment and yielded recoveries ranging from 97.9-102% for 3 QC levels. The lower level of quantitation was 0.2 ng/mL. Specificity was evaluated and no interference was observed for serum spiked with hepcidin-20 and hepcidin-22 at 200 ng/mL each. Dilution linearity was verified to be acceptable up to 8x. The reference interval was verified to be 3.1-43.5 ng/mL for males, 1.1-25.7 ng/mL for pre-menopausal women, and 2.0-46.9 ng/mL for post-menopausal women. Stability was established for up to 2 days at ambient temperature and up to 4 days at refrigerated temperature (2-8°C). Freeze-thaw stability was established for 4 cycles at both -70°C and -20°C. Long-term frozen stability was established for up to 4 months at both -70°C and -20°C.

Conclusion: We have developed and validated a sensitive and specific UHPLC-MS/MS method for the quantitative measurement of hepcidin in clinical serum samples. The method is capable of quantitating hepcidin from 0.2-100 ng/mL. The method utilizes solid phase extraction for sample preparation. The chromatography is carried out on a reverse phase sub-2 µm particle size column using ultra-high pressure liquid chromatography. The total chromatographic run time is 7 minutes. The mass spectrometry is carried out on an AB Sciex QTRAP 5500 instrument. The predominant precursor ion for hepcidin was determined to be the quintuple charged species, [M+5H]5+.

Tuesday, July 29, 2014

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

Animal Clinical Chemistry

A-428

Estimated complete blood count (CBC) reference ranges for aged adult male rhesus monkeys (Macaca mulatta) as measured on the Beckman Coulter HmX analyzer

N. M. Nwaoduah, C. D. Lang, J. S. Daviau, L. J. McCloskey, D. F. Stickle. *Thomas Jefferson University, Philadelphia, PA*

Introduction: The clinical laboratory was asked by veterinary services to provide CBC's for routine checkups on a small number of aged adult male rhesus monkeys. As we had no reference values for this population, results were analyzed as if from a control group representing a well population. We report these results obtained using the Beckman HmX analyzer, and compare reference range estimates to available literature.

Methods: Whole blood K-EDTA samples were collected from 12 male rhesus monkeys (age: 23.1 ± 1.3 years; age range: 21 to 25 years). CBC analysis was performed within 3 hours of collection using the Beckman Coulter HmX analyzer. Results were analyzed according to expectation of a normal distribution by correlation of data with the normal distribution predicted by the calculated mean (x) and standard deviation (s). Outliers were defined as samples deviating from x by more than 3s when excluded from the group. Parametric reference ranges were defined by convention as the central 95% of results (x-2s to x+2s).

Results: shown in Table. Despite low sample number, distributions for all CBC component tests were consistent with normal distributions (expected r^2 for n= 12: r^2>0.9). Calculated uncertainty in the width of the estimated reference ranges was ±20% for n=12. Mean values for WBC, MCV, MCHC and platelets were substantially different from literature data (|z|>2). These differences may be due to the substantially advanced age of the animals (life expectancy of 4 years in the wild).

Conclusions: Reference ranges for CBC component tests using the Beckman Coulter HmX analyzer were estimated based on a small dataset for an aged adult population of male rhesus monkeys. Datasets for each test were well characterized as normal distributions. Estimated reference ranges for four CBC component tests (WBC, MCV, MCHC and platelets) for this population differed substantially from available literature.

A. Test	B. UNITS	C. RR	D. n	E. r^2	F. LIT RR	G. z
WBC	10^9/L	2.8-7.4	11	0.970	12.5-18.9	* -6.63
RBC	10^12/L	4.7-6.4	12	0.940	4.9-5.8	0.98
Hb	g/dL	11.9-14.8	12	0.925	11.8-13.8	1.15
HCT	%	36.0-46.9	12	0.958	39.1-44.9	-0.38
MCV	fL	67.8-80.8	12	0.981	75.0-82.2	* -2.39
MCH	pg	21.6-26.2	12	0.911	22.9-25.1	-0.18
MCHC	g/dL	31.0-33.4	12	0.965	29.6-31.4	* 3.86
RDW	%	11.5-14.4	12	0.912	12.3-13.7	-0.15
PLT	10^9/L	118-317	11	0.954	287-431	* -3.95

A. CBC Component Test
C. Parametric reference range (RR)from data
F. Literature reference range (LIT RR): Chen et al., Xenotransplantation 2009;16:496-501
G. z value of data mean relative to mean and standard deviation of LIT RR; *|z|>2.0

A-430

Modulation of the inflammatory response induced by carragenan in a murine model by Effect of Jungia sellowii Less.

T. S. Frode, M. Nader, G. Vicente, J. S. da Rosa, T. C. Lima, A. M. Barbosa, M. W. Biavatti. *University Federal of Santa Catarina, Brasil, Brazil*

Background: Jungia sellowii Less. is a native plant from Brazil used in traditional medicine to treat inflammatory diseases.

Objective: The aim of this study was to evaluate the anti-inflammatory effect of the crude extract(CE) from Jungia sellowii Less. its derived aqueous fraction(Aq), and isolated compounds, succinic acid(SA) and lactic acid(LA) on leukocytes, exudation, myeloperoxidase(MPO) and adenosine-deaminase(ADA) activities and nitric oxide(NOx), interleukin-1β(IL-1β), tumor necrosis factor-α(TNF-α) and interleukin-17A(IL-17A) levels, using a murine model of pleurisy induced by carrageenan(Cg,1%).

Methodology: Fresh Jungia sellowii Less leaves were extracted with ethanol/water to obtain the CE, which was partitioned with solvents of increasing polarity, yielding a residual Aq fraction. The compounds, SA and LA, were isolated from this fraction and their structures were determined by nuclear magnetic resonance(1H NMR). Swiss mice were used throughout the experiments (Brit.J.Pharmacol.183.811-19.1996). The study was approved by Committee for Ethics in Animal Research of Federal University of Santa catarina (protocol: PP00757). Different groups of animals (n=5) were treated with CE(10-50mg/kg), Aq fraction(1-25mg/kg), SA(0.5-2.5mg/kg) or LA(0.5-2.5mg/kg) administered by intraperitoneal route, 0.5h prior to the intrapleural injection of Cg to analyze the effect of the herb on leukocytes and exudation. A group of animals received a gingival injection of Evans blue dye(25mg/kg) 10min before herb treatment to evaluate the exudation. The Evans blue dye was measured by colorimetric assay on enzimaimmunoassay (ELISA) plate reader. The leukocytes were determined on veterinary automatic counter. Other groups of animals were pretreated (0.5h) with CE(25mg/kg), Aq(5mg/kg), SA(1mg/kg) or LA(1mg/kg) to evaluate the effect of the herb on MPO and ADA activities, NOx , IL-1β, TNF-α, and IL-17A levels. The MPO and ADA, and NOx, were analysed in accordance with methods described by Giusti and Galanti, 1984; Rao et al., 1993, and Green et al., 1982, respectively. The IL-1β, TNF-α, and IL-17A levels, were determined using commercially available ELISA kits. All the inflammatory parameters were analyzed after 4h of pleurisy induction. Statistical differences between groups were determined by ANOVA complemented by Newman-Keuls test. Values of $p<0.05$ were considered significant.

Results: The herb inhibited leukocytes (CE:42.8±2.9% to 66.8±5.8, Aq:47.4±5.0% to 60.7±5.2, SA:24.9±6.8% to 54.4±2.9% and LA:31.2±4.3% to 66.3±5.3%), neutrophils: (CE:40.3±3.4 to 65.8±6.0%, Aq:45.9±5.3% to 59.8±5.2, SA:25.6±6.3% to 53.2±3.3%, and LA:31.8±4.2% to 66.2±5.2%), and exudation (CE:31.2±3.8 to 51.4±3.3%, Aq:41.4±2.7 to 73.4±3.2%, SA:15.0±2.6% to 42.9±4.8%, and LA:23.4±2.9% to 52.6±3.4%)($p<0.05$). Additionally, this plant inhibited MPO (CE:60.1±1.6%; Aq:67.5±1.1%; SA:58.8±3.9%; LA:65.9±2.8%), ADA (CE:45.2±2.3%; Aq:63.9±5.8%; SA:37.5±6.0%; LA:64.4±6.7%), NOx (CE:40.7±1.1%; Aq:70.4±0.8%; SA:73.8±2.6%; LA:76.5±1.4%), IL-1β (CE:78.3±2.0%; Aq:74.2±1.5%; SA:24.6±1.2%; LA:14.9±1.3%), TNF-α (CE:61.9±3.4%; Aq:55.1±2.7%; SA:82.4±2.3%; LA:63.3±2.7%), and IL-17A (CE:64.0±6.4%; Aq:54.3±2.6%; SA:41.93±4.0%; LA: 21.2±5.4%)($p<0.05$).

Conclusion: J.sellowi less.showed an important modulation of the inflammatory response induced by carrageenan into the mouse pleural cavity by inhibiting the leukocytes content and the degree of exudation. These inhibitory effects were associated with the decrease of MPO and ADA activities and NOx, IL-1β, TNF-α and IL-17A levels.

A-431

Effects of the IL-10 gene deficiency on Mouse liver function

J. DU, L. JI, D. ZOU. *Peking University Shenzhen Hospital, Shenzhen, China*

Background: Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Numerous investigations suggest that IL-10 plays a major role in chronic liver diseases. Our aim is to investigate the effect and mechanism of IL-10 deficiency on mouse liver.

Methods: Using the automatic biochemical analyzer to analyze serological biomarkers of liver function between IL-10 gene knockout mice and IL-10 wild type control. The pathological morphological changes were observed with the light microscope. The levels of iNOS and IL-1βgenes in liver tissues were determined by real-time

fluorescence quantitative PCR and enzyme-linked immunosorbent assay(ELISA).

Results: Compared with the wild type, the serum levels of ALB,TP,TBIL and DBIL of IL-10 deficient mice were significantly decreased(P<0.05), no obvious differences were found in AST,ALT and liver pathological morphology(P>O.05). The expression of iNOS and IL-1 βgenes, the serum levels of iNOS and IL-1β were significantly higher in IL-10 deficient mice than in wild type mice (P<0.05).

Conclusion: Endogenous IL-10 deficient mice can significantly decreased serum ALB and BIL. The effect may be related to the upregulated expression of iNOS and IL-1β.

A-432

Analytical Evaluation of an Assay Kit Incorporating New Ready to Use Liquid Stable Reagents for the Determination of Glucose, Through Conversion by Hexokinase, in Different Biological Fluids

K. Gilmore, S. Baxter, Z. Burnside, K. Piper, S. McElhatton, M. Rodriguez, J. Campbell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Carbohydrates provide the human body with glucose, a simple sugar used as source of energy by the cells. The body must maintain proper glucose levels to ensure that a person remains healthy. Glucose determination is useful in the diagnosis and monitoring of carbohydrate metabolism disorders (i.e. diabetes mellitus, hypoglycaemia, pancreatic islet cell carcinoma) as well as in research and drug discovery processes. This study reports the evaluation of an assay for the determination of glucose by hexokinase-mediated reaction in serum, plasma, urine, cerebrospinal fluid (CSF) samples. This assay is applicable to automated systems and incorporates new ready to use liquid stable reagents, which facilitates the application in test settings by simplifying the experimental procedure and reducing handling errors. Methods: The assay involves a series of steps, initiated by the conversion of glucose to glucose-6-phosphate by hexokinase. The glucose-6-phosphate is then oxidized by glucose-6-phosphate dehydrogenase, causing the reduction of oxidized nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH). The absorbance of NADH is measured as endpoint reaction at 340/410 nm. The assay is applicable to a variety of analysers. The reagents are liquid stable and ready to use. On-board and calibration stabilities were tested by storing two lots of reagents uncapped on the analyser for a period of 60 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 2 replicates of each sample were assayed twice a day for 10 days. Correlation studies were conducted using a commercially available assay system. Results: The reagents presented an on-board stability of 60 days and calibration frequency of 60 days. The assay was linear from 4 mg/dL up to 700 mg/dL for serum, plasma, urine and CSF. The within-run and total precision for different concentration levels, expressed as %CV, was ≤ 2.0. In the correlation studies 99 serum patient samples, 88 plasma samples (lithium heparin), 87 plasma samples (potassium EDTA), 51 urine samples and 113 CSF samples were tested and the following linear regression equations were achieved: y = 1.001x + 0.3; r = 1.0 (serum, range 5-676 mg/dL), y = 1.001x + 0.2; r = 1.0 [plasma (lithium heparin), range 5-686 mg/dL), y = 1.002x + 0.0; r = 1.0 [plasma (potassium EDTA), range 5-676 mg/dL), y = 0.989x - 0.3; r = 1.0 (urine, range 4-664 mg/dL), y = 1.005x - 0.1; r = 1.0 (CSF, range 20-654 mg/dL). Conclusion: The results of this evaluation indicate that this assay is applicable to the determination of glucose in different biological fluids. This assay kit exhibits good correlation with existing commercial assay systems for all the analysed matrices. Furthermore, it incorporates liquid stable reagents, which simplifies the experimental procedure and reduces handling errors.

A-433

Effects of Chronic Ozone Exposure on the Oxidant-Antioxidant System of Brain Tissue

m. ozler, N. Ersoz, t. topal, A. H. Apaydin, S. Oter, A. Korkmaz. *Gulhane military medical faculty, Ankara, Turkey*

Background: Ozone treatment entails exposing a body cavity or circulation system to a mixture of oxygen and ozone, and has been used in conjunction with other therapies to treat various pathologies. Although repeated ozone treatment has been purported to stimulate an antioxidant response, research characterizing long-term oxidant-antioxidant homeostasis has not yet been reported. As a therapeutic treatment for peritoneal adhesion, rats were exposed to ozone by one of two regimens. During this study, we evaluated oxidant and antioxidant parameters from brain tissues retrieved from these rats.

Methods: For this study, 24 Sprague-Dawley male rats were separated into three groups. The adhesion model for this study was established by making an incision in the cecum of the rats, followed by suturing. Two groups were administered ozone treatment for 15 days; however, each group was subjected to a different regimen: one group was treated with ozone immediately following surgery, whereas the other group was treated 24 h post-surgery. After 15 days, and while anesthetized, surgery was performed to open the abdomen in order to evaluate the adhesion site and to excise the brain tissue. Malondialdehyde (MDA), superoxide dismutase (SOD), carbonilized protein (PCO), and glutathione peroxidase (GSH-Px) levels were measured in the excised brain tissues.

Results: Brain tissues from rats immediately treated with ozone following surgery exhibited higher levels of SOD activity compared with the other two groups. By contrast, the MDA levels observed in this group were significantly lower. There was no difference between groups in terms of PCO levels. We determined that both groups exposed to ozone (i.e., 0 h and 24 h post-surgery) exhibited significantly higher GSH-Px activities in comparison with the control group.

Conclusion: Our findings indicate that long-term ozone treatment supports the antioxidant system in brain tissue. Furthermore, it should be noted that the time it takes to receive treatment is critical, as quicker ozone treatment more effectively stimulated an antioxidant response.

A-435

Evidence of the anti-inflammatory properties of Ageratum conyzoides L. in a murine model of pleurisy induced by carrageenan

T. S. Frode, S. V. G. Vigil de Mello, B. M. Facchin, A. B. G. Luz, C. F. Bosi, D. W. Rosa, M. Biavatti. *University Federal of Santa Catarina, Brasil, Brazil*

Background: A.conyzoides L. is used in Brazilian folk medicine as analgesic and anti-inflammatory agent. The aim of this study was to evaluate the anti-inflammatory effect of the Crude Extract(CE), and it's derived fractions: Ethanol(EtOH) and Hexane(HEX), and isolated compounds: Methoxy Nobiletin(MeONOB), 1,2-Benzopyrone(BP) and Eupalestin(EP) from A.conyzoides on: leukocytes, exudation and myeloperoxidase(MPO) and adenosine-deaminase(ADA) activities, and nitrate/nitrate(NOx) and cytokines (TNF-alpha and IFN-gamma) levels in a murine carrageenan-induced pleurisy.

Methodology: The aerial parts of A.conyzoides were air-dried at 50ºC, crushed and stored at 8ºC. The CE was prepared by maceration with ethanol, concentrated in rotary evaporator. The EtOH and HEX fractions were obtained by, extraction of CE, with different solvents of increasing polarity: ethanol and n-hexane. EtOH was partitioned with solvents of increasing polarity: n-hexane, dichloromethane and ethyl acetate. The dichloromethane fraction obtained from EtOH was chromatographed on silica gel "flash" column using dichloromethane and methanol gradient as eluent, being collected 100 fractions. Fractions 27-34 resulted in BP and fractions 35-69 yielded a mixture of methoxylated flavonoids were re-chromatographed on silica gel "flash" column using dichloromethane and methane gradient as eluent to obtain EP and MEONOB. Swiss mice were used though the experiments (Br.J.Pharmacol.183.811-19.1996). This study was approved by the local Ethical Committee (protocol: PP00757/CEUA/2012). Different groups of animals (n=5/group) were treated with CE(10-200mg/kg), EtOH(5-25mg/kg), HEX(25-50mg/kg), MeONOB(2.5-10mg/kg), BP(2.5-10mg/kg) or EP(1.0-10mg/kg) administered by intraperitoneal route, 0.5h before carrageenan-induced pleurisy(Cg,1%) administered by intrapleural route(i.pl.). The inflammation was analyzed after 4h. The leukocytes were analyzed using an automatic counter. A group of animals was previously challenged with Evans blue dye (25mg/kg, i.v.) to evaluate the exudation. The doses of CE(50mg/kg), EtOH(10mg/kg), HEX(50mg/kg), MeONOB(5mg/kg), BP(5mg/kg) or EP(5mg/kg) administered 0.5h before were selected to evaluate the effect of the herb on MPO and ADA activities, and NOx level which were analyzed by colorimetric assays. Analysis of cytokines were conducted using mouse inflammation cytometric bead array kit (BD Biosciences) only in EtOH and its isolated compounds. Statistical differences were determined by ANOVA and Student-Newman-Keuls post-hoc analysis. Values of p<0.05 were considered significant.

Results: CE(50-200mg/kg), EtOH(10-25Mg/kg), HEX(50mg/kg), MeONOB(5-10mg/kg), BP(5-10mg/kg) or EP(5-10mg/kg) inhibited leukocytes(CE:35.0±6.8 to 79,3±1.8%; EtOH:57.0±6.6 to 75.6±0.3%; HEX:56.7±7.5%; MeONOB:58.5±2.2 to 60.0±6.2%; BP:59.6±3.2 to 60.9±5.8%; and EP:64.7±1.6 to 69.5±1.4%), neutrophils(CE:37.4±7.1 to 80.1±1.7%; EtOH:68.3±2.2 to 77.0±0.2%; HEX:54.3±8.1%; MeONOB:63.1±2.0 to 63.8±5.5%; BP:64.3±5.1 to 71.9±2.2%; and EP:69.7±1.8 to 74.1±0.8%), exudation(CE:26.5±2.8 to 68.2±2.9%; EtOH:58.5±3.9 to 63.3±2.9%; HEX:33.3±2.4%; MeONOB:51.3±7.2 to 54.3±2.7%; BP:40.8±3.6 to 44.1±3.6%; and EP:39.9±8.1 to 42.1±6.9%), MPO(CE:34.7±5.6%;

EtOH:31.6±5.3%; HEX:29.6±4.2%; MeONOB:28.6±2.3%; BP:22.4±2.9%; and EP:18.3±1.3%), and ADA activities(CE:70.5±6.3%; EtOH:71.0±4.9%; HEX:72.1±4.8%; MeONOB:67.3±2.1%; BP:27.4±8.4%; EP:54.6±3.7%) and NOx level (CE:79.1±7.2%; EtOH:63.2±12.9%; HEX:71.2±9.4%; MeONOB:55.5±13.9%; BP:53.8±14.1%; EP:80.8±0.3%). Also EtOH and its isolated compounds inhibited TNF-alpha(CE:24.7±1.6%; EtOH:25.9±4.5%; MeONOB:26.6±2.2%; BP:31.5±1.3%; and EP:21.4±5.4%) and IFN-gamma(CE:15.7±1.6%; EtOH:11.0±1.4%; MeONOB:13.6±0.5%; BP:6.4±2.0%; and EP:11.0±0.6%)($p<0.05$).

Conclusion: A.conyzoides presented important anti-inflammatory properties not only by inhibiting leukocytes migration but activated neutrophils. This effect was also associated with the decrease of exudation and NOx and pro-inflammatory enzymes (MPO and ADA). This effect appears to be mainly related to the EtOH fraction and its isolated compounds: MeONOB, BP, and EP which inhibited all the inflammatory parameters, including TNF-alpha and IFN-gamma.

A-436

Effects of methanolic leaf extract of African mistletoes (Loranthus micranthus) on male sexual function in streptozotocin-induced diabetic Wister rats

B. O. Idonije[1], O. O. Festus[1], H. B. Osadolor[2]. *[1]Ambrose Alli University, Ekpoma, Nigeria, [2]University of Benin, Benin, Nigeria*

Background: The leaves of African mistletoes (*Loranthus micranthus*) have been shown in traditional African setting to improve sexual function in diabetic males but data on scientific proofs of this therapeutic action of these leaves is scanty hence this study. In this study, the effect of methanolic extract prepared from the leaves of *L. micranthus* on serum testosterone levels, sperm count and motility in diabetic male wistar rats was studied.

Method: The animals were randomly divided into four (4) groups made up of six (6) rats each and diabetes was induced in the rats by the administration of alloxan (100mg/kg) for 7 days. Group A served as the control (untreated diabetes), groups B and C were treated with 150mg/kg and 300mg/kg respectively of the extract while group D received the 100mg/kg of the standard antidiabetic drug (chlorpropamide). The duration of substance administration was fourteen days. On the fifteenth day, all the animals were lightly anaesthetized with ether and their blood collected for testosterone analysis. The rats were further dissected and the caudal epididymis of each incised and seminal fluid collected for sperm count and motility tests.

Results: Table 1 showed diabetes to decrease male sexual functions (group A) when compared with standard reference value. Also, significant increase ($p<0.05$) in the level of serum testosterone, sperm count and sperm motility which was dose depended was showed with the extract administration (groups B and C) compared with control (group A). Chlorpropamide treated rats (group D) also showed a significantly increased male sexual function compared with the control, however, mistletoe was more potent.

Conclusion: From the findings of this study, we suggest that leaf extract of African mostletes be studied in detail so as to know its therapeutic dose for it possible use as a therapeutic agent in the treatment of male infertility secondary to testosterone/sperm abnormalities.

Table 1: Effects of leaf extract of African mistletoes (*Loranthus micranthus*) on serum testosterone level, sperm count and sperm motility of diabetic male wister rats

Group	Testosterones level	Sperm count (x10)	Sperm Motility (%)
A (n=6)	1.21± 0.22	8.25±2.00	67.50±1.73
B (n=6)	6.63 ± 2.15*	9.90 ± 2.46*	92.50 ±22.64*
C (n=6)	7.76 ± 2.29*	10.90 ± 2.62*	105.00± 25.45*
D (n=6)	2.53 ± 0.67*	10.85 ± 2.89*	95.00± 22.66*

Values are mean ± S.D; * = significantly difference from the control (Group A) at $P < 0.05$.

A-437

Effects of Uvaria chamae Extracts on Blood Glucose,Inflammatory Markers,Hematological and Renal Status in Streptozotocin-induced Diabetic Rats

F. E. Olumese[1], I. O. Onoagbe[1], F. O. Omoruyi[2]. *[1]University of Benin, Benin City . Nigeria, Benin, Nigeria, [2]Texas A&M University, Corpus Christi, USA, Corpus Christi, TX*

Background: Uvaria chamae is a medicinal plant that is used in some regions of the world in the treatment of diabetes, and as an antifungal, antimalaria, and bacteriostatic herb. The chemical constituents of Uvaria chamae, include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. Traditionally, a decoction of the root is used in the treatment of many diseases, including diabetes. However, the use of this plant extracts in the treatment of diabetes have not been scientifically validated. In this study, we determined some blood analytes in streptozotocin-induced diabetic rats administered aqueous or ethanolic extract of the root of Uvaria chamae. Methods: Thirty six (eighteen adult normal and eighteen streptozotocin-induced diabetic rats) Sprague rats were administered aqueous or ethanolic extract (300 mg/kg body weight) of Uvaria chamae for 35 days [6 rats per group, average body weight (265.23 ± 7.20 g)]. The six groups were composed as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal rats receiving ethanolic extract (Normal plus Ethanolic Extract); Diabetic rats receiving de-ionized water (Diabetic Control); Diabetic rats receiving aqueous extract (Diabetic plus Aqueous Extract); and Diabetic rats receiving ethanolic extract (Diabetic plus Ethanolic Extract). Diabetes was induced using a single injection of streptozotocin (Sigma-Aldrich, 60 mg/Kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. Animals were euthanized by decapitation on day 35 after commencement of the feeding trial. Blood was collected for assays. Results: There was a significant ($p<0.05$) decrease in blood glucose level in the treated diabetic groups compared to the diabetic control. We also noted significant ($p<0.05$) increase in BUN in the diabetic control compared to the normal control. The administration of aqueous or ethanolic extract to the diabetic rats did not restore the level of BUN to that of normal control group. The diabetic groups administered aqueous or ethanolic extract showed increasing trend in the level of MCV toward the normal control group compared to the diabetic control. The levels of MCHC and WBC were significantly ($p<0.05$) lower in the diabetic groups administered aqueous or ethanolic extract compared to diabetic control. The levels of RBC, Hgb, PCV, platelets, monocytes and granulocytes were not significantly ($p>0.05$) altered among the groups. We noted reducing trend in the levels of IL-6 and IL-β in the diabetic groups administered aqueous or ethanolic extract compared to the diabetic control. However, serum creatinine level was slightly elevated in the diabetic group administered ethanolic extract. Conclusion: Overall, the consumption of aqueous or ethanolic extract of Uvaria chamae lowers blood glucose level which may be beneficial in the management of diabetes. The increasing trend in MCV level due to the administration of ethanolic or aqueous extract may protect against the development of anemia that is associated with diabetes. The inflammatory cytokine (IL-6) normally up-regulated in diabetes was depressed by the aqueous or ethanolic extract administration. However, the increased serum creatinine level is indicative of the potential adverse effect of the ethanolic extract on renal function.

A-438

Validation of Automated Immunoglobulin A, G, and M in Non-human Primate Serum to Support Pre-Clinical Toxicology Studies

S. E. Wildeboer, J. H. Bock, J. L. Wisniewski, R. P. Giovanelli. *Pfizer, Groton, CT*

Background: Immunoglobulins are glycoprotein molecules that are produced during an immunogenic response, and are divided into five classes, based on the differences in the amino acid sequence found on the heavy chains. Immunoglobulin A (IgA), Immunoglobulin G (IgG) and Immunoglobulin M (IgM), are the three most common immunoglobulins found in serum. Measurement of immunoglobulin concentrations are used to aid in the diagnosis of immune response or abnormal protein metabolism. Here, we validated automated human IgA, IgG and IgM assays for use in pre-clinical primate studies.

Methods: Siemens IgA (02194102), IgG (02193432), and IgM (02193483) assays are PEG-enhanced immunotubidimetric assays performed on the Siemens Advia 1800. Serum samples are pre-diluted by the instrument, then mixed with specific antiserum to form a precipitate that can be measured turbidimetrically at 340/694 nm. The measured absorbance is then compared to an established calibration curve (Advia Chemistry Liquid Specific Protein Calibrator, 07711199) and the concentration is determined and reported as mg/dL. Bio-Rad Liquid Assayed Multiqual® (Level 1: 694, Level 2:695, Level 3: 696) quality control (QC), AUDIT® MicroCV™ Protein Linearity (K702M-5), and colony primate (*Macaca fascicularis*) serum were analyzed to test the performance and dynamic range of the assays.

Results: Intra-assay precision testing was performed using 4 primate serum samples, and 2 levels of QC. Samples were analyzed a minimum of 5 replicates in a single assay run. All samples demonstrated %CV ≤ 3.6. Accuracy and inter-assay precision testing was performed using 3 levels of QC run in triplicate for 5 runs. Mean and imprecision were calculated and fell within the manufacturer's established 2SD range, and demonstrated %CV ≤ 3.0. Three primate serum samples were analyzed in duplicate over 4 separate assay runs, demonstrating %CV values ≤ 3.6. Commercially available linearity standards were analyzed and demonstrated reportable assay

analytical ranges of 31.4 - 669.4, 140 - 3009, and 17.4 - 393.8 mg/dL for IgA, IgG and IgM, respectively, confirming the manufacturer's stated analytical range. Dilutional linearity was performed with 3 primate serum samples with high concentrations. Samples diluted starting at a 1:2 dilution in 0.9% saline and analyzed in duplicate. Dilutional linearity was established as 1:16, 1:8, and 1:4 for IgA, IgG and IgM, respectively. Spike recovery was performed with pooled primate serum spiked with 10%, 7.5% and 5% of the highest level of linearity material. All spiked sample results were within 20% of the expected value. Sample frozen stability and freeze/thaw stability (-80°C) was performed with eight primate serum samples with varied IgA, IgG and IgM results. The samples were assayed neat then analyzed after 1, 3, and 6 months and after 1, 2, and 3 freeze/thaw cycles. All samples had appropriate percent recovery (80 - 120%).

Conclusion: The Siemens IgA, IgG, and IgM assays met all outlined criteria for validation and are appropriate for use in non-human primate serum samples to support pre-clinical toxicology studies.

Wednesday, July 30, 2014

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

Automation/Computer Applications

B-001

Reference values study for the urinalysis parameters measured in the sysmex uf1000

N. Z. Maluf, C. F. d. Pereira, E. V. V. Oliveira, L. L. Jaques, S. B. Ferreira, R. Balherini, M. d. Menezes, S. D. S. Vieira. *DASA, Barueri, Brazil*

Background: The urinalysis department of our Laboratory implanted the Sysmex UF1000 for the urinary cells analysis. The designed new sample flow includes the Roche Urisys 2400 for biochemical analysis and UF1000 for the urine sediment analysis. The methodology used by this equipment is flow cytometry. It performs the analysis and counting of erythrocytes, leukocytes, epithelial cells (EC), cylinders, crystal, mucus, sperm, bacteria (Bact) and yeasts. Currently, all the literature reference values for these parameters are based on the analysis by optical microscopy, which was the previous methodology used in the lab. Thus, we need to review and standardize the references values of these parameters in our laboratory. The objetive is to evaluate and standardize the reference values of the UF1000 measured parameters.

Methods: We evaluated 100 healthy individuals with no disease and medications or vitamins, to standardize the reference values for erythrocytes, leukocytes, cylinders, epithelial cells and bacteria. These individuals are FTEs from the laboratory and we ask them to participate in this study. For the statistical analysis we use dispersion graph and Gaussian distribution.

Results: After analyzing the results and statistics through the ABC curve, we found the results that is on the table. We also noted that the RBC values between 13.4 to 28.0 x 10^3/µL should be reassessed and compared with optic microscopy.

Conclusion: We concluded that the reference values for the UF1000 were higher than the obtained by the optical microscopy methodology. It uses larger amounts of sample and counts a greater number of cells, what allows it to be more accurate. After this study and with the continuous experience of monitoring patients in the last months, the new reference values got a good acceptance by the Lab professionals and physicians.

Parameters	Value - UF1000
RBC	13,3x10^3 /µL
WBC	31,5x10^3/µL
EC	3,6/µL
CASTS	1,07/µL
BACT	26,4x10^3/µL

B-003

QMS Tacrolimus Assay for the Beckman Coulter AU480, AU680, and AU5800 Clinical Chemistry Analyzers

C. Wong, D. Cheng, A. Thao, L. Ye. *ThermoFisher Scientific, Fremont, CA*

Background: The objective of this study is to evaluate the performance of Beckman Coulter AU480/AU680/AU5800 clinical chemistry analyzers for the quantitative determination of tacrolimus in human whole blood used in the management of kidney, heart, and liver allograft patients receiving tacrolimus therapy. Monitoring for tacrolimus is important for effective use to prevent allograft rejection following organ transplantation. The measurement of tacrolimus concentrations in whole blood in conjunction with other laboratory data and clinical evaluation can optimize immunosuppressive effect and minimize adverse side effects for patients.

Methods: The QMS Tacrolimus assay is a liquid stable particle-enhanced turbidimetric inhibition immunoassay. The assay is based on competition between free tacrolimus in the sample and tacrolimus derivative coated onto a micro-particle for anti-tacrolimus antibody binding sites. The tacrolimus-coated micro-particle reagent is rapidly agglutinated in the presence of anti-tacrolimus antibody reagent and the rate of agglutination is inversely proportional to the tacrolimus concentration in the sample. The rate of absorbance change is measured photometrically and is directly proportional to the rate of agglutination of the particles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the tacrolimus concentration in the sample. The assay consists of two reagents and an extraction solution for sample pretreatment. The calibrators contain tacrolimus in the human whole blood matrix at concentrations of 0, 2, 5, 10, 20, and 30 ng/mL.

Results: The performance of the QMS Tacrolimus Assay was evaluated on the Beckman Coulter AU480/AU680/AU5800 analyzers. All studies were evaluated using CLSI guidelines. On the AU480 and AU5800, four levels of Tacrolimus controls were used in the studies. The precision ranged from 4.3 %CV to 4.2 %CV for within-run and 7.2 %CV to 4.8 %CV for total run. Linearity was measured and confirmed over a range of 1.0 ng/mL to 28.7 ng/mL. The functional sensitivity was observed at 1.0 ng/mL. Patient correlation studies: AU480=1.0(AU680) - 0.08 (N=107, r=1.00), AU5800=1.02(AU680) + 0.23 (N=108, r=1.00). On the Beckman Coulter AU680 analyzer, three levels of Tacrolimus spikes and patient pools with lowest concentration at 2.9 ng/mL and highest at 25.0 ng/mL were tested twice per run, two runs per day for 20 days. The precision ranged from 1.8 %CV to 4.9 %CV for within-run and 3.9 %CV to 7.5 %CV for total run. Linearity was measured and confirmed over a range of 0.4 ng/mL to 30 ng/mL. The functional sensitivity was observed at 0.9 ng/mL. Patient correlation studies: AU680=1.14(LC-MS/MS)+0.50 (N=266, r=0.97).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the QMS Tacrolimus Assay on the Beckman AU480/AU680/AU5800 analyzers, and will provide an effective monitoring system for patients receiving Tacrolimus therapy.

B-004

Comparative study between ELISA and Chemiluminescence (CLIA) methods for the analysis of ENA-screening and specific ENA.

E. Melguizo[1], C. González-Rodríguez[1], G. Ávila García[1], Á. Fernández-Hermida[2], P. Falcó-Pegueroles[2]. *[1]Virgen Macarena University Hospital, Sevilla, Spain, [2]Menarini Diagnostics, S. A., Barcelona, Spain*

Introduction: The anti-cellular antibodies are autoantibodies directed against a variety of cellular structures (DNA, ribonucleoproteins ...). The group of specific antibodies directed against specific cellular proteins, anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-RNP/U1RNP, anti-Scl-70/topoisomerase I and anti-Jo-1 / histidyl-tRNA synthetase are clinically important in patients with autoimmune diseases (Sjögren's syndrome, systemic lupus erythematosus (SLE), scleroderma, dermatomyositis and polymyositis among others).

Objetive: Our aim was to analize the degree of agreement between chemiluminescence (CLIA) Zenith-RA from Menarini Diagnostics (Florence, Italy) and the habitual ELISA from Inova Diagnostics (San Diego, USA) for anti-ENA screening, anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-RNP/U1RNP, anti-Scl-70 and anti-Jo-1.

Material y method:

Serum samples from 496 patients with positive anti-cellular antibodies (title 1/160 or higher) were selected. ENA screening tests for specific antibodies were measured and in positive results, specific antibodies (anti-SSA, anti-SSB, anti-Sm, anti-RNP, anti-Scl-70 and anti-Jo-1) were measured by ELISA (INOVA diagnostics) and CLIA (Menarini, Zenit RA). Samples we classified as positive or negative according to the manufacturer cut-offs (20 U/mL for ELISA and 10 U/mL for CLIA assays, except CLIA ENA-screening wehere cut-off is 1) and the agreement degree was obtained using SPSSv19 statistical program.

Results: The results are shown in table 1. It was not possible to calculate the Kappa index for anti-Jo-1 since all samples were negative by CLIA. The rest of determinations show a good correlation between the two methods, and showed a good classification of patients with systemic autoimmune disease.

Conclusions: Both methods show a good degree of agreement in the analysis of specific anti-ENA. Given the advantages of CLIA techniques in front of ELISA (master curve for each lot of calibrators and controls, linearity and continuous access of samples) it could be a valid option for the analysis of specific anti-ENA in the clinical laboratory.

cut-offs and degree of agreement (measured by kappa index) ENA screening and specific ENAs.

	Cut-offELISA (UI)	Cut-offCLIA (UI)	kappa index	ELISA -	CLIA -	ELISA +	CLIA +
ENA-screening	20	1	0.769	295	328	201	167
anti-SSA (Ro)	20	10	0.947	82	85	126	123
anti-SSB (La)	20	10	0.914	133	153	75	55
anti-RNP	20	10	0.876	145	165	63	43
anti-Sm	20	10	0.917	190	193	18	15
anti-Scl-70	20	10	0.976	200	196	8	12
anti-Jo-1	20	10		201	208	7	0

B-005

Comparative evaluation of four assays for the automated determination of glycated hemoglobin

N. Z. Maluf, C. F. d. Pereira, C. Rosin, M. R. Ribeiro, L. K. Tirado, E. F. Moreira, A. N. Camilo, F. S. Fukuoka. *DASA, Barueri, Brazil*

Background: Recently, it has been discussed, among endocrinologists, pathologists and general physicians, which is the most accurate method for automated determination of glycated hemoglobin (HbA1c). Attempting to answer this question, in this study, we compared HbA1c results obtained from two distinct methodologies in four different analyzers to evaluate their performance and the impact in clinical monitoring of diabetes mellitus.

Materials and methods: We selected 73 samples, 16 with a percentual ≤5% of HbA1c, 20 borderline samples (upper normal range result between 5.5 and 7%), 37 samples with high percentual of HbA1c (≥7%) to perform on the following analyzers for HbA1c determination: TOSOH HLC®-G7, Bio-Rad VARIANT II, both based on the principle of High-Performance Liquid Chromatography (HPLC), ROCHE Cobas 6000 and Siemens ADVIA 2400, both based on turbidimetric methods. Among the selected samples, it is possible that some presented anomalous hemoglobin.

Results: The correlation among the analyzers is summarized in the table below.

Conclusion: There was good agreement among results of HbA1c when comparing different analyzers and distinct methodologies. Our study suggests that determination of HbA1c for clinical monitoring of diabetes mellitus can be performed using any of the automated assays systems evaluated.

HbA1c, %	Regression Equation	R^2
Tosoh, vs		
Bio-Rad	0.971x - 0.126	0.9952
Siemens	1.076x - 0.653	0.9867
Roche	1.130x - 0.526	0.9889
Bio-Rad, vs		
Siemens	1.050x - 0.770	0.991
Roche	1.095x - 0.585	0.9934
Siemens, vs		
Roche	1.047x + 0.145	0.9826

B-006

Reporting Critical Laboratory Values (PANIC) - Identifying Problems and Improving Processes

D. Weinstein, E. Neumark, J. Goldman, T. Tohami, A. Shalom, E. Weiss, G. Rashid. *Meir Medical center, Kfar Saba, Israel*

Background: Reporting critical laboratory test values to hospital wards in real-time is essential to provide immidiate treatment to critically ill patients. It is challenging for the laboratory staff due to the workload and the differences in critical values between regular departments and specialized departments, like dialysis, oncology, etc.

A routine monitoring of panic values reported by phone documented on the LIMS (Laboratory Information Management System) revealed a 40-60% gap between the number of results that should have been reported in real-time and the actual implementation. Therefore, the laboratory management initiated a major improvement project in order to minimize this gap.

Purpose: To increase reporting of real-time critical lab values in order to improve the quality of patients care. Improvement targets were reporting at least 80% of critical laboratory results in 2012 and at least 90% in 2013.

Methods: During 2012, an Excel program was developed in the lab in order to determine the percentage of the reported panic values from each laboratory. The introduction of a simple periodic report in an easy and automated manner revealed several problematic laboratories on one hand, and on the other, increased the awareness among laboratory staff and their commitment to report panic values. This new parameter was chosen to be one of the quality criterions in laboratory surveys. After implementation of this program and increased awareness among laboratory staff, panic values reporting by each lab increased gradually and steadily to >80% in 2012 with a continuous increase in 2013.

Conclusions: The availability of the report and the ability of the managers and staff to present it quickly improved quality, and allowed real-time monitoring of failures in reporting critical results. This report allowed us to know exactly- where, when and who did not report critical results and to address each problem. Results during 2012-2013 indicated that this led to a fundamental change in the conduct of the lab staff and their commitment to report the PANIC values in real-time.

As an outcome of this project, and due to its importance, this feature will be implemented in all laboratories of Clalit Health Care Services as a new module in the LIMS software. This implementation will take into account the lab's experience and knowledge. Such a module will allow control of the rules for complex alarms, managing alerts via pop-up windows, and producing statistics reports for different sectors in a convenient and flexible way.

B-007

Performance Evaluation of Siemens Dimension EXL 200 Integrated Chemistry System for a Regional Medical Center.

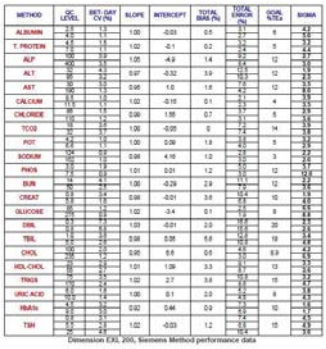
A. KHAJURIA, B. Robeson, J. Hafermann, G. Palmer, T. Walters. *Marshfield Clinic, Marshfield, WI*

***Background*:** The Dimension® EXL™ 200 Integrated Chemistry System offers LOCI® advanced chemiluminescent technology and automated, productivity features for the smaller-sized laboratory. Chemistry and immunoassay integration allows simultaneous processing to maximize workflow efficiency. Manufactures design their analytical systems to achieve certain performance characteristics for intended use and all instruments from a particular vendor may not have equivalent performance specifications. In a clinical laboratory risk management should begin with the verifications of those characteristics based on the performance goals to assess the suitability for the intended use.

***Methods*:** A test menu of 22 analytes was evaluated on Dimension EXL 200 for precision, reportable range, accuracy, detection limit and analytical specificity. The patient comparisons were done with another Dimension EXL 200 to have method comparability for the analytes. Assay performances were evaluated against performance criteria established in our laboratory. The statistical analysis was done by Analyse-it and method performance was calculated as Sigma metrics value [(Tea-Bias)/CV]. A Sigma 3.0 value is the minimum performance and 6.0 Sigma is considered world class performance.

***Results*:** Method performances evaluated on Sigma scale against our established quality performance criteria showed Glucose meeting a Sigma >6.0, five assays were <3 and rest varied between >3 and ≤5.5 Sigma. Chloride, BUN, creatinine and triglycerides showed <3 Sigma at low concentrations. HbA1c assay was unable to meet the performance goal requirement of 6% proposed by CAP & NGSP.

***Conclusion*:** Estimation of performance of a method is greatly influences by the stability of method under routine operating conditions and may not always mimic the controlled testing environment of vendors. Sigma metric performance assessment tool is a good measure to evaluate the performance of analytical processes. The data shows that most of methods on Dimension EXL 200 perform in the scale of 3 to 5.5 Sigma.



Dimension EXL 200, Siemens Method performance data

B-008

Diagnostic paths - towards computational evidence

Z. Liniger, G. Lindner, G. M. Fiedler, A. B. Leichtle. *Inselspital - Bern University Hospital, Bern, Switzerland*

Background: For the establishment of rapid, efficient, and financeable laboratory diagnostics in an ICD-10-funded hospital environment, defined diagnostic paths are of ever-growing importance. Usually they are set up as hierarchical trees or flow charts, leading to altered diagnostic suggestions depending on the outcome of previous tests (step-by-step diagnostic schemes). These guidelines are designed to focus diagnostic efforts on the most selective analytes, thereby avoiding unnecessary testing and providing a test panel sufficient to cover the most important side diagnoses. By overall reducing the number of recommended tests, they can help to improve cost effectiveness - especially in a health care compensation system that includes all diagnostic testing in a flat charge (as e.g. the recently introduced Swiss DRG system).

Diagnostic paths arise from different sources: single publications, recommendations of the different medical associations, and from the (inter-)national societies of Laboratory Medicine. However, all of these recommendations bear a severe drawback: they are agreements of experts in the field and therefore reflect opinions, not evidence.

Methods: Along with the rapid evolution of computational tools for parallel, GPU-based and grid- or cloud-based computing and the development of powerful statistical strategies an appealing resolution for this aforementioned lack of evidence emerges: To utilize already collected laboratory data, to merge it with diagnostic information and to derive which analytes are selective and likewise superior for the setup of a diagnosis. The conventional diagnostic path recommendations can be replaced by diagnosis-specific models inferred from the laboratory and classification data, a process that does not imply prior "expert knowledge" in terms of opinions which parameter to measure, but that is based only of the numerical evidence contained in the dataset and that delivers disease probabilities, assisting the physician to balance decisions for further diagnostic and therapeutic procedures. In a proof-of-principle study we utilized laboratory data and diagnostic information of n>15'000 patients of the Inselspital's Department of Emergency Medicine and computationally determined, which lab tests are indispensable and which ones are unnecessary for establishing the top ICD-10 coded diagnoses via the estimation of posterior inclusion probabilities with bootstrapped confidence intervals of a set of lab tests.

Results: Our results clearly show the feasibility of our new approach: For myocardial infarction e.g. our algorithm without any prior knowledge of the disease nor any pathophysiological basis suggests a panel of lab test similar to current guidelines - solely based on computational principles, our patient population, and laboratory data already generated thereof.

Conclusion: In a highly digitalized hospital environment, the present lack of evidence for diagnostic paths is unjustifiable: All diagnosis-related classification of all patients - hospitalized or out-patient - are electronically registered, and usually all lab tests are also electronically available. These data, stored away and laid untouched for decades, could improve and streamline diagnostic testing and implicitly generate benefit for the patients - the tools therefor are ready.

B-009

New Instrument Interface Standard to Enable Improved Interoperability with Integrated Information Systems.

J. B. Jones[1], E. Heierman[2], R. Bush[3], E. Olson[4]. *[1]Geisinger Health System, Danville, PA, [2]Abbott Diagnostics, Irving, TX, [3]Orchard Software, Indianapolis, IN, [4]Siemens Healthcare, Raleigh, NC*

The In Vitro Diagnostic (IVD) Industry Connectivity Consortium (IICC) has worked with several standards organizations to develop a new interoperability (i.e. instrument interface) standard that provides plug-n-play connectivity between IVD analyzers and IT systems, eliminating the need for unique analyzer interfaces. Currently, the Clinical and Laboratory Standards Institute (CLSI) LIS1 and LIS2 specifications (also known as ASTM) provide limited guidance on the structure and content of the data being exchanged in instrument interfaces. These older standards are highly flexible and have been implemented in many different ways thus creating barriers to integration and interoperability. In addition laboratories must validate these unique interfaces every time a new analyzer is installed, and often encounter lengthy implementation cycles before they can go "live".

IICC established partnerships with the CLSI (Clinical Laboratory Standards Institute), IHE (Integrating the Healthcare Enterprise), and HL7 (Health Level 7) standards organizations in order to leverage existing work, accelerate the creation of a plug-n-play standard, and promote worldwide adoption.

The resulting IICC standard is documented in an IHE Laboratory Analytical Workflow (LAW) profile. This profile provides the following capabilities, most of which are not supported by the LIS2 (ASTM) standard:

1) Support for Immunoassay, Chemistry, Hematology, and Microbiology testing

2) Unique identification of each order request at the test or test panel level

3) Improved query for orders

4) Selection of query as the default mode

5) Simplified order download

6) Ability for an analyzer to accept/reject orders

7) Improved device identification for test logging

8) Contributing substance identification for test logging

9) Basic and enhanced message interface to support IVD instrument rule evaluation

10) Support for LOINC to identify test requests and observations

11) Unique identification of runs

12) Support for hematology images, graphs, and plots

13) Support for transmission of raw values

To confirm that the LAW profile could support plug-n-play connectivity, vendors representing seven IVD analyzer and three IVD IT systems have participated in the 2012 European IHE and 2014 North American IHE "Connectathons." The IHE team defined ten test cases representing major LAW scenarios and focused on immunoassay and clinical chemistry orders. Each IVD analyzer tested interoperability with each IVD IT system through the execution of the test cases. The testing was monitored by IHE independent representatives. Each IVD IT system used the same interface implementation to communicate with each of the seven instruments.

All 13 testing events were successfully completed, allowing all participating vendors to register an IHE Integration Statement documenting that their implementation successfully integrated with the other vendors through the use of the LAW profile.

This new IICC instrument interface standard is now available for adoption by IVD instrument vendors (ivdconnectivity.org). Its use should greatly simplify interoperability between different IT systems in the more integrated healthcare continuum that is currently evolving under federal guidelines of "Meaningful Use".

B-010

Measuring Reproducibility of analysis in a proficiency testing (PT) scheme using modified control materials. A novel approach using big data analysis.

O. Panagiotakis[1], D. Rizos[2], K. Makris[3], A. Haliassos[1]. *[1]ESEAP Greek Proficiency Testing scheme for Clinical Laboratories, Athens, Greece, [2]Hormone Laboratory, Aretaieion Hospital, Medical School, University of Athens, Athens, Greece, [3]Clinical Biochemistry Department, KAT General Hospital, Kifissia, Greece*

The evaluation of reproducibility in proficiency testing (PT) schemes is based on the analysis of the same sample multiple times during a cycle. This can be tempting for participants to detect the replicated samples and to report already known target values. In order to overcome this possibility ESEAP designed a study involving the analysis of two replicates of a sample and two other samples, derived from the initial, but diluted or concentrated by 10% by modification of the serum volume lyophilized per vial.

The aim of our study was to investigate the interference of samples' modification to the estimation of reproducibility.

The 290 laboratories of ESEAP from Greece and Cyprus participated in this blind study during the 2013 cycle of Clinical Chemistry scheme. We excluded the results from the laboratories that haven't reported all four samples, from those excluded from the normal bias analysis and from the laboratories that reported a method change during this cycle. We evaluated 4 results from 209 laboratories for 21 different parameters, the two as received (samples 1&2) and the other two after correction with the appropriate factor for the dilution (sample 3) or concentration (sample 4) and we calculated the mean value for each sample. We evaluated the 6 possible comparison couples for each parameter using t-test. Our results are presented at the followinf table:

Couples of Samples	Analytes																					
	Glucose	Urea	Creatinine	Sodium	Potassium	Total Protein	Albumin	Cholesterol	HDL Cholesterol	Triglycerides	Uric Acid	Total Bilirubin	Calcium	Phosphate	Magnesium	Iron	SGOT (AST)	SGPT (ALT)	γ-Glutamyl Transferase	Creatine Kinase	Amylase
1 & 3	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-
1 & 4	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
1 & 2	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+
3 & 4	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
4 & 2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
2 & 3	-	+	-	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-

(- = statistically non-significant difference, + = statistically significant difference)

A pattern (**in bold**) emerged for the majority of the analytes showing that the first unmodified and the diluted sample, as also the two unmodified and the concentrated and the second unmodified showed statistically non-significant difference in contrast to the other three combinations that where statistically different.

Our data show that the possible interference due to the modification of the samples is smaller than the uncertainty of measurements of the identical samples (1&2) thus our approach can be used for the estimation of reproducibility.

B-011

Rapid Consistent Turnaround time (TAT) of Lab Results through an Innovative Centrifugation Protocol

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

More than a billion laboratory tests are performed each year in the United States influencing 64% to 74% of the medical decisions. Labs have to provide accurate results and as quickly as possible for the STAT tests. So, turnaround time (TAT) of test results is a critical component in patient care.

MultiCare Health System (Tacoma, WA) laboratory has been providing excellent TAT for several critical lab tests over the past several years. We have recently achieved a "Received to Result" mean TAT time of 23.1 minutes (N=2743, SD=7.2 minutes, median=21 minutes) using "Abbott ARCHITECT Enzymatic Creatinine test" as a proxy for our Comprehensive Metabolic Panel (CMP) This test requires the longest analytical time in a CMP, thus equating to 90% of CMP results reported in 30 minutes. In case of cardiac assays, 90% of all Troponin results are reported within 35 minutes and 85% of all BNP results are reported within 35 minutes.

Centrifugation time is a major bottleneck and becomes the rate-limiting step and greatly reduces the laboratory specimen throughput. To improve TAT, we switched from the conventional slow spin procedure (6 minutes spin at 3500 RPM) to the new fast spin procedure (1 minute spin at 12,000 RPM). There was no noticeable difference in the results obtained after a fast spin compared to the slow spin when we tested 41 different patient samples in 16 different tests. After this off-automation track high speed centrifugation step for STAT samples, the samples are placed on the TLA (Total Lab Automation) track system for processing as usual. Thus the STAT TAT target is met with the advantages of TLA.

Manufacturers of blood collection tubes typically recommend slower speeds and times for centrifugation. Using a fixed rotor refrigerated centrifuge (Beckman Coulter Allegra Model X30R and F1010 rotor assembly) allows faster speeds and shorter times for separation of plasma from cells. Gel barrier plastic collection tubes are highly resistant to breakage or failure during centrifugation at this higher speed and no statistical change in hemolysis is present. However, balance tubes in the centrifuge should be replaced monthly as a precautionary step as they show visual bending of the tube after this period of centrifugation.

Most laboratories are constantly pressured to deliver results more quickly. A viable option to improve sample handling in the laboratory is total laboratory automation which has been shown to dramatically improve laboratory TAT and clinical throughput. However, even when using automation, centrifugation is still a major bottleneck for STAT specimens and becomes a rate limiting step. Performing off-automation track centrifugation of STAT specimens using this Innovative Centrifugation Protocol (with 2 centrifuges each with 6 tube capacity) has allowed MultiCare Health System to achieve quick and consistent turnaround times for stat chemistry results.

B-012

Simoa HD-1: a fully automated digital immunoassay analyzer capable of single molecule counting, sub-femtomolar sensitivity, and multiplexing

D. H. Wilson, C. W. Kan, A. J. Rivnak, T. G. Campbell, T. Piech, D. M. Rissin, P. Patel, E. Frew, G. Provuncher, A. Schroff, R. E. Meyer, M. Fishburn, L. Chang, D. R. Fournier, D. C. Duffy. *Quanterix Corporation, Cambridge, MA*

Objective: The aim of this work was to develop the next generation immunoassay analyzer capable of several orders of magnitude greater sensitivity than current best-in-class conventional immunoassay systems. The technology utilizes single molecule array (Simoa) technology to usher in fully automated digital immunoassay and multiplexing capability to the clinical laboratory. Simoa technology isolates individual paramagnetic beads in arrays of femtoliter-sized wells and detects single enzyme-labeled proteins on these beads using sequential fluid flows in microfabricated polymer array assemblies for ultra-sensitive signal measurements. These array assemblies have been incorporated into a low cost disk consumable. The array approach for assay signal quantification allows for rapid digital data acquisition and high throughput, enabling development of a fully automated system for low-cost measurement of clinically relevant biomarkers with high precision and unprecedented sensitivity across a broad dynamic range.

Methods: Detection of single molecules using Simoa has been reported previously. In brief, proteins are captured on antibody-coated paramagnetic microbeads (2.7-mm diameter) and labeled with single enzymes, followed by partitioning single beads into arrays of femtoliter-sized wells and sealing the arrays in the presence of a fluorogenic substrate. We developed a low cost disk consumable that enables standard fluidics handling instrumentation to load and seal assay beads into the arrays using only fluidic flow. Beads with single enzyme label molecules are isolated in single wells in the presence of a substrate, and fluorescent product is allowed to build up within the 40 femtoliter confines of the wells. The fluorescence signal quickly concentrates in such a small volume, allowing detectable signal from a single enzyme label in only 30 seconds. Depending on the analyte concentration, hundreds to many thousands of single molecule signals are counted simultaneously using a fluorescence microscope optical system and image analysis software. Next we integrated this array and imaging module together with a standard fluidics-handling platform that performs sequential cuvette processing of paramagnetic bead-based ELISA reagents. The reagents employ antibody-coated capture beads, biotinylated detector antibodies, and streptavidin-ß-galactosidase as the signal enzyme. A standard bead-based immunoassay is performed, and then the beads are transferred to the Simoa module for signal development and digital quantification.

Results: Prototype single-plex digital immunoassays were developed for PSA, Troponin, IL-6, and Aβ42. A prototype cytokine 6-plex was also developed. LoD's ranged from 0.002 to 0.05 pg/mL. The LoQ of the PSA assay was estimated as 0.037 pg/mL. These sensitivities ranged to over 1000-fold greater than conventional immunoassay. Imprecision for the prototype assays was evaluated over 10 runs across five days in a CLSI format. CVs were generally less than 10%. Spike recovery and linearity met standard criteria for acceptability. The system throughput is 68 tests/hour, and over 4 logs of dynamic range were demonstrated. The prototype 6-plex gave equivalent precision and sensitivity performance to single-plex versions of the same assay.

Conclusion: The data indicate we have developed a next generation fully automated immunoassay analyzer capable of orders-of-magnitude greater sensitivity than conventional state-of-the-art immunoassay systems.

B-013

Capability Analysis for Procalcitonin Assay Performed with the miniVidas® Method.

V. M. Genta[1], R. Murray[1], E. Ravago[1], B. Boston[1], S. Spingarn[2], W. Tang[1]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Norfolk General Hospital, Norfolk, VA*

Background: Procalcitonin blood values have been linked to both increased risk and severity of sepsis. Consequently, in-hospital assays assure availability of timely results for prompt medical intervention. In our institution we evaluated the capability of the miniVidas method with phase1 (short-term) and phase2 (long-term) precision verification studies. **Methods:** After a miniVidas instrument (IVD1210422, BIOMERIEUX) was installed by the manufacturer's representative, a phase1 study was performed by assaying two levels of QC material (level1 lot#1031940; level2 lot#103150, BIOMERIEUX) with five independent assays for five consecutive days.

Phase2 study was performed by assaying two levels of control material once a day for 100 days. The observations were transferred to Minitab® (Version 16, Minitab Inc.) statistical software. The observations were analyzed with descriptive, exploratory, inferential and diagnostics univariate and multivariate statistical techniques. For the process capability analysis the UCL and LCL were calculated using the mean of phase1 study +/-(0.5xtotal error allowed by CAP). **Results:** Phase1 study. Descriptive statistics: Level1 mean=17, s=0.7, C.V.=3.9%, min=16, Q1=16.8, median=17, Q3=17.8, max=19; Level2 mean=1.57, s=0.05, C.V.=3.2%, min=1.4, Q1=1.55, median=1.58, Q3=1.59, max=1.64. For level1 histogram, normality plot and Anderson-Darling (A-D) test (level1 P=0.4) showed quasi-normal distribution; for level2 A-D test showed non-normality (P=0.005) due to a possible outlier (obs. #14 = 1.4 ng/mL), the histogram and the normal probability plot showed a quasi-normal distribution. Tests for equality of variances showed discordant results; for both levels Bartlett's test P<0.05, Levene's test P>0.5. Estimates of daily s with Bonferroni's 95% C.I. showed that the values of s for level1 of day 2 and for level 2 of day 14 were larger than the others and had larger 95% C.I. Parallel boxplots and ANOVA with Tukey's multiple comparisons showed that while for level 1 there were statistically significant differences between daily means (P=0.004), for level 2 there were no statistically significant differences(P=0.06). However, the maximum mean difference for level1 = 1.4ng/mL was not significant for either QC or clinical practices. The plots of the autocorrelation function showed statistically significant autocorrelation for the first two observations only. The plots of Hotelling's T-square and generalized variance did not show either parallelism or non-randomness. The individual point QC charts for level 1 and 2 were constructed using the estimates of mean and s (for s = s/0.98, corrected for bias) with LCL= mean-3s, UCL= mean+3s. Phase 2 study: Level1 mean=16.5, s=0.8, C.V.=4.8%, Level2 mean=1.6, s=0.07, C.V.=4.3%. The individual points charts for both levels of contol did not show trends, shifts,outliers or autocorrelation. There were no statistically significant differences between either means or s for Phase1 and Phase2 studies (P>0.05).The capabilities indexes Cp (level1=2.4, level2=2.3) and Cpk (level1=2.8, level2=2.7) were similar indicating centering of the mean, their values (> 2) indicated acceptable six-sigma performance. **Conclusion:** These studies showed that the phase1 study design was adequate to estimate mean and s for the individual points QC charts.Furthermore, phase2 studies indicated that the method's reproducibility and capability were adequate to monitor the variability within the total error specifications. Finally, appropriate statistical software was essential for the analysis of the observations.

B-014

Performance Evaluation of three URiSCAN series for routine urinalysis

K. Ko, M. Kwon, H. Woo, H. Park. *Kangbuk Samsung Hospital, Department of Laboratory Medicine, Seoul, Korea, Republic of*

Background: Urinalysis is one of the important diagnostic screening tools in clinical practice. Correct urinalysis results offer information of the renal and genitourinary system. The URiSCAN devices (Yeongdong Diagnostics, Seoul, Korea) are one of the most commonly used urine analyzers in Korea. Our study aimed to evaluate the analytical performance of the three URiSCAN devices for routine urinalysis in comparison with Roche urine analyzers.

Methods: A total of 1,273 urine specimens were enrolled in this study between June 2013 and November 2013. We performed urinalysis using three URiSCAN devices; Optima, Pro II and Super+, and compared to other urine analyzers (Roche Diagnostics, Switzerland); Urisys 1100, Cobas u411 and Urisys 2400. Each Roche analyzer was selected with consideration of complexity of each URiSCAN device. The results of three analyzers for blood, bilirubin, urobilinogen, ketone, protein, nitrite, glucose and leukocyte were considered concordant if they were within ±1 grading difference in comparison with the results by Roche analyzers. Moreover, the screening of leukocytes and erythrocytes using both systems were compared with microscopic examinations.

Results: Good correlation between three URiSCAN devices and their corresponding methods were observed (range of correlation coefficient: 0.602 to 0.989, p<0.001). Overall agreement rates for eight test items were acceptable: 84.9% - 100% for Optima *vs.* Urisys 1100; 96.8% - 100% for Pro II vs. cobas u411; and 99.3% - 100% for Super+ vs. Urisys 2400. The sensitivity and specificity of the URiSCAN Optima were 62.7% and 95.4% for leukocytes, 91.4% and 78.1% for erythrocytes; for URiSCAN Pro II were 79.6% and 86.4% for leukocytes, 62.2% and 96.9% for erythrocytes; for URiSCAN Super+ were 82.5% and 87.4% for leukocytes, 92.9% and 83.8% for erythrocytes.

Conclusions: The three URiSCAN devices showed high agreement rates with the corresponding Roche urine analyzers and microscopic examination. Therefore, these three URiSCAN series would be useful for clinical laboratory performing in routine urinalysis.

B-015

Improvement of work processes in the laboratory after introduction of the Automate 1250 System

E. Neumark, E. Weiss, J. Goldman, A. Shalom, G. Rashid. *Meir Medical center, Kfar Saba, Israel*

Background: In early 2012, the automated "Automate 1250" system (Beckman Coulter) was introduced to the Chemistry, Immunology and Endocrinology Laboratories at Meir Medical Center. This computerized robotic system sorts and archives laboratory samples. It is designed to treat pre-analytical processes (such as decapping, sorting and if necessary aliquoting samples, followed by samples distribution to designated workstations) and post-analytical processes (archiving samples). Prior to introducing the system, work processes in the 3 laboratories were mapped. Immunology Laboratory processes were different from other laboratories since most of the blood samples that arrived were aliquoted to several tubes in order to run on different analyzers. This resulted in delays and results were only available 1-3 days after receiving the samples, depending on how often the equipment was operated. We present in this work the improvement that was made in the Immunology Laboratory.

Aim: This study investigated several workflows to decrease immunology TAT (Turn Around Time). This was accomplished by using the Automate 1250 to route samples for immediate testing on analyzers that operated daily in the original tubes, and then to send the aliquoted samples to analyzers that operated less frequently.

Results: Analyzing for several months how the Immunology tests were processed, the various tests requested for each blood sample, and their distribution between analyzers, was the basis for the improvement in the lab. If the analyzer on which the most tests were performed was run daily, more than 50% of the samples would not be aliquoted, and the test results would be available within a day. Due to these observations, Immunology Laboratory staff began working on a method whereby they analyzed samples immediately upon their arrival in the first round of the Automate 1250. All the original samples were sent to the analyzer that ran most of the tests. This change in procedure affected other processes in the lab, which were not directly linked to the Automate 1250 system, and all together it resulted in a significant decrease in turnaround times:

Before the improvement: Average result availability before the use of Automate 1250 was 2.7 days _ 17% longer than the specified result time.

After the improvement: Average result time decreased to 1.1 days, only a 2% deviation in the specified result time.

Conclusions: Using the Automate 1250 system increased Immunology Laboratory efficiency, resulting in shorter TAT times and improved service. TAT time reduced from 2.7 days before the introduction of the new system to the lab to 1.1 days, affecting 60% of the samples. This resulted in safer patient care and improved service quality. Following the above results, the use of the Automate 1250 system was extended to other laboratories.

B-016

QMS Everolimus Assay for the Beckman Coulter AU480, AU680, AU5800 Clinical Chemistry Analyzers

C. Wong, D. Cheng, H. Naqvi, K. Yokobata. *ThermoFisher Scientific, Fremont, CA*

Background: The objective of this study is to evaluate the performance of the Beckman Coulter AU480/AU680/AU5800 clinical chemistry analyzers with the QMS Everolimus Assay for the quantitative determination of everolimus in human whole blood used in the management of organ allograft transplant patients receiving everolimus therapy. Monitoring for everolimus is important for effective use to prevent allograft rejection following organ transplantation. The measurement of everolimus concentrations in whole blood in conjunction with other laboratory data and clinical evaluation can optimize immunosuppressive effect and minimize adverse side effects for patients.

Methods: The QMS Everolimus assay is a liquid stable homogeneous particle-enhanced turbidimetric inhibition immunoassay. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the everolimus antibody reagent. The everolimus-coated microparticle reagent is rapidly agglutinated in the presence of the anti-everolimus antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically. When a sample containing everolimus is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. The concentration-dependent classic agglutination inhibition curve can be

obtained with the maximum rate of agglutination at the lowest everolimus concentration and the lowest agglutination rate at the highest everolimus concentration. The assay consists of ready-to-use reagents, calibrators (value-assigned concentrations at 0, 1.5, 3, 6, 12, and 20 ng/mL) and controls (value-assigned concentrations at 4, 8, and 15 ng/mL).

Results: The performance of the QMS Everolimus Assay was evaluated on the Beckman Coulter AU480/AU680/AU5800 analyzers. All studies were evaluated using CLSI guidelines. Three levels of everolimus controls were used in the studies. The precision ranged from 7.1%CV to 6.4%CV for within-run and 10.5%CV to 8.1%CV for total run. Linearity was measured and confirmed over a range of 1.5 ng/mL to 20 ng/mL. The least detectable dose on the AU480/AU680/AU5800 yielded 0.3 ng/mL. Patient correlation studies: AU480=0.90(Hitachi 917) + 0.18 (N=107, r=0.99), AU680=0.94(Hitachi 917) + 0.0 (N=100, r=0.99), AU5800=0.99(Hitachi 917) + 0.13 (N=106,r=0.98).

Conclusion: All measured studies demonstrated acceptable performance, validating the use of the QMS Everolimus Assay on the Beckman Coulter AU480/AU680/AU5800 analyzers, and providing an effective monitoring system for patients receiving everolimus therapy.

B-017

Error Rate Testing for the Accelerator p540 Preanalytical Sample Processor Vision System

A. DeFrance, B. Smith, K. Reed, Y. Smith. *Abbott Laboratories, Irving, TX*

Introduction: The ACCELERATOR *p*540 is a fully automated perianalytical sample processor that performs sample loading, identification, decapping, aliquoting, and sorting operations. A Vision System was added to the p540 aliquoter unit to identify cap color, if present.. Parameters link the cap color to the specimen type. Middleware determines compatibility of specimen type for the tests ordered. Depending on that determination the tube can either be further processed, or moved to an error location subject to user preference.

Methodology: The vision system library contains 35 common cap colors. Additionally, it can be trained to read other cap colors. The vision system consists of a digital camera that acquires images of racks as they are presented to the aliquoter. This picture is available for operator viewing. The cap color as detected by the camera and the pixel data for each cap is sent to the p540 software. The software analyzes the data and determines whether the cap matches a trained color. In the error rate test, 12 different caps were tested on two different systems. These caps consisted of a mixture of rubber, plastic and screw caps of various colors. Some caps were part of the library and some were trained.

Results: The table below summarizes the p540 Vision system error rate testing.

p540 Error Rate Testing								
	System 1				System 2			
Cap Type	Trained/ Library	Original error rate	Retrain	Error rate	Trained/ Library	Original error rate	Retrain	Error Rate
Greiner: lavender	Trained	0%	No	0%	Trained	0%	No	0%
Greiner: white	Library	100%	Yes	0%	Library	100%	Yes	0%
Terumo: red	Library	12%	Yes	0%	Library	8%	Yes	0%
Terumo: green	Library	20%	Yes	0%	Library	0%	No	0%
BD-Plastic: lt green	Library	0%	No	0%	Library	0%	No	0%
BD-Plastic: gold	Library	0%	No	0%	Library	0%	No	0%
Sekisui: tan	Library	0%	No	0%	Library	0%	Yes	0%
Sekisui: gray	Library	16%	Yes	0%	Library	36%	Yes	0%
BD-Rubber: red	Library	0%	No	0%	Library	0%	No	0%
BD-Rubber: blue	Trained	0%	No	0%	Trained	0%	No	0%
Sarstedt: orange	Library	100%	Yes	0%	Library	80%	Yes	0%
Sarstedt: lavender	Library	0%	No	0%	Library	4%	Yes	0%

Conclusion: The error rate of the p540 vision system is low. As seen in the retrain column, if the cap color exists in the library the system may or may not initially identify the correct color. However, it is a simple process to train any cap color. This ability to train any cap color keeps the error rate low and allows for changes in the color manufacturer's caps without having to reconfigure the camera.

B-018

Utilising Information Technology (IT) to improve work processes at the Satellite Laboratories

S. Lim, C. Yeo. *Singapore General Hospital, Singapore 169608, Singapore*

Background: Singapore General Hospital (SGH) Clinical Biochemistry Laboratory has under its charge; 9 satellite laboratories that are situated in polyclinics (public-funded primary healthcare facilities). These satellite laboratories offer phlebotomy services and some onsite laboratory tests, although the bulk of the specimens are sent back to the central laboratory for analysis. HbA1c (Glycated Haemoglobin A1) constitutes a significant 38% of the onsite testing repertoire. The objective of this study was to utilise information technology to improve the otherwise manual processes for handling HbA1c results and to achieve a standardised workflow across the 9 satellite laboratories. Laboratory turnaround time (TAT) for HBA1c was used as a performance indicator to measure the success of this endeavour.

Methods: HbA1c analysers were connected to the Laboratory Information System by the first quarter of 2012. Standard procedures for specimen registration and processing and result verification were instituted at the laboratories in the last quarter of 2012 and autoverification (AV) of HbA1c test results was piloted at 2 laboratories in July 2013. TAT, defined as the time taken from registration at the lab to result verification.

Results: Significant improvement in TAT was observed at all the satellite laboratories after the introduction of LIS connectivity and a standardised workflow. By the end of 2012 >90% of HbA1c results were completed within 30 minutes, a betterment over the pre-study rate of 50% completion within 30 minutes. This improvement was also sustainable from 2013. AV was piloted in July 2013 at 2 of the satellite laboratories to explore its effect on the TAT. Data showed that TAT slipped initially due to operator unfamiliarity but improved in the following months, enabling both laboratories to complete >80% HbA1c testing within 15 minutes.

Conclusion: Online connectivity of the analysers and standardisation of work processes have definitely improved the efficiency of onsite HbA1c testing at the satellite laboratories. Auto-verification of test results, with careful planning, can also improve TAT. Our next steps will be to sustain the work process improvements, implemented AV for the remaining 7 satellite laboratories and study the impact of our enhanced practices on the overall operations of the polyclinics.

B-019

Implementing the "Integrated Laboratory" tool for ANF tests in a high-functioning laboratory

F. B. Flumian[1], R. Mansur[1], R. J. Marani[1], A. Cintra[2], F. O. Paiva[1], G. Lakos[3], R. Vrunski[2], M. D. Freire[4], N. Gaburo[1]. *[1]DASA, Sao Paulo, Brazil, [2]Werfen Medical, Sao Paulo, Brazil, [3]INOVA Diagnostics, San Diego, CA, [4]DASA, Rio de Janeiro, Brazil*

Background: The "gold standard" method used to screen for antinuclear factor (ANF) is Indirect Immunofluorescence (IIF) on HEp-2 cells. However, this methodology presents some problems, such as subjectivity in interpreting the standard reading, a lack of trained technicians and the test's low standardization. On the other hand, ANF diagnosis has great clinical importance, which justifies the increasing demand, year after year. Given this scenario, laboratories processing a large number of ANF screening tests on a daily basis require alternatives that meet this high test volume while not compromising diagnosis quality and accuracy.

Objective: To present the implementation of "Integrated Laboratory" system and the Quanta-link® software that integrates the Quanta-Lyser® and NOVA View® equipment with the laboratory's LIS.

Methods: The validation of the equipment used to prepare the HEp-2 slides (QUANTA-Lyser®) utilized 40 samples, 20 positive and 20 negative, and the positive samples were from different titers and standards. Quanta-Lyser®'s reproducibility was verified using 4 positive samples and 4 negative samples. The samples were replicated 5 times in the same session. Both the AFN automated reading equipment, NOVA View®, and the training to read AFNs were used every day. Therefore, 200 AFNs/day were visualized, with comparative readings between the NOVA View® reading equipment and the manual microscope reading, which was carried out by a team of 8 technicians trained to read AFN.

Twenty one collaborators from different areas of the company were involved in

configuring the systems, infrastructure and participated in the technical-diagnostic process in order to make this validation possible.

Results: The implementation process lasted 5 months. In the first phase, the presentation of the project and the installation and validation of Quanta-Lyser® were carried out, followed by the installation, calibration, training and validation of NOVA View®. In the second phase, the implementation of the DAPI reading and the learning curve was carried out, as well as the implementation and configuration, training and validation of QUANTA Link®. In the third and last phase, the training and validation of the system as a whole was developed. When compared to the previous method used, the results found were: a 2.64% reduction in repetitions, a 10% reduction in false positive results, a 70% reduction in

the use of paper and a 53.6% reduction in the release time of negative samples and 7.1% reduction for positive samples. Qualitative results can also be highlighted: Improved standardization of the results release, greater precision in diagnosis, significant improvement in the traceability of samples, reduction in the manual entry of results, ensuring reduction in possible transcription errors, and reduction in the causes of repetitive strain injury.

Conclusion: Implementing the "Integrated Laboratory" was a great challenge for DASA's manual immunology sector, as it was a project that involved many collaborators from different sectors and companies, and broke paradigms in the traditional diagnosis of AFN. It provided important gains for the department's costs and productivity, with an important gain in analytic quality. The project also led to the integration of various departments within the company and produced knowledge that can be used in future projects

B-020

Evaluation of Calibrator and System Stability for Beckman Coulter Access 2 System

N. Akbas[1], A. Algeciras-Schimnich[1], N. A. Baumann[1], D. R. Block[1], J. R. Budd[2], S. Gaston[2], G. G. Klee[1]. *[1]Mayo Clinic, Rochester, MN, [2]Beckman Coulter, Chaska, MN*

Introduction: It is standard practice in clinical laboratories to calibrate assays prior to use and recalibrate at the pre-established calibration expiration time. All automated assays have a time frame for calibration curve stability set by manufacturers which are monitored with quality control and assurance systems to ensure calibration integrity in clinical labs.

Objective: In this research, we investigated the stability performance of calibrators and the system for three automated assays (human luteinizing hormone (hLH), total triiodothyronine (TT3) and vitamin B12) performed on the Beckman Coulter Access 2 analyzer.

Methods: Thirty data sets were collected over forty-three days for six levels of calibrator by testing as unknown and analysis of results was compared to acceptance criteria. Each data set consisted of four replicates of calibrator levels S0, S1, S4, and S5 and three replicates of calibrator levels S2 and S3. Three levels of serum quality control pools for the same three assays also were analyzed for comparison. In addition, one replicate for three levels of quality control materials (QC1 through QC3) were performed. The maximum number of days before the calibration exceeds the defined calibration limits were calculated using a time regression analysis program (provided by Beckman Coulter) for calibrator levels and QC levels. Two separate models (percent change versus day model and concentration change versus day model) were used for analysis. The initial calibration curve was constructed using total number of twelve calibrator measurements. Percent coefficient of variation (% CV) for each calibrator and QC levels were calculated for each data set.

Results: Time regression analysis of hLH showed stability that was beyond the manufacturer's stated stability limit (28 days) for all calibrator and QC levels using both analysis model. The observed CVs were less than 3.5% for all calibrator and QC levels except QC3 which had CV of less than 5.5%. Results of analysis showed extended stability compared to manufacturer's suggested stability (14 days) for TT3 at all levels of calibrators and QCs using both analysis models. TT3 assay had CVs of less than 5% for higher levels of calibrator (S2 through S4) and QC (QC2 and QC3) while CVs were less than 10% for lower levels (S1 and QC1). Most of the results for vitamin B12 were beyond the recommended 21 day stability: 6 of 7 analyses using percent versus day model and 4 of 7 analyses using concentration versus day model had greater stability. The observed CVs were less than 7% for calibrator levels and less than 16% for QC levels.

Conclusions: Results indicated that measured stability was increased for all three assays when data was analyzed using percent change as opposed to the concentration change model. hLH and TT3 assays were stable longer than the manufacturer's recommendations. Results of four analyses for vitamin B12 did not meet the manufacturer's suggested stability limit. It is important to recognize the current research study includes both calibrator and system stability in a manner not typical of the manufacturer's intended use.

B-021

A multicenter study on the performance of Grifols' Erytra®, a fully-automated high throughput analyzer, for Kell grouping in US population

J. Roback[1], S. Barclay[1], J. M. Moulds[2], G. Denomme[3]. *[1]Emory University Hospital, Atlanta, GA, [2]LifeShare Blood Centers, Shreveport, LA, [3]BloodCenter of Wisconsin, Milwaukee, WI*

Background: After the antigens of the ABO and Rh blood groups, Kell antigen (K) is the next most immunogenic. The Kell locus is highly polymorphic and gives rise to many antigens. The most important ones, K and Cellano (k), are produced by two major codominant allelic genes. Exposure to K antigen can stimulate an IgG type antibody that can trigger transfusion reactions and hemolytic disease of the fetus and newborn. However, pre-transfusion K antigen typing is not routinely performed in the US. The objective of this study was to test Erytra® (Grifols, Barcelona, Spain), a fully-automated high throughput analyzer for pre-transfusion testing, to establish its performance for K grouping versus comparative method.

Methods: K typing was performed on 2669 samples selected from routine workload representing a very diverse population of donors and patients (46% and 54%, respectively) in 2 US sites. The 8-column DG Gel® 8 ABO/Rh+Kell cards (Grifols) containing monoclonal antisera were used for test procedures. Comparative method was traditional tube testing. Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and OPA (Overall Percent Agreement) between the Erytra and the comparative method were calculated at the 95% confidence level. At least a 99% concordance was considered acceptable.

Results: Of the 2669 tests performed, the Erytra detected 222 K positive and 2447 K negative. The concordances with the comparative method were 99.09% for PPA with a 95% lower confidence bound (LCB) of 96.75%; 99.84% for NPA (99.58% LCB); and 99.78% for OPA (99.20% LCB). Of the 6 discrepancies found (0.22 %), 4 samples were positive by the Erytra and negative by the reference system (all of them were confirmed as true positives by the Erytra), and 2 samples were negative by the Erytra and positive by the reference system (all of them were concluded to be true negative in favor of Erytra). Further investigation revealed that the 2 false negatives were apparently due to clerical or technical error in performance or predicate test.

Conclusion: The Erytra test performance in the Kell determination with its DG Gel 8 cards was safe and effective, consistently obtained the expected results in all the repetitions and was substantially equivalent to the FDA-licensed reagents and FDA cleared instruments used in the study. The Erytra system can be acceptable for K antigen detection by routine pre-transfusion tests in US centers.

B-022

Workflow efficiency of Erytra® in a hospital transfusion service environment

L. C. Winn[1], S. Leonard[1], C. Sánchez[2], S. Solé[2]. *[1]Grifols, Emeryville, CA, [2]Diagnostic Grifols, S.A., Parets del Vallès, Spain*

Background: Automated blood grouping systems for hospital blood banks, transfusion services and donor centers should demonstrate high loading capacity and self-organization to provide maximum processing power. The Erytra® (Grifols) is the newest generation fully automated blood bank system for blood group determination and pre-transfusion compatibility testing using the gel agglutination technique that has been recently approved by the FDA. The objective of the study was to assess the workflow efficiency of the Erytra in a hospital transfusion service environment in terms of turn-around times from process start to finish, efficiency and advantages of the 8-column cards and the ease of use and acceptance by the laboratory staff.

Methods: Patient samples collected in ACD, EDTA or sodium citrate were tested for ABO/Rh+K (Grifols DG Gel® 8 ABO/Rh + Kell card) and antibody screening (Grifols DG Gel Anti-IgG card and Search Cyte 0.8%) using the Erytra automated blood banking system. The Erytra was loaded with increasing number of samples. The following performance metrics were assessed: time to first result (TTFR), turnaround time from first result to last result (cadence), manual "hands on" time required and walk-away time. "STAT" (urgent) samples could be inserted at different times during the regular testing. Operators followed the Package Insert Instructions for Use for the Grifols DG Gel 8 cards, Red Blood Cell Reagents and for the Erytra system. For the ease of use and acceptance evaluation, the following activities were tracked: Set-up, QC, sample preparation, sample sort and loading, routine testing, post-run procedures, consumables used, IT/data review, space requirements, and ergonomics.

Results: Consecutive loads of 96, 120, 144, 168 and 192 samples for the ABO/Rh+Kell typing and antibody screening in the Erytra gave similar values of cadence from 42 to 46 samples per hour. This means that increasing loads had no negative effect on the Erytra performance. STAT samples did not modify the cadence of the routine samples. Most valued feature of Erytra by the staff was related to cards and reagents continuous loading and traceability tracking. Cards and reagents were considered easy to load, thus shortening time to start processes. In addition, software allowed accurate and timely notification of cards tracking and reagent status. Being a see-through instrument was also a valued feature since its well lighted interior provided a clear view of all operating processes.

Conclusion: High workflow efficiency Erytra was demonstrated through its increasing performance with increasing sample loads. Erytra was particularly valued for its ease of loading and tracking cards and reagents, which lead to efficiency and time savings.

B-023

Optimization of Sample Workflow with Total Laboratory Automation: The experience of a 4,5 million tests/month Clinical Laboratory

S. V. L. Argolo, M. L. Moreira, C. F. A. Pereira, O. F. Silva. *DASA, Rio de Janeiro, Brazil*

Background: Central laboratories today face significant challenges: increasing number of samples and tests; the need to find ways to reduce costs; limited technical staff; to minimize laboratory errors; and, to provide results in lower turnaround times (TAT). Laboratory automation can be the solution for most of these issues, but in an operational cenario of 4 to 5 million tests/month, the developed solution has to be carefully and individually studied.

Objective: To evaluate the improvements in efficiency and productivity obtained by the implementation of a high volume laboratory automation in a large clinical laboratory.

Methods: We installed, with support from Roche Diagnostics Brazil, two FlexLab® System 3.6 from Inpeco, included in each system an Input Output Module, Rack Output Module, Bulk Input Module and Decapper Module. In the first one, were connected the following platforms: 2 Cobas 775, 3 Modular EEE, 2 UniCel DxI and 1 ImmunoCap 1000. In the second were connected the following platforms: 1 Cobas 775, 1 Cobas 777, 1 Cobas 8000 EEE, 3 Modular EEE, 2 UniCel DxI and 1 ImmunoCap 1000. We then compared pre versus post implementation data, regarding number of tests processed by tube, TAT, and number of tests by technician.

Results:There was a significant decrease of time consuming in load platforms and all the actions related to it, increasing productivity, reduction of number of tubes collected with important gain in costs and a decreased TAT for most parameters. We had an increase in number of tests per tube by 34%, saving around 48.000 dollars per month. The productivity per technician was increased in 15% and TAT was reduced, in average, by 32%.

Conclusion: At the time we started the studies to implement this solution, we were processing, at the serum area, 2,6 million tests/month using equipments from different suppliers, and also used a sorter to manage the destination of the tubes. All equipments were loaded manually by the technicians. This new technology implemented at our large clinical analysis laboratory allowed us to improve TAT, productivity and reduce production costs, and to deal with almost 3,2 million tests/month. With the new platform we were able to absorb an increase of 20% in number of tests, without increases in personnel costs. Reorganization of the laboratory using automation can be the solution to support the growing of large laboratories.

B-024

Comparison between the determination of glycated hemoglobin through automation system and front-loaded on a clinical chemistry analyzer

N. Z. Maluf, L. K. Tirado, M. D. C. Freire. *DASA, Barueri, Brazil*

Background: Automation systems provide cost efficiency and increased productivity, by integrating test menu and automating manual steps. However, some assays present specificities on sample handling. The determination of Hemoglobin A1c (HbA1c), a diabetes marker, requires mixing the blood samples immediately before testing. Generally, accurate results will be achieved if the sample is tested within 10 minutes after mixing. If the sample sits for too long, the red blood cells will settle, only plasma will be aspirated and false lower results could be reported. In this context, laboratories discuss if performing HbA1c through automation system would be appropriated. Here, we compare HbA1c results when samples are front-loaded on chemistry analyzer with samples loaded on automation system, considering the 10-minute limitation.

Methods: 166 blood samples were tested for HbA1c using the automated pretreatment kit on Siemens ADVIA Chemistry 2400. The samples were first mixed and loaded on a STAT tray on Siemens ADVIA LabCell (100 capped tubes and 66 decapped). Samples were run in batch, without any other samples on the track. The aspiration time of the samples were obtained from CentraLink Data Management System. Then, these samples were mixed and front-loaded on Siemens ADVIA Chemistry 2400. The test was performed in triplicate, mixing the samples between each run. The time to aspirate all the samples in the tray was measured

Results: The correlation data is summarized in the table below

Conclusion: Correlation results between compared measurements were satisfactory. However processing through automation system took longer than the manufacture recommendations (10 min. after mixing). Our study suggest that is possible to process HbA1c tests in batch on ADVIA LabCell under ideal circumstances of sample homogenization and system monitoring.

Table 1 - Linear regression and correlation coefficients between automation vs. front-loaded measure

Sample Handling	N	Total Aspiration Time (min)	Linear Regression	Correlation coefficient (R^2)
capped tubes	100	19	1,0516x-0,7496	0,9434
deccaped tubes	66	12	0,9782x-0,1449	0,9726

B-025

Manual Verification of Aldolase Reference Materials and Validation of an Aldolase Assay on an Automated Chemistry Analyzer

J. Wiencek[1], E. Olson[2], K. Lembright[2], S. Lawson[2], S. Wang[2], E. Reineks[2]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH*

Background: Aldolase (ALD) is a useful marker of muscle damage and is still frequently ordered in conjunction with creatine kinase. A commercial ALD kit (Roche Diagnostics, Indianapolis, IN) is available as a manual method. The test principle of this kit utilizes the disappearance of NADH UV absorbance in a coupled reaction. Although we were interested in employing the reagents of this kit for use on an automated platform, initial calibration materials from third parties were found to be unreliable for quality control(QC) and calibration purposes. Using a validated spectrophotometer and the manual method, we verified a set of QC and calibration materials. These materials, along with method comparison studies, were important in transferring the ALD test to an automated platform.

Methods: A Beckman DU800 spectrophotometer (DU800) was first used to asses ALD QC (Roche) following their kit's established protocol. Patient samples (n=16) and a third party calibrator were further assayed and compared to values obtained by an outside reference laboratory. After this analysis, the kit was modified to work with the P800 automated analyzer (Roche). Calibration of the analyzer was required to establish a *k*-factor in its system. The average value of replicate ALD measurements (n=6) determined by the DU800 was assigned to the calibrator to be used in this procedure. Once this parameter was defined, studies were performed to determine linearity, precision and method comparison of ALD activity in patient samples (n=42).

Results: Roche QC (PreciNorm and PreciPath) assayed on the DU800 fell within the recommended ranges of ALD activity of 12.4 ± 0.8 and 24.4 ± 1.8 units/L, respectively. Deming regressions of 16 patients analyzed with both the DU800 and a reference laboratory showed a slope of 0.998, an intercept of -0.12 units/L, and a correlation coefficient of 0.9632. The labeled value of a third party calibrator stated the product's lot contained 18.7 units/L of ALD; however the DU800 and a reference laboratory reported values of 21.2 and 21.7 units/L, respectively. Using this data, the *k*-factor was established on the P800 and the automated method was determined to be linear over a range of 1.8 to 53.1 units/L. Also the analysis of inter-day precision over 20 days revealed a CV of 4.6% for PreciNorm and 2.2% for PreciPath. Final method comparisons between patient samples (n=42) with the P800 and a reference laboratory showed a Deming regression with a slope of 1.070 an intercept of -0.19 units/L, and a correlation coefficient of 0.9136.

Conclusion: We established confidence in the third party calibration material and vendor QC materials by verification on a manual platform. Our data established that the manual Roche ALD kit can be adapted to an automated method valid for routine clinical service.

B-026

Automated Band Neutrophil Counts by the CellaVision DM96 Digital Blood Imaging System

H. Broome[1], H. Bengtsson[2]. *[1]University of California, San Diego, La Jolla, CA, [2]CellaVision AB, Lund, Sweden*

Background: Early recognition of sepsis is critical for instituting life-saving therapy. Recent studies shows that the neutrophil band count remains one of the most predictive, readily-available tests for positive blood cultures (1, 2). CellaVision DM96 images blood smears and pre-classifies nucleated cells into leukocyte differential categories including band neutrophils. Before posting results from the DM96, clinical laboratory scientists (CLS) must confirm or re-classify the cells in the differential categories (3). The objective of this study was to compare pre-classification and post-reclassification results of DM96 blood smears from emergency department patients to evaluate the reliability of the DM96 reliably to screen for increased band neutrophils.

Methods: From a total of 180 consecutive complete blood counts plus differentials performed on ED patients, 64 smears were selected that had DM96 pre-classification band counts ranging from 0% to 48%. After two CLS independently reclassified the DM96 results from these 64 blood smears, the pre-classified and post-reclassified results were analyzed for correlation and agreement using post-reclassification band percentages as the reference method.

Results: Correlation coefficient (R^2) = 0.66 with a linear regression equation of: pre-classification band % = 1.06 x (average post-reclassification band percentage) + 0.09. Using a cutoff of 25% bands for the pre-classification DM96 and 15% bands for the average of the two CLS post-reclassification results, the positive agreement was 71%, and the negative agreement was 100%. There were two discrepant values with high band % by the DM96 pre-classification (26%; 28%) but normal by the two CLS post-reclassification (0.9% and 1.7%; 6.1% and 0.9%).

Conclusion: This preliminary study suggests that DM96 pre-classification band counts reliably screens for increased band counts using a cutoff of 25%. Additional studies need to be done with more samples with high band counts to determine the optimum cutoff and to determine the sensitivity, specificity, and predictive value of the DM96 pre-classification band percentage. These results suggest the DM96 pre-classification probably is more sensitive than specific since the two discrepant samples had higher DM96 pre-classification results and since pre-classification showed a positive bias compared to post-reclassification. High sensitivity with a reasonable specificity is acceptable for a screening test.

References:

1. Chase M et al. Predictors of bacteremia in emergency department patients with suspected infection. American Journal of Emergency Medicine. 2012;30:1691-1697.

2. Drees et al. Bandemia with Normal White Blood Cell Counts Associated with Infection. American Journal of Medicine. 2012 125:1124.e9-1124.e15

3. Kratz et al. Performance Evaluation of the CellaVision DM96 System. American Journal of Clinical Pathology 2005; 124:770-781.

B-027

Automated review of laboratory information system quality assurance (QA) reports using text analysis

B. P. Jackson, J. M. Toohey, M. S. Berntsen, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Laboratory accreditation requirements include regular review of results reporting. At our hospital, a quality assurance (QA) report generated by the laboratory information system (LIS) is printed and reviewed daily to verify proper reporting of all critical results, linearity failure results, and delta checks. In most cases, the review process is entirely algorithmic -- there is a fixed series of steps and rules by which most results either "pass" or "fail" review. Such a process was amenable to automation. Our objective was to produce a computer program to "read" and assess all pertinent text elements of the QA report so as to eliminate manual review of as many results as possible.

Methods: Programming was conducted using Visual Basic (VB). The LIS QA report for a given day was produced as a text file sent to a computer hard drive. The executable VB program (QADR.exe, named for QA Data Reduction) read the QA report so as to extract and group cases of critical results, linearity failures, or delta checks. Information content related to assessment of each condition was extracted from the original report as defined by non-null characters in fixed locations in the original text file. For critical results, the program verified whether results were called back according to standardized comment codes. For linearity failures, the program verified that results were reported as appropriate for the individual analyte (e.g., not reported as ">" for analytes that should have been repeated on dilution). For delta checks, results were excluded from further consideration by a variety of rules, such as if the time between the LIS-generated delta check results exceeded 72 hours. Results not meeting acceptance criteria were assigned a "non-verified" code and automatically printed for manual review. The results of automated review were recorded in a summary text file for all results, and both the original LIS QA report and the summary text file were archived in electronic form.

Results: After VB program development, a validation period of one month was used to compare manual and automated reviews of the LIS QA reports. With program refinement, automated review contained no comparison errors. Following this period, automated review was adopted as the routine procedure for QA review. Prior to adoption of automated review, printed QA reports were 20-30 pages, and approximately 30 minutes were required daily for QA review. After adoption, summary printed reports for non-verified results were 1 page maximum, and less than 10 min total was required for daily QA review. In projection, it is estimated that automated QA review will save more than 3 man-weeks per year in labor. Moreover, automated QA review is a definitively "green" undertaking, in that it will eliminate printing and archiving of more than 7000 pieces of paper per year.

Conclusions: Automated review of LIS QA reports was accomplished using a custom computer program performing text analysis. The program was successful in assessing common "pass/fail" criteria to an extent that greatly reduced manual effort and costs associated with daily results review.

B-028

Characterization and stability of time-of-day patterns of running averages as potential inputs to patient-based quality control algorithms: examples for basic metabolic panel analytes in a university hospital

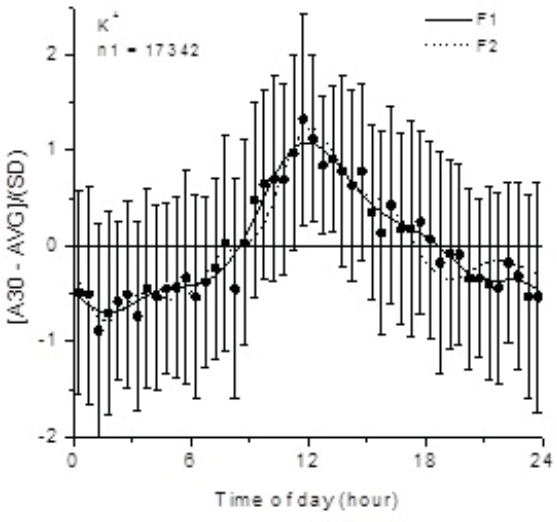
B. P. Jackson, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Regular patterns of time-of-day (TOD) variation of running means of patient data are potentially useful as inputs in patient-based quality control (PBQC). Characterization of TOD patterns and their stabilities are initial steps to determine whether use of patterns might improve PBQC. For 7 analytes of a Basic Metabolic Panel (BMP) at a university hospital, we mathematically characterized average TOD patterns (t = 0-24 hours) of running means of patient data for one month intervals, and assessed the stability of such patterns across successive months' data.

Methods: Successive one-month datasets (M1, M2) for patient measurements were obtained for BUN, Ca2+, Cl-, CO2, creatinine (Cr), K+ and Na+. Running means of length 30 (A30) were calculated across M1 and M2, with data restricted to samples within each analyte's reference range. Independently for both M1 and M2, average A30 as a function of time-of-day (TOD) was calculated for 48 half-hour intervals across 24 hours. From these data, TOD-dependence of A30 was characterized mathematically using low-frequency ($\omega \leq 12\pi$/day) Fourier transforms to produce continuous, smooth functions F1(t) and F2(t). Stabilities of TOD patterns were evaluated by correlation (r2) between F1(t) and F2(t).

Results: An example TOD pattern is shown for K+ in Figure. The TOD-dependence of A30 for K+ was relatively stable, as demonstrated by high correlation between F2(t) and F1(t) (r2 = 0.929). Across the remainder of BMP analytes, there was marked variation in apparent cross-month stability of patterns as assessed by r2: Cr (0.299), BUN (0.765), Na+ (0.811), Cl- (0.849), CO2 (0.870), Ca2+ (0.973).

Conclusions: Methods presented here exemplify an approach to characterization of TOD patterns in running means and assessment of their stability. The stability assessment results provided a logical order among BMP analytes for further evaluation of the potential utility of TOD patterns as inputs to PBQC algorithms at our institution.



B-029

Optimization of Sample Work-Flow and Testing Efficiencies with a Paradigm Shift in Automated Systems for Clinical Molecular Diagnostics Laboratories

C. Newhouse[1], T. W. Myers[1], E. Smith[1], S. Boyle[1], V. Namasivayam[1], A. Nelson[1], D. Schoener[1], S. L. Moseley[2], P. Rodriguez[2], M. Lewinski[1], J. Osiecki[1], S. Roehrig[1]. *[1]Roche Molecular Systems, Inc., Pleasanton, CA, [2]Roche Diagnostics Corporation, Inc., Indianapolis, IN*

Introduction: Similar to trends faced by clinical chemistry laboratories 20 years ago, today's molecular diagnostics laboratories are increasingly challenged with balancing volume growth across an expanding menu of tests, while maintaining meaningful productivity and efficiency gains. The challenges currently faced by clinical molecular diagnostic labs extend from complexities in tubes standardization; increasing demand for testing from multiple sample types; fluctuations in demands for specific tests; subjectivity in result reporting to increased demands on labs to streamline workflows with less hands-on time for applications. With the constant pressure of these variables, there is potential for work-flow inefficiencies to increase costs and potential risk for human error associated with result generation.

Objective: To detail a paradigm shift in design features for automation in clinical molecular diagnostic testing. A description of how innovative integrated analyzer design features could address the current challenges faced by labs responding to volume growth in molecular testing.

Methodology: Design features of two fully automated, integrated Real-Time PCR testing systems, the **cobas**® 6800 System* and **cobas**® 8800 System* will be detailed. These systems include automation of sample transfer, processing and target detection, including onboard assay specific reagent cassette storage and handling. A dedicated lane for urgent bypass testing accommodates the need for sample prioritization. The **cobas**® 6800 System and **cobas**® 8800 System can accept multiple primary and secondary tube types with no pre-sorting or "batching" of tubes or racks. Through universal sample preparation and PCR profiles (**cobas omni** process) the instrumentation can process samples for 3 different assays simultaneously for detection of HIV, HBV, HCV, and CMV. By taking up to 3 aliquots from one specimen, up to 3 assays can be run from a single patient sample. Sample input parameters include 200uL and 500uL EDTA plasma for testing of HIV-1. A user defined channel for lab developed tests provides flexibility and benefits of automation using the **cobas omni** process. The instrumentation requires very little user interaction and maintenance.

Results & Conclusion: The first 96 results are available in less than 3.5 hours with an additional 96 results every 90 minutes for **cobas**® 6800 System (or 30 minutes for **cobas**® 8800 System). The **cobas**® 6800 System can report up to 384 patient results in 8 hrs. The **cobas**® 8800 System accommodates higher volumes with up to 960 patient results in 8 hrs. User interactions are limited to loading and unloading and periodic removal of waste, reducing hands on time and risk for human error. Collectively, the combination of the new system design and assay design enhancements provide a level of automation that approaches systems used in clinical chemistry, facilitating a paradigm shift for routine clinical molecular diagnostics laboratories and advancements for both laboratorians and patients.

*The **cobas**® 6800 System and **cobas**® 8800 System are in development and not available for sale in the US.

B-030

Laboratory Automation with the Beckman-Coulter Power Express produces improved turnaround times for stat and routine chemistries

C. P. Yeo, D. S. L. Lee, C. S. P. Chiong, J. S. Y. Wong, P. R. Chincholkar, K. S. L. Lim, C. H. C. Tan, W. Y. Ng. *Singapore General Hospital, Singapore, Singapore*

Background The Singapore General Hospital (SGH) Clinical Biochemistry Laboratory, with its need to provide 24/7 coverage for stat and routine chemistry tests to SGH and its affiliated cancer and heart centers, has constantly strived to achieve optimal performance and service delivery. In 2007, the laboratory introduced laboratory automation in the form of the Beckman-Coulter Power Processor Laboratory Automation System (PP LAS). With its 2007 workload of 5.94 million tests increasing at a rate of 5-7% a year, the laboratory quickly outgrew its LAS's capacity. In 2013, in tandem with plans to move into new purpose-built facilities, design considerations were made to assimilate further refined workflow and specimen routing strategies and to install the latest generation of LAS - now coined the Beckman-Coulter Power Express (PE). **Method** The LAS inlet was placed next to the pneumatic tube station which receives deliveries from Emergency, Intensive Care and other campus sites. The PE LAS with three centrifuges, decappers, aliquoters and two 5000-tube refrigerated stockyards was linked to 2 DxI800s, 1 AU680 and 2 AU5822s. Turnaround time (TAT) was defined as that between specimen arrival in the laboratory and result release (sans auto-verification) to EMR. TAT for stat specimens from Emergency and Intensive Care was the key performance indicator for the laboratory. **Results and Discussion** Pre-PE-LAS TAT for stat specimens was 95% in 45 minutes and 70% in 35 minutes; a good performance achieved through expending much effort into manually moving stat specimens ahead of routine specimens on the LAS tracks. With PE LAS, TAT was sustained but with significantly less manual effort. Data also showed that the PE LAS provided faster turnaround for routine test orders from the specialist outpatient clinics and wards, hitting >80% in 45 minutes (a vast improvement over previous <20% in 45 minutes). The improvement in TAT and workflow could be attributed to: (1) priority identification design layout of the stat/routine laboratory that places the PE LAS close to the specimen reception area, (2) an improved LAS and the much higher capacity of the online analysers. The important features of the Power Express LAS are: (a) a dynamic inlet that allows seamless input of up to 4 types of specimens (stat, routine, pre-centrifuged and archival) without the need to initiate the pause or standby mode, (b) ability to support up to 4 centrifuges (we installed 3 and have space for a fourth) and therefore significantly reducing the occurrence of oft-cited bottlenecks at the LAS centrifuges, (c) 4-laned specimen transportation track that allows stat specimens to bypass routine specimens, (d) online RFID specimen identification that circumvents previous issues with barcode readers and (e) online high-throughput analysers. **Conclusions** With close proximity to specimen reception, workflow refinements and favourable new features of the latest Beckman-Coulter Power Express LAS, quicker result reporting has been shown. In addition, provision for a fourth centrifuge and high capacity of the online analysers permit further growth in the coming years.

B-031

Look before you leap: Developing optimized automated rule sets for reporting hemolyis, icterus and lipemia based on a priori outcomes analysis

J. M. Boyd[1], R. Krause[1], G. Waite[2], W. Hui[2], E. Yadzi[2], D. Wilmik[2], I. Seiden Long[1]. *[1]Department of Pathology and Laboratory Medicine, University of Calgary and Calgary Laboratory Services, Calgary, AB, Canada, [2]Gamma Dynacare Medical Laboratories, Brampton, ON, Canada*

Objective: The new CLSI C56A guideline directs labs to generate their own policies for hemolysis(H), icterus(I) and lipemia(L) reporting by automated methods. Here we describe a process to review and optimize reporting policies for test results with known interference prior to implementation.

Methods: Table 1 describes proposed commenting and cancelling policies generated using CLSI C56A vs baseline policies from two high volume community laboratories. Identical Proposed Rules were applied to the test result data sets from each lab. Test Data: Lab A, Roche Modular platform, IBM AS400 Custom LIS reporting 541236 tests, for 72 analytes over 1 week. Lab B, Roche Cobas C8000 with Cerner Millennium/Roche PSM reporting 391515 tests for 45 analytes over 2 weeks. Any test receiving >5 flagged results was considered for generation of Optimized Rules including analyte level and clinical significance. Number of Commented and Cancelled tests was counted for each lab and rule set.

Results: Optimized rules were only required for hemolysis. Comment and cancellation test counts at baseline were compared to optimized rules for each lab. Hemolysis

rules for Lab A: baseline(comment:3656, cancel:NA) vs optimized(comment:155, cancel:19) reduced hemolysis flagging by 96%, while for Lab B: baseline(comment:0, cancel:277) vs optimized(comment:107,cancel:2) reduced result cancellation 99%. Icterus rules for Lab A: baseline(comment:492) vs. optimized(comment:0) reduced flagging 100%, while in Lab B: baseline(comment:0) vs optimized(comment:13) increased flagging. For lipemia Lab A: baseline(comment:2383) vs optimized(comment:24) reduced flagging 99%, while in Lab B baseline(comment:74) vs optimized(comment:193)increased flagging 62%.

Conclusions: Implementation of identical rule sets in Labs A and B indicated that the outcomes of automated HIL reporting are significantly lab dependent . This process of testing and optimization of HIL reporting rules prior to implementation by *a priori* outcomes analysis demonstrates the clear benefit of impact assessment for reporting policies with automated HIL rule sets.

Table 1: Baseline, proposed and optimized reporting rules

		Baseline Rules	Proposed Rules	Optimized Rules
Hemolysis	Lab A	Test result reported, Manual visual inspection of sample with application of HIL flag, no comment	Test result reported, Automated HIL detection and application of rules, comment on direction of interference up to H of 600, cancel H>=600	Test result reported, Automated HIL detection & application of rules, comment on direction of interference up to H of 600, cancel H>=600, only when CLINICALLY SIGNIFICANT*. * defined as a degree of interference sufficient to generate a test result outside of the normal range for the test
	Lab B	Test result not reported, Automated HIL detection and application of rules with test cancellation		
Icterus	Lab A	Test result reported, Manual visual inspection of sample with application of HIL flag, no comment	Test result reported, No cancellation, Automated HIL detection and application of rules, comment on direction of interference	Same as proposed rules
	Lab B	Test result reported, no flag, no rules in place		
Lipemia	Lab A	Test result reported, Manual visual inspection of sample with application of HIL flag, no comment	Test result reported, no cancellaton, Automated HIL detection with comment AFTER Manual ultracentrifugation process applied	Same as proposed rules
	Lab B	Test result reported, Automated HIL detection with comment AFTER Manual ultracentrifugation process applied		

B-032

Identification of Erroneous Potassium Results Using a Laboratory Data-Derived Machine Learning Algorithm

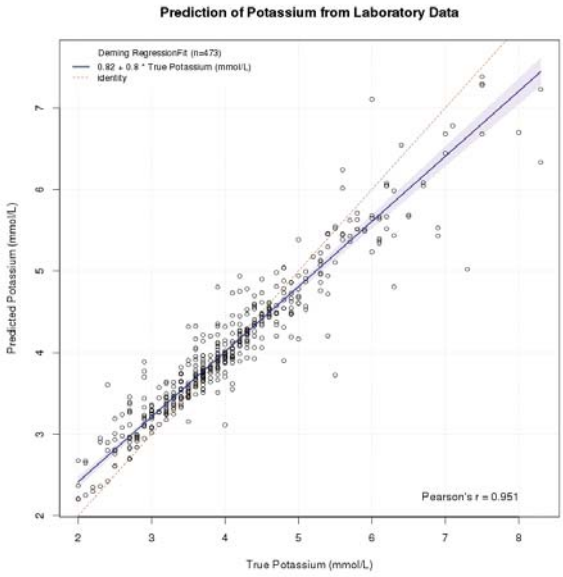
C. R. McCudden, M. Henderson. *The Ottawa Hospital, Ottawa, ON, Canada*

Background and Objectives: Potassium is a critically important analyte with many preanalytical considerations. While laboratories have procedures to avoid reporting erroneous results, it is difficult to identify if a given potassium result is accurate. The objectives of this study were: 1) to use commonly available laboratory data to develop a machine learning algorithm to predict potassium concentrations 2) determine the performance of the algorithm for error detection.

Methods: LIS data was used to develop a random forest regression model to predict potassium results. The prediction algorithm was trained using known, accurate potassium concentrations with commonly available hematology (complete blood count) and biochemistry (basic metabolic panel) data. Data included 2876 result sets (80% for model training, 20% for testing) from 1642 patients with encounters at 174 different hospital/clinic locations over a two-year period. 'Accurate potassium' was defined as values where blood gas (GEM4000) and chemistry analyzer (Vista 1500) values were within +/- 0.4 mmol/L. Error simulation (introduced bias from 0.5-2.0 mmol/L) was used to determine the performance of the algorithm to detect inaccurate results, which were identified by the difference between observed and predicted potassium.

Results: A comparison of the predicted and accurate potassium concentrations is shown below. The most important predictors were creatinine, urea, sodium, anion gap, and WBC. Based on simulations, the algorithm detected an error of 0.5 mmol/L with a sensitivity of 78% and specificity 74% and an error of 1.0 mmol/L with a sensitivity of 86% and specificity of 96%.

Conclusions: The model described herein represents a powerful new quality tool whereby predicted concentrations could be used to prevent reporting grossly inaccurate potassium results. This is particularly valuable given the clinical importance of potassium and abundance of preanalytical considerations. Routine application of the model (e.g. as a autoverification rule) could prevent reporting of inaccurate potassium results due to unforeseen preanalytical factors.



B-033

Differentiation between glomerular and non-glomerular hematuria by an automated urine sediment analyzer

P. V. Bottini, B. D. Andreguetto, K. Krempser, J. Lauand, C. R. Garlipp. *Univesity of Campinas, Campinas, SP, Brazil*

Background: Differentiation between glomerular and non-glomerular hematuria by observation of the erythrocytes morphology using phase-contrast microscopy has been very well established for almost 35 years. However, it is a time-consuming and labor-intensive procedure that requires skilled personnel. Some years ago, an automated urine sediment analyzer based on the KOVA® method with on-screen review of the images was introduced. The aim of this study was to evaluate the performance of this image based automated sediment analyzer (UriSed, also called sediMAX® in some countries) as an alternative to the phase-contrast microscopic analysis of erythrocytes morphology.

Methods: We studied 312 urine samples with hematuria (erythrocytes>5/hpf). Samples were analyzed by UriSed and all the images reviewed by an experienced analyst. Parallelly the urine samples were centrifuged (10 mL, 5 minutes, RCF = 400) and the sediment (0.5 mL) was placed on a slide and examined under a coverslip by phase-contrast microscopy. Erythrocytes morphology was analyzed by both methods by different observers. Based on the presence of codocytes and/or acanthocytes, samples were classified as non-glomerular (absence of codocytes or acanthocytes) and glomerular (presence of codocytes, acanthocytes or both). Kappa correlation was used to assess the agreement between both methods.

Results: Our data showed an agreement of 97.4% between erythrocytes morphology analyzed by both methods (kappa=0.9484, p<0.001). From 312 samples, 140 of them (45%) presented isomorphic erythrocytes and hematuria was classified as non-glomerular by both methods whereas in 164 samples (52.5%) we observed the presence of codocytes and/or acanthocytes by phase contrast microscopy and by UriSed being classified as glomerular hematuria. Only 8 samples (2.5%) had discordant results. Five

of them revealed the presence of codocytes by phase contrast microscopy which were not displayed on UriSed. On the other hand, 3 samples classified as non-glomerular by phase contrast microscopy presented codocytes on UriSed images.

Conclusion: UriSed is a precise and accurate alternative to the gold standard phase-contrast microscopy that allows a better workflow and may significantly improve turnaround time.

B-034

Moving Patient Averages: A Pilot Study Using Error Simulation

R. Ybabez, J. Boysen, A. Zblewski, B. Hendrix, C. Wittwer, L. Rossini, S. Chanakarnjanachai, L. Duncan, D. Block, N. Baumann. *Mayo Clinic, Rochester, MN*

Background: Robust quality control (QC) processes in clinical laboratories are required to ensure stable operation of analytical systems and to provide reliable test results. There is potential added value in using real-time patient data to supplement traditional statistical QC. The concept of monitoring moving averages of patient results has been discussed for decades, and recently middleware programs that can calculate moving averages continuously and in real time have become commercially available. The objectives of this study were to evaluate Moving Averages software by: (i) collecting moving average patient data for three common analytes (calcium, chloride and creatinine) in a high volume clinical laboratory, (ii) configuring analyte-specific protocols and (iii) testing the protocols using simulated systemic errors.

Methods: All patient data were generated using Roche Cobas 8000 reagents and analyzers. Moving Averages (Data Innovations, Inc) protocols were configured for calcium, chloride, and creatinine. For each analyte, the mean of patient results and Sp (standard deviation of the patient population) were calculated over a 2 week time period. Sa (standard deviation of the analytic method) was obtained from the standard deviation (SD) of in-use QC at concentrations near the patient population mean concentration. Sp/Sa was calculated and power function charts (Cembrowski, GS et al. (1984)) were used to estimate the appropriate number of patient test results to average (N). Exclusion criteria were applied to calcium and creatinine protocols to exclude values >4SD from the mean and results from patients in dialysis units were excluded for creatinine. Systemic 2SD errors were simulated by analyzing consecutive patient samples with results approximately 2SD above the patient mean. The concentrations of analyte in the simulated error samples were: calcium, 9.5-9.8 mg/dL; creatinine, 1.3-1.4 mg/dL; chloride 104-106 mmol/L. The number of patient samples with simulated errors needed to trigger an error warning was calculated.

Results: Sp/Sa and suggested number of patient test results to average (N) were 9.8 and N=200 for calcium, 4.6 and N=50 for chloride, and 6.4 and N=70 for creatinine. Error simulation showed that 2SD error warning thresholds were triggered after 140 patients for calcium, 50 patients for chloride, and 47 patients for creatinine. A second simulation study was performed using protocols with different N for each analyte (calcium, N=100; chloride, N=30; creatinine, N=30) and yielded 2SD error warnings after 75, 30, and 26 patients for calcium, chloride and creatinine, respectively.

Conclusion: Moving Averages may help laboratories detect systemic errors in real-time. Three analytes (calcium, chloride, and creatinine) were used to evaluate the program and optimize parameters for detecting a simulated 2SD error. Errors simulated using preliminary estimates of the number of patient test results to average (N) suggested that a smaller N (fewer patient results averaged) may allow errors to be detected earlier and before large numbers of patient results are affected. The ability to detect a shift in patient results on a continuous basis and in real time may complement existing QC processes in the laboratory.

Wednesday, July 30, 2014

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

Infectious Disease

B-035

Comparative Study of Granada and ChromoID StreptoB media for identification of Group B Streptococcus

N. Z. Maluf, D. C. Santos, C. L. Chacon, J. Silva, C. F. A. Pereira. *DASA, Barueri, Brazil*

Background: BIOMÉRIEUX StreptoB Granada and ChromoID media are used to identify group B Streptococcus. Microbiological screening of these bacteria is crucial since it can lead to neonatal septicaemia in pregnant women. Generally, it appears in the first 24 hours of life and often results in fulminant septicaemia, meningitis, or pneumonia associated with high morbidity and mortality. The most effective strategy to reduce the incidence of GBS in newborns is a pre-natal tracking of all pregnant women between 35-37 weeks of gestation time. This is done using culture methods to determine the necessary intrapartum antibiotic prophylaxis. The objective of this study was to compare the effectiveness of the Granada medium at identifying Group B Streptococcus in samples of vaginal and perianal openings.

Methods: A 100 samples of vaginal and perianal openings of women with 35-37 weeks of gestation were collected and transported in Stuart and AIMES transport at room temperature. The procedure was carried according to the package insert. After 24 hours of incubation at 35 °C, macroscopic analyses in the broth were carried out to detect any orange coloring, and if so, samples were positive for group B Streptococcus. Those samples were seeded in the ChromoID StreptoB biphasic Granada broth to compare both tests and verify which one provides more effective and precise results. Inoculated plates were incubated in micro-aerobic culture systems at 35 °C for a duration of 12 hours.

Results: More than half of the 100 samples analyzed showed incompatibilities between the results, especially the Granada ID medium presenting an average of less than 45% positivity when comparing to the ChromoID StreptoB.

Conclusion: In the presented data, three types of tones were considered: strong, intermediate, and weak. A weak-colored orange still resulted in a clinical evaluation of the sample and the patient. If the result returned positive, the patient was returned to isolation and identified as positive for group B Streptococcus. The Granada ID test was shown unreliable if done alone. In order to achieve better accuracy, this test should be done with another identification technique, such as the camp-test or even Chromo ID (STRB). The latter presents excellent specificity and is relatively easy to execute. Its only limitation is that it takes12 hours longer than Granada ID to achieve results.

B-037

Automated Sample-to-Results Analysis of Clinical Specimens for Sexually-Transmitted Infections

G. Spizz[1], C. McGuire[1], K. Kowitski[1], W. Hungerford[1], R. Yasmin[1], R. A. Montagna[2]. *[1]Rheonix, Inc., Ithaca, NY, [2]Rheonix, Inc., Grand Island, NY*

Background: The global burden of sexually-transmitted diseases (STDs) is considerable with an estimated 340 million new cases occurring each year. Although many of these new cases could potentially be effectively cured with modern antibiotic therapy, the early stages of the infections can often go unnoticed. Females are disproportionately affected, in whom untreated STDs can proceed to disabling pelvic inflammatory disease which, in turn, can lead to infertility, infant mortality and infant blindness. Complications in untreated males, although rarer, can proceed to urethritis, epididymitis, as well as infertility. In order to streamline testing, we have developed a fully automated molecular detection system to simultaneously detect N. gonorrhoeae (NG), C. trachomatis (CT) and T. vaginalis (TV) in an unattended manner from a variety of specimens.

Methods: An injection molded disposable CARD (Chemistry & Reagent Device) was developed that, when inserted into the EncompassMDx™ workstation, can automatically lyse cells, extract and purify DNA, multiplex PCR amplify rRNA genomic targets in NG and TV and cryptic plasmid DNA of CT. In order to confirm that all steps of the assay were performed correctly by the system, three separate chimeric plasmids were designed that harbor unique DNA sequences that can be amplified by the same primer pair sets designed to amplify the individual targets of CT, NG, and TV. Hybridization of the control amplicons can be detected and distinguished from hybridization of target amplicons on the integrated DNA array.

Results: Several different clinical reference laboratories provided us with approximately 100 diverse specimens (vaginal swabs, endocervical swabs, and urine specimens), previously tested using FDA-cleared devices in their facilities. Evaluation of the same samples with the Rheonix CT/NG/TV CARD assay yielded similar results. Moreover, since the FDA-cleared test was only able to test for the presence of CT and/or NG, a number of samples were also found to be co-infected with TV. In addition, the use of the chimeric plasmid controls yielded positive signals on all runs, thus confirming that each step of the fully automated assay was properly conducted by the unattended system. Furthermore, to confirm that the DNA arrays were properly orientated in the CARD, spotting controls that display signals were placed on the DNA array in a defined pattern. In order to assure that the proper organisms were detected, the imaging software was designed to only accept results that displayed the proper spotting control orientation, thus confirming that the microorganism(s) detected were correctly scored.

Conclusion: The ability to analyze specimens in a fully automated, sample in_result out format, will allow detection and identification of three sexually transmitted infections to be performed by individuals of varying skill level. The automatic performance in an unattended manner of all sample preparation, DNA purification, amplification, end-point detection, analysis and readout functions makes the platform suitable for central lab, point-of-care, as well as non-traditional healthcare settings. Clinical studies intended to gain FDA clearance are expected to be undertaken in 2014

B-038

A Novel Enzyme-Linked Immunosorbent Assay for the Detection of Nontreponemal Antibodies in the Sera of Patients with Syphilis.

A. R. Castro[1], M. R. Shukla[2], J. Deustch[1], K. Karem[1], H. C. Mody[3]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Arlington Scientific, Inc., Springville, UT, [3]Arlington Scientific, Inc., Sprinville, UT*

Background: We describe an enzyme-link immunosorbent assay (EIA) for the detection of nontreponemal antibodies. This assay is ideal for the automation of high throughput screening of sera

Methods: The nontreponemal (cardiolipin) antigen was chemically modified and it was attached covalently to amine functionalized micro titer plates.

Results: A total of 1,006 banked serum samples were evaluated and the results compared to a quantitative rapid plasma regain (RPR) test. The accumulative reactive concordance of the nontreponemal EIA was 93.3% when the RPR titer of the sera was 1:1, 96.2% at 1:2, 98.5% at 1:4, 99.3% at 1:8 and 100% at ≥ 1:16. The nonreactive concordance was 100%. Also 50 samples with known stages of syphilis and 158 from diseases other than syphilis were included.

Conclusion: These results indicate that the nontreponemal EIA test can be used for the screening of large volume of samples using the traditional syphilis testing algorithm of screening with a nontreponemal test and confirming the results with a treponemal test.

B-039

Evaluation of the Alere Determine™ HIV-1/2 Ag/Ab Combo Kit for the Rapid Determination of Antibody/Antigen Status in STAT Specimens in a Busy Metropolitan Hospital Setting

M. Eskandari, S. Barden, K. W. Simkowski, Y. Posey, E. Sykes. *Department of Clinical Pathology, 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 Alere Clearview® STAT-PAK® point-of-care device for HIV-1/2 Antibody STAT testing (needle stick; Labor & Delivery cases). The Abbott ARCHITECT® 4G immunoassay (IA) with reflex to BioRad Multispot HIV-1/HIV-2 Rapid Test kit is used for routine testing. When the Multispot does not "confirm" a reactive ARCHITECT result, serum is referred for further HIV-1 RNA qualitative testing.

OBJECTIVE: The Alere 4G Determine HIV-1/2 Ag/Ab Combo device was recently approved by the FDA. The ARCHITECT reports a signal-to-cutoff ratio for combined HIV-1/2 antibodies and p24 antigen (HIV-1), whereas the Determine distinguishes the HIV-1/2 antibody result from the HIV-1 p24 antigen result. We were, therefore,

interested in evaluating the Determine as a replacement for the current Clearview method for STAT testing.

STUDY DESIGN: 111 serum samples from 106 patients, previously tested for HIV status, were re-tested by the ARCHITECT/Multispot and then by the Clearview and Determine rapid tests. Reactive samples had been stored frozen for up to 18 months, however, there was no significant difference between original and repeat results. Most non-reactive samples had been refrigerated for up to 5 days. ARCHITECT algorithm results and chart review were used for definitive HIV diagnosis.

RESULTS: Two samples from a HIV-2 antibody positive patient were reactive by all methods. Sensitivity and specificity for HIV-1 testing are shown:

Test Systems	Sensitivity (%)	Specificity (%)
Abbott ARCHITECT 4th Generation IA (n=109)	100	77
Alere Determine HIV-1/2 Ag/Ab Combo kit (n=109)		
HIV-1 Antibody	90	93
p24 Antigen	17	98
p24 and/or Antibody	98	92
Alere Clearview HIV-1/2 STAT-PAK (n=108)	83	100
BioRad Multispot HIV-1/HIV-2 Rapid Test (n=109)	83	100

CONCLUSIONS: The Alere Determine HIV-1/2 Ag/Ab Combo kit had an overall sensitivity of 98% for detection of HIV-1 p24 antigen and/or antibody while the Clearview, which does not detect HIV-1 p24 antigen, had a sensitivity of 83%. Therefore, the Determine Combo would be an improvement over the current method for the detection of HIV-positive patients for STAT testing.

B-040

Evaluation of Analytical Sensitivity and Workflow of the VERSANT Hepatitis C Virus Genotype 2.0 Assay (LiPA)

A. Lal, P. Lau, H. Huang, D. Monga, U. Vajapey, R. Nandkeshwar, G. Kritikos, J. Surtihadi, G. Gorrin. *Siemens Healthcare Diagnostics, Berkeley, CA*

Background: The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a reverse hybridization line probe assay that uses sequence information from both the 5' untranslated region (UTR) and the core region to accurately distinguish between HCV genotypes 1 to 6 and subtypes 1a and 1b. Prior studies have shown that the assay can genotype 96% of HCV samples with 99.4% accuracy.(1) Assay steps have been automated to improve efficiency and decreased time to results. This study evaluates assay workflow and analytical sensitivity.

Methods: The VERSANT HCV Genotype 2.0 Assay (LiPA) is run in three steps: extraction, amplification, and genotyping. Viral RNA is extracted from plasma or serum using the VERSANT Sample Preparation 1.0 Reagents. The 5' UTR and core regions of HCV are amplified using RT-PCR and the VERSANT HCV Amplification 2.0 Kit (LiPA). Biotinylated amplicons are hybridized to immobilized oligonucleotide probes on nitrocellulose strips and visualized using reagents in the VERSANT HCV Genotype 2.0 Assay (LiPA) Kit. Processed strips are interpreted using the optional LiPA Scan software to yield the HCV genotype. Assay intermediates from each step can either be processed immediately or stored at defined conditions. Analytical sensitivity was evaluated using one specimen for each genotype (1a, 1b, 2, 3, 4, 5, and 6) diluted separately in serum and plasma. Dilution series were prepared at concentrations ranging from 50 to 2000 IU/mL, and each target concentration was tested in multiple replicates and runs with multiple reagent lots on different days. These data are analyzed using a regression method with probit link function.

Results: Automation of extraction and strip processing allows for simultaneous processing of 94 samples. Extraction and loading of the PCR plate have been optimized with the VERSANT kPCR Sample Preparation module, a fully automated instrument for isolation and purification of nucleic acids using magnetic-bead extraction technology. Genotyping has been optimized on the automated Auto LiPA 48 Genotyping Instrument (Strip Processor), which can process up to 46 samples and 2 controls per run. Assay times for 94 samples are 3.5 hours for extraction, 4 hours for amplification, and 4 hours for genotyping (with two Auto LiPA 48 processors), which includes a hands-on time of 2 hours. Initial assessments of analytical sensitivity, measured as the limit of detection for individual genotypes/subtypes, was less than or equal to 500 IU/mL. Further assessments are underway to confirm the analytical sensitivity.

Conclusion: The VERSANT HCV Genotype 2.0 Assay (LiPA) is a sensitive and reliable HCV genotyping assay. Automation of the VERSANT HCV Genotype 2.0 Assay (LiPA) workflow results in higher throughput, improved efficiency, and a decreased time to results.

References: 1.Verbeeck J, Stanley MJ, Shieh J, Celis L, Huyck, E, Wollants E, Morimoto J, Farrior A, Sablon E, Jankowski-Hennig M, Schaper C, Johnson P, Ranst MV, Brussel MV. J Clin Microbiol. 2008;1901.

VERSANT HCV Genotype 2.0 Assay (LiPA) is CE-marked in Europe and for research use only (RUO) in the U.S.

B-041

Quantitation of IFN-γ and IFN-γ induced chemokine mRNA expression levels in active pulmonary tuberculosis patients for effective monitoring of anti-TB therapy

H. Kim[1], S. Kim[2], J. Cho[3], S. Hong[1], Y. Kim[1], G. Kim[1], S. Ahn[1], S. Park[1], J. Kim[1], D. Lee[4], H. Jin[5], S. Park[6], S. Cho[7], H. Lee[1]. *[1]Yonsei University, Wonju, Korea, Republic of, [2]Institute for Life Science and Biotechnology, Seoul, Korea, Republic of, [3]Daegu Health College, Daegu, Korea, Republic of, [4]Hyejeon College, Hongseoung, Korea, Republic of, [5]Catholic University of Pusan, Busan, Korea, Republic of, [6]Daegu Hanny University, Daegu, Korea, Republic of, [7]Yonsei University, Seoul, Korea, Republic of*

Background: Tuberculosis (TB) that is mainly caused by *Mycobacterium tuberculosis* (MTB) remains a major global health problem, with approximately 9 million new TB cases annually, leading to 1.4 million in 2011. The results of several previous studies showed numerous cytokines have been implicated in the pathogenesis, diagnosis and control of MTB infection. Especially, interferon gamma (IFN-γ)-specific response to the MTB specific antigens can be used as biomarker for differentiation of active TB and latent tuberculosis infection (LTBI). However, there is an urgent need of prognosis markers for tuberculosis (TB) to determine the response to therapy and improve treatment strategies. **Methods:** In this study, the messenger RNA (mRNA) expression levels of IFN-γ and IFN-γ induced chemokines (MIG, IP-10 and I-TAC) were quantitatively measured by using real-time RT-PCR. For effective anti-TB therapy monitoring, blood sampling, MTB specific Ag stimulation, and molecular assay were performed with a total of 32 active PTB patients at the time of diagnosis (before therapy) and after therapy completion (6 months later). **Results:** The target genes (IFN-γ, MIG, IP-10 and I-TAC) mRNA expression levels were significantly changed and showed a statistical significance at the time of therapy completion from the initial diagnosis active PTB (IFN-γ; p=0.0387, MIG; p<0.001, IP-10; p<0.001, I-TAC; p<0.001). **Conclusion:** In conclusion, data show that the analysis of IFN-γ and IFN-γ induced chemokine mRNA expression levels after MTB specific Ag stimulation could provide useful information during the anti-TB therapy of active PTB patients group.

B-043

Proficiency Test for Laboratory Identification of *Corynebacterium diphtheriae* in Health Care System at Lower Northern Thailand

R. Wiriyaprasit, V. Srisakul, D. Boonyod, P. Ratanakasetsin. *Clinical Pathology, Regional Medical Sciences Center 2 Phitsanulok, Phitsanulok, Thailand*

Background: Diphtheria is an acute, communicable infectious disease of upper respiratory tract caused by toxigenic lysogenized strains of *Corynebacterium diphtheriae. The incident* in Thailand often found in June to February. The immunized vaccination can only reduce the violation of diphtheria, but cannot be used for extremely protection from re-emerging of diphtheria. Therefore, the rapidly accurate identification of *Corynebacterium diphtheriae* for diagnosis is important for prevention and control. Rehabilitation, practical training laboratories were not enough for confidential results. The proficiency test (PT) program was an assurance implement to use for evaluation of efficiency and quality of laboratory.

Methods: Eighteen hospitals in nine provinces of Lower Northern from Thailand were enrolled in the PT program. The accuracy Identification of *Corynebacterium diphtheriae* was evaluated by statistically from three different PT samples which were homogeneous and stable and were prepared from the same matrix of throat swab in Amies transport medium .

Results: One hundred percentages (16/16) of PT results have been reported and returned to PT provider within the period prescribed. Accuracy identification of the three PT samples was calculated and revealed for 87.5 % (14/16). However, there was 13.5% (2/16) represented inaccuracy with uncorrected results. The satisfaction of PT program was rated for 90.0 %.

Conclusion: The PT program for quality laboratory identification of *Corynebacterium diphtheria*e in health care system at Lower Northern from Thailand was the effectively implement for the sixteen- laboratory participants .

B-044

Screening on Sexually Transmitted Infections among pregnant women

O. Aliyeva. *Skin-venereal health center, Ust-Kamenogorsk, Kazakhstan*

Background:STI constitute a serious threat to reproductive health of the person in the form of possible complications or increase in risk of transmission of HIV.50 - 60% of STI in female organism proceed without symptoms, causing serious consequences such as pelvic inflammatory disease, tubal infertility,ectopic pregnancy. Children and pregnant women are especially vulnerable concerning STI.The low health index of women of reproductive age and the complicated pregnancy period because of transferred STI, lead to the birth of newborns with a low and very low mass of a body, and is at the bottom of 15% of cases of early neonatal mortality. Prenatal screening of pregnant women can prevent development of the listed above complications. In Kazakhstan protection of motherhood and the childhood is one of the priority directions of a strategic course of development of health care. According to the resolution of the president of Kazakhstan Republic "About Motherhood and Childhood Protection" from 12.12.2003r, one of additional expenses for rendering the state volume of free medical care is inspection of pregnant women on pre-natal infections, congenital anomalies and STI.

Methods:Within the program - screening in clinic laboratory of Skin - venereal specialized health center (East - Kazakhstan region) since 2004 to 2012 examination of pregnant women on DNA STI identification - Chl.trachomatis, Ureaplasma spp., Mycoplasma hominis, Trichomonas vag., Gardnerella vag. Patient material: the fence of a material for researches of pregnant women was spent from an urethra and vagina by disposable urogenital probe in special transport medium. Used method nucleic acid amplification tests (NAATs) - Polymerase chain reaction (PCR),"AmplySens" (Russia).

Results:From 2004 to 2012 were surveyed 19116 pregnant women. Screening was carried out during different terms of pregnancy. The majority of women passed screening on early terms. The percentage of detect ability on 5 infections has been analyzed. On the first place on detect ability is conditionally pathogenic flora - Ureaplasma spp. - 41,8 %, Gardnerella vaginalis - 32%, Mycoplasma hominis - 17%. Among pathogenic flora on first place on detect - Chl.trachomatis - 10% of and Trichomonas vag. - 4%. The share of mono-infection has made - 38%, mixed - infection - 62%. The highest percentage of occurrence of different commensal belongs to the association - Ureaplasma spp - Gardnerella vag. - 45%, Ureaplasma spp. - Mycoplasma hom. - Gardnerella vag. - 17%, Chl.trachomatis - Ureplasma spp. - 8%, Chl.trachomatis - Trichomonas vag. - 3%.

Conclusion:Factors which strengthen potential pathogenicity are: violation of the immunological reactivity. Change of hormonal background. All these factors promote development of diseases of a small pelvis with massive colonization of the urogenital tract. Prenatal screening of pregnant women can prevent the development of premature placental abruption, uterine inertia fetal hypoxia, placentation abnormalities of the fetus in STI.

B-045

Traditional clinical laboratory tests for Dengue fever diagnosis in a children´s hospital, São Paulo, Brazil.

L. R. Almeida. *DASA, São Paulo, Brazil*

Background Dengue fever (DF) and dengue hemorrhagic fever (DHF) the more severe form of dengue illness. Dengue viruses are transmitted though the bite of an infected mosquito *Aedes aegypti* is the primary mosquito vector, however other species can also be vectors of Dengue virus. Illness caused by dengue viruses can range from nonspecific febrile illness, as the most DF cases, to more severe illness with bleeding, thrombocytopenia, and plasma leakage in cases of DHF. Dengue incidence and prevalence are rising in endemic areas of the tropical and subtropical regions. Dengue infections occur in more than 100 countries in the Asia-Pacific region, the Americas, the Middle East, and Africa, and cases of infection continue to rise worldwide.3-5Approximately 50 million infections are estimated to occur each year.3 Dengue incidence rates are increasing mainly in tropical and subtropical regions of the world, and in the Americas, a dramatic increase of cases has been reported during the last decades.

Methods: We establish a classification of the results according with the clinical laboratory findings by comparing the results of the tourniquet test and the platelets of samples from all children attended on a Children´s Hospital in São Paulo, SP, Brazil.

Results: 249 tests were analyzed from January to December 2013, March, April and May had the highest number of tests, we divided the classification of the results in four groups, group A negative tourniquet test with normal platelets count, group B negative tourniquet test with low platelets count (thrombocytopenia), group C positive tourniquet test with normal platelets count and group D positive tourniquet test with low platelets count (thrombocytopenia). Group A had the highest combination 201 tests, negative tourniquet test with normal platelets, this result shows that for all suspected cases we have a low incidence of positivity, group B 24 combination (negative tourniquet test with low platelets count), group C 16 combinations (positive tourniquet test with normal platelets count) and group D 4 combination (positive tourniquet test with low platelets count (thrombocytopenia).

Conclusion: Traditional tests for diagnose Dengue Fever is not the gold standard, we expected to have a high number of combination os group D (positive tourniquet test with low platelets count (thrombocytopenia) which shows more evidence to diagnose DF however group A showed us that both tests are still very much solicited by clinical and is not helpful for the diagnose. This way laboratory did not contribute to the DF diagnose though It´s available other tests that can substitute and be helpful such as serology (had number of tests during this period), rapid test and also PCR. There are currently more advanced tests for the diagnosis of dengue fever of which confer higher sensitivity and specificity and contrite to more accurately diagnosing the disease.

B-046

A Multiplex Real-time PCR Assay for the Rapid Detection of the *mec*A Gene with Staphylococci Directly from Positive Blood Cultures

J. Kim[1], H. Kim[1], H. Wang[2], S. Kim[3], Y. Kim[1], S. Park[4], E. Choi[4], G. Kim[1], S. Ahn[1], S. Park[1], H. Jin[5], S. Park[6], Y. Kim[7], Y. Uh[4], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [3]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of, [7]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of*

Background : Sepsis causes increasing morbidity and mortality, particularly in elderly, immunocompromised patients, and it represents one of the greatest challenges in intensive care medicine. Staphylococci are the most commonly isolated organisms accounting for almost 50% of sepsis. In addition, Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most prevalent cause of sepsis and is recognized as a major nosocomial pathogen. This study aimed to evaluate a newly designed multiplex real-time PCR assay capable of the simultaneous detection of *mec*A, *S. aureus,* and coagulase-negative staphylococci (CoNS) in blood culture specimens.

Methods : The Real-MRSA® and -MRCoNS® multiplex real-time PCR assay (M&D, Republic of Korea) uses the following TaqMan® probes which were labeled with different fluorophores (FAM, HEX, and Cy5, respectively): 16S rRNA for *Staphylococcus* species, the *nuc* gene for *S. aureus*, and the *mec*A gene for methicillin resistance. For blood culture, two or three pairs of culture bottles for aerobes or anaerobes were incubated in the BacT/Alert 3D (bioMérieux, Marcy, France), BACTEC™ 9240 system (Becton Dickinson Diagnostic System, Spark, MD, USA), or the BACTEC™ FX (Becton Dickinson) blood culture systems for 5 days after inoculating with blood drawn from the patient at the bedside. The identification of organisms and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux, Durham, NC, USA).

Results : The multiplex real-time PCR assay was evaluated using 118 clinical isolates from various specimen types and a total of 350 positive blood cultures from a continuous-monitoring blood-culture system (CMBCS). Cycle threshold (C_t) values were used to determine the limit of detection. The detection limit of the multiplex real-time PCR assay was 10^3 CFU/mL for each gene target. The results from the multiplex real-time PCR assay for the three targets were in agreement with those of conventional identification and AST methods except for one sample. The sensitivities of the multiplex real-time PCR kit were 100% (166/166), 97.2% (35/36), and 99.2% (117/118) for 16S rRNA, *nuc*, and *mec*A genes, respectively, and the specificities for all three targets were 100%.

Conclusion : The Real-MRSA® and -MRCoNS® multiplex real-time PCR assay is very useful for the rapid and accurate diagnosis of staphylococcal blood stream infections (BSIs). Moreover, the multiplex real-time PCR assays may provide the essential information to accelerate therapeutic decisions for earlier and adequate antibiotic therapy based on detection of the *mec*A gene.

B-047

Field Test of the Dynex M² Multiplexed Assay System in the Democratic Republic of Congo Using Dried Blood Spots

S. Higgins[1], A. Karmali[1], M. Poncheri[1], A. Fusellier[1], P. Mukadi[2], J. Ngamboli[2], N. Kavira[2], R. Doshi[3], N. Hoff[3], E. Okitolonda-Wemakoy[4], J. Muyembe-Tamfum[2], A. Rimoin[3]. *[1]Dynex Technologies, Inc., Chantilly, VA, [2]Institut National de Recherche Biomédicale, Kinshasa, Congo, Democratic Republic of the, [3]UCLA Fielding School of Public Health, Los Angeles, CA, [4]Kinshasa School of Public Health, Kinshasa, Congo, Democratic Republic of the*

Background: The Dynex Technologies, Inc. M²® multiplex chemiluminescent immunoassay platform was selected as the processing platform for an MMRVT immunity assessment in support of the 2013 Democratic Republic of Congo Demographic Health Survey (DRC-DHS). Within five months and in collaboration with University of California, Los Angeles, Fielding School of Public Health (UCLA-FSPH) Dynex was able to deliver a fully functional automated processing system, reagents and adequate assay plates to process 10,500 dried blood spot (DBS) samples to Kinshasa, DRC.

Methods: Polystyrene beads coated separately with antigen to Measles, Mumps, Rubella, Varicella-Zoster Virus and Tetanus were immobilized within 54-well M² assay strips with 10 beads per well. Three separate within-well positive control beads were coated with horseradish peroxidase, total human IgG, and polyclonal goat anti-human IgG. Two negative control beads were coated with MRC-5 and E6 cell lysate. 423 dried blood spots were anonymously collected from children visiting Kinshasa health centers during the pilot study and more than 8,500 samples collected during the nationwide principal DHS survey. Positive control DBS were made using a 5-donor pool of normal defibrinated serum. Negative control DBS were made from pooled normal IgG-stripped serum. Each DBS was extracted into 1ml of PBS, 0.5% tween20, 5.0% dried milk and processed on a modified Dynex automated DS2® ELISA processing system. Optimization runs in the DRC included examination of DBS spotting order, DBS extraction time, two different anti-human IgG-HRP conjugates, PBS vs. Tris-NaCl wash, room temperate vs. 37°C sample/conjugate incubation temperatures, and 30 vs. 60 minute sample/conjugate incubation times. Duplicate DBS reference sets were made using a 32 previously-characterized plasma samples and a 7-point 4-fold dilution series of pooled positive control into negative serum. The duplicate reference sets were processed independently in Kinshasa and the Dynex labs.

Results: During the initial optimization of the M² testing platform all samples were tested in replicate and gave excellent concordance of clinical calls regardless of processing variables used. Currently, all 423 pilot samples and 1000 DHS samples have been processed. Sensitivity and specificity of the M² system in Kinshasa was shown to be equivalent to that at Dynex based on extraction of the 32-member reference set as well as to fresh dilutions of the control sera. Extraction of the 7-point DBS calibration series in the DRC shows an equivalent assay response to the same set extracted in the Dynex labs. Samples tested in replicate during optimization runs in Kinshasa gave 92% concordance of clinical calls regardless of processing variables used, with discrepant results found within the indeterminate range.

Conclusion: As shown by the speed of assay development, having been deployed to a substantially resource-limited environment, and agreement of replicates regardless of processing conditions the Dynex M² multiplex immunoassay system has shown itself to be a very robust assay platform with excellent sensitivity and specificity. The use of this system in conjunction with DBS processing offers a very cost-effective automated multiplexed immunoassay processing system in challenging environments.

B-048

Laboratory diagnosis of viral respiratory tract infections in a Children's Hospital in São Paulo, Brazil, one year study

L. R. Almeida. *DASA, São Paulo, Brazil*

Background Viruses are recognized as the major cause of respiratory tract infections, particularly in children. Emerging virus, such as Influenza H1N1, Metapnenumovirus and Bocavirus are detected by Molecular Biology methods, with high sensitivity and specificity. Frequently more than one virus are detected in the same sample and considered responsible for these infections.

Methods: Data from the results of laboratory tests for viral respiratory infections were collected, from January to December 2013, for patients attended a children´s hospital in the city of São Paulo Brazil. The test utilized was the RT-PCR Microarray: CLART® Pneumovir virus panel that detects Influenza A, Influenza A H1N1 strain 2009, Influenza B, Parainfluenza1, 2, 3 and 4, Syncycial Respiratory Virus (SRV) A and B, Adenovirus, Bocavirus, Metapneumovirus, Coronavirus, Enterovirus and Rhinovirus.

Results: 1394 respiratory samples were tested by the respiratory virus panel and 67% of the tests were positive for at least one virus. The months with higher positivity were from March to July corresponding to the beginning of autumn and winter, respectively, in Brazil. April showed the major positivity, 87% when compared with the other months. The most frequent viruses identified in this period of time were SRV 36%, Bocavirus 14%, Metapneumovirus 7,2% and Adenovirus 6,3%. The samples were collected from children aged 0 to 14 years and the positivity was higher in young children under 2 years old with 80% of the positive samples. Influenza A H3N2 was detected in two samples during the year 35 samples were positive for H1N1. Most of he results were provided to the physician in two days after collection.

Conclusion: The viral molecular panel detected a wide range of respiratory virus with high sensitivity, including more than one virus in the same sample. The rapid result, two days, is important for the etiologic diagnosis of respiratory infections and infection control measures for the patients admitted to the hospital.

B-049

The First Isolates of the Emerging New Delhi Metallo-β-lactamase in a Laboratory in Rio de Janeiro, Brazil

A. Chebabo[1], M. G. Quiles[2], T. T. Rocchetti[2], L. C. Fehlberg[2], E. J. U. Kusano[1], D. Thielmann[1], R. M. G. Pereira[1], O. Fernandes[1], A. C. Gales[2], A. C. C. Pignatari[2]. *[1]DASA, Rio de Janeiro, Brazil, [2]Laboratório Especial de Microbiologia Clínica. LEMC/ALERTA. Departamento de Medicina. Universidade Federal de São Paulo, São Paulo, Brazil*

Background: Antimicrobial resistance is a growing global challenge to human health. The emerging New Delhi metallo-β-lactamase (NDM), an acquired class B carbapenemase has gain public attention due to its extended hydrolysis of β-lactams including carbapenems. Objective: We report the first isolates of this emerging resistance mechanism in our hospitals in Rio de Janeiro, Brazil. Methods: Between September and October 2013, four carbapenem-resistant strains, three Enterobacter cloacae and one Providencia rettgeri were isolated in our clinical Microbiology Lab from distinct patients hospitalized at different hospitals located at two distinct cities of Rio de Janeiro. MIC was determined by CLSI broth microdilution method. Specific primers were used for PCR detection of blaNDM, blaKPC, blaGES, blaSPM, blaGIM, blaSIM, blaCTX-M, blaSHV, blaTEM, blaOXA-48, armA, rmtA, rmtB, rmtC, rmtD, rmtG, npmA followed by DNA sequencing. Clonal relatedness among E. cloacae isolates was examined by PFGE. Plasmid extraction was performed by Kieser protocol. Conjugation with E. coli J53 (LacZ− Nalr Rifr) and hybridization with specific probes were used to determine transfer of carbapenem resistance. The species identification was confirmed by MALDI-ToF MS® and 16sRNA DNA sequencing. Results: Of the four isolates, three were from public hospital and one from a private hospital. Enterobacter cloacae were isolated in blood, ascitic fluid and rectal swab and one Providencia rettgeri was isolated in urine. All strains showed higher resistant rates to carbapenems and to broad-spectrum cephalosporins and P. rettgeri was resistant to polymyxin B, as expected. blaNDM-1 and blaTEM-1 were identified in all isolates. All E. cloacae also produced blaCTX-M-15, and showed different PFGE pattern. blaKPC-2 was identified in one E. cloacae isolate (isolate E134) and armA gene was detected in E. cloacae gentamicin and amikacin resistant (isolate E133). Conjugation of blaNDM-1 was achieved in 2/4 isolates, E. cloacae (isolate E134) and P. rettgeri (isolate E132). The hybridization revealed that blaKPC-2 was located in the E. cloacae chromosome. The genetic location of blaNDM-1 is being confirmed among the isolates evaluated. Conclusion: Those isolates in Rio de Janeiro, showed the importance of correct molecular study of isolates that express carbapenenem resistance in the clinical Microbiology Lab, as, although the blaNDM-1 has been previously reported in Enterobacteriaceae clinical isolates in our country, this study constitutes the first one that identified the co-association of blaNDM-1 and blaKPC-2 in E. cloacae. The results can lead to improve infection control measures to avoid its spread in hospital environment.

B-050

New materials for Hepatitis A and Hepatitis B IgM immunoassay calibration and quality control

A. Doty[1], L. OCallaghan[2]. *[1]EMD Millipore, Billerica, MA, [2]Merck Millipore, Cork, Ireland*

Purpose: We propose that human monoclonal antibodies can be used as replacements to human serum as controls and calibrators in diagnostic assays for Hepatitis A IgM or Hepatitis B core IgM immunoassays . These *in-vitro* produced, standardized products offer an unlimited and consistent supply of antibody for calibration and quality control of infectious disease immunoassays. Relevance: Despite advances in public health and medicine, infectious diseases are persistently counted as a significant cause of human illness and economic loss. Assays developed for diagnosis and monitoring infectious diseases require robust, stable and readily available control and calibration materials. Traditionally manufacturing of these vital materials has depended upon discovering source plasma units from naturally infected individuals. This material is increasingly difficult to find. We present data on a new source of material for the manufacture of controls and calibrators for Hepatitis A Virus VP1 IgM or Hepatitis B virus core p22 IgM antibodies from immortalized human lymphocytes. Method: Human lymphocytes from individuals expressing the antibody of interest are isolated from fresh whole blood by Ficoll. Following *in vitro* immortalization with Epstein-Barr virus, primary B cells expressing the antibody of interest are fused with a hybridoma partner. Following an extended growth period hybridomas are screened to determine if antibody is being secreted. Monoclonality is assessed by the limiting dilution method. Stability of hybridoma cell lines is assessed and validated through extended cell culture & passage. Results: Recovery parallel to human serum, antibody specificity, lack of cross reactivity and performance in several analytical techniques are shown. Conclusion: These new materials can be used in the formulation of both calibrators and positive controls by manufactures of diagnostic kits for the detection of IgM antibodies to Hepatitis A or Hepatitis B. :

B-051

Vitamin D and Vascular Endothelial Growth Factor Levels in Hepatitis B Infection

A. F. Tuncel, H. Pasaoglu, O. Gumusay, G. Yilmaz, S. Ozenirler. *Gazi University School of Medicine, Ankara, Turkey*

Background: In addition to its well-known effect on calcium metabolism, vitamin D has various roles such as regulation of inflammatory processes, immune response, cell proliferation and differentiation. Hepatitis viruses can cause inflammatory liver disease and vitamin D deficiency was reported in patients with hepatitis C virus (HCV) infection. Vitamin D deficiency is a common finding in chronic liver disease patients but there is not much data on serum vitamin D levels of hepatitis B virus (HBV) infected patients. Angiogenesis may be observed during inflammatory processes and vascular endothelial growth factor (VEGF), which is an important mediator in angiogenesis, is found to increase in mesentery and liver tissue in cirrhotic patients. There are different findings on the effect of vitamin D on VEGF expression in viral hepatitis patients and we aimed to evaluate the relationship between these two markers in HBV infected patients.

Methods: The study included 57 patients with HBV infection and 19 age-matched healthy controls. Serum 25-OH vitamin D levels were measured by Advia Centaur XP chemiluminescence assay (Siemens AG, Germany). Serum VEGF levels were measured by enzyme linked immunosorbent assay (R&D Sytems, MN, USA). Two pathologists evaluated liver tissue samples from HBV infected patients. All data were analyzed using MYSTAT version 12 (SYSTAT, CA, USA). Data is presented as mean ± standard deviation. Spearman's rho and Mann-Whitney U tests were used as appropriate. A test result of $p<0.05$ was considered statistically significant.

Results: Mean serum vitamin D levels were lower in HBV infected patients by 3.79 ng/mL (27%) compared to the controls ($p<0.037$). Serum VEGF levels did not show significant difference between groups. There was no correlation between VEGF and vitamin D levels in patient population, however, control group showed an inverse correlation between these markers ($r2=0.228$, $p<0.039$).

Conclusion: Vitamin deficiencies are common in various viral hepatitis types and we showed that the serum vitamin D levels of HBV patients were lower than controls. Vitamin D is suggested to decrease viral replication so maintaining normal vitamin D levels might be beneficial in viral hepatitis. Both HBV and HCV genes can lead to an increase in the expression of VEFG. Vitamin D and its analogs modulate angiogenesis in viral hepatitis and suggested to be a regulatory factor of VEGF production. The negative correlation, which is found between serum vitamin D and VEGF levels in controls, was not observed in our patient group. This discrepancy might be caused by low vitamin D levels. We advocate measuring vitamin D levels in HBV infected patients; furthermore, we suggest vitamin D supplementation in deficient individuals.

B-054

Use of the MagArray Immunoassay System as a Platform for Pathogenic Escherichia coli Detection

L. M. Clotilde, A. Juang, H. Yu, L. Carbonell. *MagArray Inc, Sunnyvale, CA*

Background: In recent years, pathogenic Escherichia coli have been causing numerous foodborne outbreaks leading to mild to bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and even death of patients. Many foods with short shelf life are related to the public before a negative testing for E. coli is confirmed. Currently, the process by which regulatory agencies screen for pathogenic E. coli in foods takes over 3 days. The MagArray immunoassay system is a low-cost chip-based platform capable of simultaneously detecting up to 80 different analytes in as little as 10 min. The reduction in detection time of pathogenic E. coli can contribute to a faster recall of contaminated foods and can therefore limit the number of individuals ingesting the contaminated food and decrease the total cost of lost productivity and treatment. The objective of this study was to demonstrate on MagArray platform the simultaneous detection of two main types of pathogenic E. coli (i.e., O157 and O145) in ground beef with high sensitivity.

Methods: MagArray chips were first spotted with E. coli O145 and O157 antibodies. The chips were then blocked and ground beef samples were spiked with E. coli O145 and O157 for incubation. After incubating with detection antibodies, magnetic particles were then applied to generate signals. Different concentrations of E. coli were spiked to establish the standard curve and determine assay sensitivities.

Results: In this 2-plex immunoassay in ground beef, detection of E. coli at a concentration as low as 2 cfu/µl was demonstrated. More specifically, for 2 cfu/uL of E. coli O145 and E. coli O157, the inter-run CVs were less than 10% for both types. And the results were compared and agree well with samples spiked to pure buffers. This sensitivity of detection was achieved using a 30-min assay. And the results showed that assay sensitivity is minimally affected by changing assay media from pure buffer to ground beef.

Conclusion: The MagArray technology demonstrated that it can provide exceptional sensitivity with reasonable reproducibility for simultaneous detection of both E. coli O145 and O157 in ground beef. Thus we believe this technology provides a good fit for detecting multiple E. coli serogroups. This assay not only accelerates identification of pathogenic E. coli, but also holds the potential to help regulatory agencies to quickly issue a product recall for contaminated foods.

B-055

Real-time PCR TaqMan assay for Rapid Screening of Sepsis using Positive Blood Cultures

J. Kim[1], H. Kim[1], H. Wang[2], S. Kim[3], Y. Kim[1], S. Park[4], E. Choi[4], G. Kim[1], S. Ahn[1], S. Park[1], S. Park[5], H. Jin[6], Y. Kim[7], Y. Uh[4], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [3]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of, [6]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [7]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of*

Background : Sepsis is a lethal medical condition that results from a harmful or injurious host response to infection. Rapid detection of pathogens in blood from septic patients is essential for adequate antimicrobial therapy and prognosis of patients. The aim of this study was the acceleration of detection and discrimination of Gram positive (GP)-, Gram negative (GN)-bacteria and *Candida* species in blood culture specimens by molecular methods.

Methods : The Real-Sepsis® real-time PCR kit (M&D, Wonju, Republic of Korea) uses the following TaqMan® probes: the bacterial 16S rRNA gene for pan-GP, pan-GN and fungal 18S rRNA gene *Candida* species, respectively. For blood culture, two or three pairs of culture bottles for aerobes or anaerobes were incubated in the BacT/

Alert 3D (bioMérieux, Marcy, France), BACTEC™ 9240 system (Becton Dickinson Diagnostic System, Spark, MD, USA), or the BACTEC™ FX (Becton Dickinson) blood culture systems for 5 days after inoculating with blood drawn from the patient at the bedside. The identification of bacteria and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux, Durham, NC, USA). For identification of *Candida* species, a VITEK-2 (bioMérieux) YST ID CARD was used.

Results : The Real-time PCR TaqMan assay was evaluated using a total of 62 bacterial reference strains representing 39 of GP, 23 of GN species and 25 fungal reference strains. Subsequently, it was evaluated with 115 clinical isolates, 256 positive blood culture specimens and 200 negative blood culture specimens, and results were compared to those of conventional identification method. The overall sensitivity of the real-time PCR TaqMan assay was 99.6% and the specificity was 89.5%.

Conclusion : The Real-Sepsis® real-time PCR assay could not only differentiate bacterial and fungal from viral and other pathogens, but also can classify Gram staining with a much shorter turnaround time than the gold standard culture method. Furthermore, it could have an important impact on choosing the appropriate antibiotic therapy based on simultaneous detection and discrimination GP-, GN-bacteria and *Candida* species.

B-057

In vitro antimicrobial susceptibility of clinical and environmental strains of *Burkholderia pseudomallei* from Brazil

T. P. G. Bandeira[1], M. G. Castelo[2], E. T. Oliveira[3], F. M. P. Ventura[3], V. P. G. Bandeira[4], R. S. N. Brilhante[5], M. F. G. Rocha[6], J. A. Sales[6], G. M. M. Guedes[5], O. V. P. Denadin[3], C. F. A. Pereira[3], D. C. C. Branco[5], J. J. C. Sidrim[5]. *[1]DASA; Postgraduate Program in Medical Microbiology, Federal University of Ceara, Fortaleza, Ceara, Brazil, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, FORTALEZA, Brazil, [4]Christus College, School of Medicine, FORTALEZA, Brazil, [5]Postgraduate Program in Medical Microbiology, Federal University of Ceara, Fortaleza, Ceara, Brazil,, FORTALEZA, Brazil, [6]Postgraduate Program in Veterinary Science, State University of Ceará, FORTALEZA, Brazil*

Background: *Burkholderia pseudomallei*, the causative agent of melioidosis, is intrinsically resistant to a wide range of antimicrobial agents [1]. Ceftazidime is the drug of choice for treating melioidosis, although carbapenems are indicated for severe infections. Following this initial treatment, an eradication phase is recommended, consisting of prolonged oral therapy with trimethoprim-sulfamethoxazole (SXT) combined with doxycycline or amoxicillin-clavulanic acid (AMC) for up to 6 months [2]. The aim of this study was to determine the antimicrobial susceptibility of clinical and environmental *B. pseudomallei* from Brazil.

Methods: Ten clinical strains of *B. pseudomallei* were included in this study, obtained from the DASA central laboratory at Fortaleza, Ceará, and the others environmental strains were obtained from bacterial collection of Federal University of Ceará. Identification of *B. pseudomallei* was confirmed using an automated VITEK® 2 system, bioMérieux, followed by sequencing of the 16S-23S spacer region. For antimicrobial susceptibility assay, five antimicrobial agents were tested by the microdilution technique according to CLSI guidelines [3].

Results: All MICs determined by the broth microdilution from 20 strains of B. pseudomallei in this study were distributed in MIC50 and MIC90 and as the percentage of sensitivity. The percentage of sensitivity for doxycycline, imipenem and sulfametol / trimethoprim were 100% each and amoxicillin / clavulanate and ceftazidime was 80% and 90% respectively (Table 1).

Conclusion: The current results were compatible with those previously reported in the literature [4,5] and corroborate those of Jenney et al. [4]. The susceptibility of the tested strains appears to be independent of the origin of the isolates (environment or clinical cases).

In conclusion, this work provides knowledge on the antimicrobial susceptibility of *B. pseudomallei* from Brazil, serving as a guide for the selection of appropriate empirical therapy, thus contributing to better medical care in addressing melioidosis.

Antimicrobials	MIC (µg/mL) ^{50}	MIC (µg/mL) ^{90}	Range	Susceptibility (%)
Amoxicilin/ clavulanate	8/4	18/8	4/2 - 32/16	80
Ceftazidime	4	16	2 - 16	90
Doxycycline	0	1	0,25 - 0,5	100
Imipenem	1	1	0,125 - 1	100
Trimethoprim-sulfamethoxazole	1	2	0,125/2,375 – 2/38	100

B-058

The performance of a highly sensitive chemiluminescent enzyme immunoassay for HBsAg.

S. Yamauchi, K. Yamaguchi, S. Kojima, H. Sugimoto, Y. Imai, T. Saito, T. Higuchi, K. Moriyama. *Fujirebio Inc., TOKYO, Japan*

[Background] HBsAg is an envelope protein of HBV and is continuously secreted into blood as not only a portion of HBV but also a secretion protein during HBV persistent infection. HBsAg level in blood is correlated with intra-hepatic covalently closed circular DNA (ccc DNA) and is useful as an indicator of HBV persistent infection, especially during treatment with anti-viral drugs.

We have developed a fully automated highly sensitive chemiluminescent enzyme immunoassay for HBsAg (new CLEIA system) which has 10 fold higher sensitivity than a commercially available HBsAg kit. We evaluated the basic performance for the new CLEIA system and here we report the results.

[Methods] The highly sensitive chemiluminescent enzyme immunoassay was run on the fully-automated CLEIA system LUMIPLUSE G1200 (FUJIREBIO INC.).

[Results] The CV for within-run reproducibility was 0.5-3.3% and for between-run reproducibility it was 0.5-1.4%. The quantitation limit was 5 mIU/mL (0.005 IU/mL). The new CLEIA system could detect 1-3 bleeds earlier than the commercial HBsAg kit in seven seroconversion panels among nine. The correlation coefficient and the slope with the commercial HBsAg kit were 0.92 and 1.30, respectively.

[Conclusion] The new CLEIA system has good reproducibility and high sensitivity. And it shows good correlation with a commercial HBsAg kit. In addition, the new CLEIA system can be easily operated to complete an assay in 30 min.

The new CLEIA system is considered quite useful for the routine quantification of HBsAg.

B-059

Utilization of Serum Total Bile Acids for the Prediction of HCV Active Infection in Anti-HCV Antibody Positive Patients

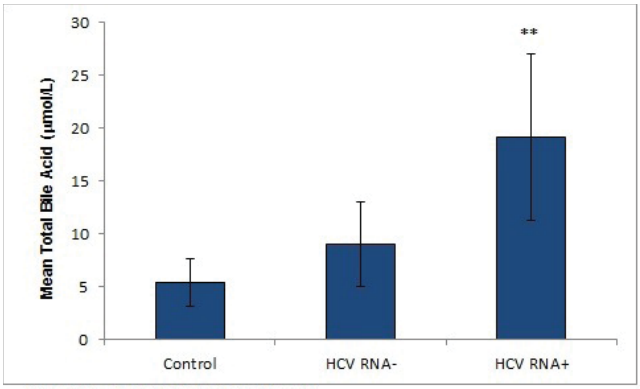
B. Chung, G. Guzman, M. Jin. *University of Illinois Hospital and Health Sciences System, Chicago, IL*

Background: Conventional liver function tests do not correlate with active hepatitis C infection or response to treatment. Recent studies have suggested that bile acids (BA) in the blood may be elevated in patients with detectable HCV RNA levels. It has also been shown that bile acids increase HCV RNA replication and has been suggested as a possible etiology for poor response to IFN therapy in patients with specific genotypes.

Methods: Total BA levels from blood samples were measured on the Beckman DxC using the Diazyme Total Bile Acids Assay. Conventional liver function tests using Beckman reagents were performed on the Beckman DxC. BA levels of 30 anti-HCV antibody positive patients with detectable HCV RNA and 30 without detectable HCV RNA were compared to 24 healthy controls. Reference range includes results < 10 umol/L. Mean values, 95% confidence intervals, and p-values from independent sample Student's unpaired t-test were calculated using MS Excel.

Results: Mean total BA values for controls and HCV RNA negative patients fell within the reference range while mean values for HCV RNA positive patients were elevated. Mean total BA levels were statistically significantly higher in patients with detectable HCV RNA levels versus controls and patients with undetectable HCV RNA levels (p-values: control, 0.09; HCV RNA-, 0.05). No statistically significant difference was observed for liver function test values between the 3 compared groups.

Conclusions: Currently, quantitative HCV viral load testing is employed to monitor treatment response but remains costly and is not practical for surveillance in chronic hepatitis C patients. Our preliminary findings suggest that total BA levels may distinguish between active and chronic hepatitis C infection. For physicians needing a non-invasive and less cost-prohibitive method for monitoring of recurrence or active infection in their hepatitis C patients, bile acid testing may prove a viable tool in their arsenal



B-061

Prevalence of Tuberculosis in Sao Paulo diagnosed by Laboratory tests in the period 2011-2013

M. C. Feres, R. Bini, N. A. Raphael, S. G. Oliveira, P. G. Campana, D. Mazzotti, A. A. Lino de Souza, S. Tufik. *Associacao Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil*

Background: According to data presented by the World Health Organization (WHO) in 2010 were diagnosed and reported 6.2 million cases of tuberculosis (TB) worldwide, with 5.4 million new cases, representing 65% of the estimated cases for the same year. Countries like China and India account for 40% of cases, and Brazil is among the 22 countries which account for 82% of TB cases worldwide.

Combating TB 2011 - 2015 follows the overall plan proposed by the WHO. Your goal is to dramatically reduce the burden of disease by 2015. The main objective to reduce TB are:1) reduce the incidence of TB in HIV / AIDS and the incidence of HIV in TB patients, prevent and control - multidrug-resistant TB and strengthen actions to meet the needs of poor and vulnerable populations, 2) strengthen the health system based on primary care, 3) engage all providers of health services, and 4) enable and promote research and others.

The Plan also has, as main targets: to reduce the incidence and mortality of TB until 2015 compared to 1990 and eliminate TB as a public health problem until 2050. With this goal it becomes increasingly important to accurate and early diagnosis of this disease and laboratory testing and higher efficiency are of great importance in this context.

Objective: To evaluate the percentage of positive diagnosis of TB for each specific laboratory test for this disease for the period 2011 to 2013, retrospectively analyzing the database of a Laboratory Oversize working in São Paulo, Brazil

Material and Methods: The authors retrospectively analyzed 44931 results of laboratory tests ordered for diagnosis and monitoring of TB originated from 42 ambulatory care of Associação Fundo de Incentivo a Pesquisa- Afip, from Sao Paulo- Brazil, (January2011-December2013). Laboratory tests analyzed were: Adenosine deaminase (ADA), Bacillus Koch (BK), BK-automated culture/Bactec , *Mycobacterium tuberculosis* PCR.

Results: The test results of ADA, BK. And BK- automated culture/Bactec from of years (2011, 2012 and 2013) with their respective percentages of positivity were: ADA for 2183 (23.5%), 2105 (21.9%), 2233 (48.8%), search for BK , 7219 (9.5%), 7563 (10.1%), 7400 (10.4%); BK-automated culture/Bactec 1926 (6.0%) 1653 (5.5%) 1535 (8.0%), and *Mycobacterium tuberculosis* PCR, 48 (4.2%) 79 (6.3%) 95 (7.4%).

The data were presented as percentage of positive prevalence of the most requested in the affiliated units of the Afip laboratory. However, these methods have limitations and many of them are carried out in more than one sample.

Conclusion: We note that the ordering patterns of these tests remained constant over the years. The PCR method showed a small increase in use and an increase of positivity but the general results showed that the most requested examination for diagnosis of TB is a direct search in lamina and culture of BK. Analyzing the percentages of positivity of the Afip tests performed, we conclude that from 2011 to 2013 there was an increase in cases of TB of nearly 0.5 to 1.5%, which is very worrying that we may achieve the government's goal to reduce TB cases until 2015.

B-062

Comparison of clinical performances among Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 assays for detection of high-risk types of human papillomavirus

S. Yu[1], K. Ko[1], M. Kwon[1], E. Lee[2], H. Woo[1], H. Park[1]. *[1]Kagnbuk Samsung Hospital, Seoul, Korea, Republic of, [2]Green Cross Reference Laboratory, Yongin, Korea, Republic of*

Background: High-risk types of human papillomavirus (HR-HPV) is an important cause of cervical cancers. Current cervical cancer screening guidelines suggest that early detection of HPV-16 and HPV-18 may prevent the progression of cervical cancer. We evaluated and compared three HPV DNA tests, Roche Cobas HPV (Roche Molecular Systems Inc., Pleasanton, CA), RFMP HPV Papillo Typer (GeneMatrix Inc., Yongin, Korea) and Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD, USA). The HC2 has been recommended for use as a reference test, Roche Cobas HPV specifically identifies HPV-16 and HPV-18 with concurrently detecting other 12 HR-HPV types and RFMP identifies 74 HPV genotypes.

Methods: A total of 861 cervical swab specimens from women over 30 years of age were classified into groups of high grade squamous intraepithelial lesion (HSIL) and non-HSIL according to cervical cytology results and analyzed by Roche Cobas HPV, RFMP HPV Papillo Typer and HC2. The results of direct sequencing or Linear array (LA; Roche Molecular Systems Inc., Pleasanton, CA) HPV genotyping test were considered true when three assays presented discrepancies.

Results: Concordance rates between Roche Cobas HPV *vs.* RFMP, RFMP *vs.* HC2, and HC2 *vs.* Roche Cobas HPV were 94.5% (814/861), 94.2% (811/861), and 95.8% (825/861), respectively. In 71 specimens with discrepant results, concordance rates between each assay and direct sequencing or LA were as follows: Roche Cobas HPV, 35.2%; RFMP, 93.0%; HC2, 25.4%. Clinical sensitivities and specificities for detecting HSIL were 80.3% and 95.8% with Roche Cobas HPV, 83.6% and 95.1% with RFMP and 90.2% and 94.8% with HC2.

Conclusion: Roche Cobas HPV, RFMP and HC2 showed high agreement rates each other. Although Roche Cobas HPV and RFMP showed lower clinical sensitivity in detecting HSIL compared to HC2, they would be clinically useful since both provide HPV genotypes.

B-063

Prevalence of fungal bloodstream infections in a tertiary University Hospital in Brazil - Comparative analysis between two periods in the last decade

L. S. Vasconcellos, L. L. Castro, D. H. Bücker, P. H. O. Mourão, J. R. Romeiro. *Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: In the last decades, the growing population of immunosuppressed hosts has dramatically increased. Therefore, the prevalence of nosocomial fungemia has increased throughout the world and mortality from this disease is high. The objective of the study is to identify the etiology of fungal bloodstream infections in a Tertiary University Hospital in Belo Horizonte, Brazil, comparing two periods in the last decade.

Methods: we retrospectively analyzed the results of all blood cultures processed in the hospital, between two periods: from 2001 to 2003 and from 2011 to 2013. For each triennium were reported the number of blood cultures collected, the number of positive cases, the percentage of fungemia and all identified fungal species. The samples were observed in the laboratory routine carried out by incubation in BacT ALERT® (bioMérieux). The positive samples were subcultivated for species identification through morphologic and biochemical assays.

Results: From 2001 to 2003, 34.822 blood culture were performed and 5,510 (15.8%) positive. Fungi were isolated in 229 (16.4%) cases. From 2011 to 2013, the number of blood cultures increased to 55,052, but the number of positive samples decreased to 4,873 (8.9%). Fungal bloodstream infections increased to 290 (6.00%) cases. Candidemias were predominant: 97.38% (2001-2003) and 91.72% (2011-2013). The isolated species are shown in Table 1.

Conclusions: The prevalence of fungemia increased in the last decade. Candidemia was responsible for more than 90% of the cases. Non-albicans Candida species increased and C. albicans decreased. Others species of fungi increased too.

Prevalence of fungal species on bloodstream infections in University Hospital in the last decade

Species	2001-2003		2011-2013		Total	
	N	%	N	%	N	%
Candida albicans	91	39.74	84	28.97	175	33.72
Candida glabrata	1	0.44	5	1.72	6	1.16
Candida guilliermondii	3	1.31	3	1.03	6	1.16
Candida kefyr	0	0	2	0.69	2	0.39
Candida krusei	0	0	9	3.10	9	1.73
Candida parapsilosis	60	26.2	76	26.21	136	26.20
Candida spp	29	12.66	24	8.28	53	10.21
Candida tropicalis	39	17.03	63	21.72	102	19.65
Cryptococcus neoformans	3	1.31	7	2.41	10	1.93
Cryptococcus spp	3	1.31	3	1.03	6	1.16
Fusarium sp	0	0	8	2.76	8	1.54
Trichosporon spp	0	0	6	2.07	6	1.16
Total	229	100	290	100	519	100

B-064

Distribution of HIV genotypes among Brazilian regions

P. Y. Nishimura, D. Nifoci, B. C. Vilanova, V. D. T. Niewiadonski, O. P. Denardin, N. Gaburo Jr.. *DASA, Sao Paulo, Brazil*

Background: Lung cancer is the most prevalent life-threatening cancer worldwide with more than 80% being non-small cell lung cancer (NSCLC). Detection of mutations of EGFR gene is critical for predicting the response to therapy with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, in patients with NSCLC. Patients that are EGFR mutants have constitutive TK activity and, therefore, a greater sensitivity to anti-EGFR inhibition.

Objective: To describe the EGFR mutations frequency found in lung adenocarcinoma samples, using pyrosequencing method.

Method: Thirty samples of lung adenocarcinoma were analyzed from January 2013 to December 2013. The test was performed on formalin-fixed, paraffin-embedded tumor specimen, after the selection of the specimen region to be analyzed by a pathologist. The DNA was extracted using the Qiaamp FFPE Tissue kit (Qiagen, Hiden, Germany). Concentration of DNA sample was measured spectrophotometrically using a NanoDrop spectrophotometer (NanoDropTechnologies, Wilmington). Codons 719, 768, 790, 858, 861 and exon 19 were amplified by PCR using the EGFR Pyro kit (Qiagen, Hiden, Germany). Successful and specific amplification of the region of interest was verified by visualizing the PCR product on capillary electrophoresis using Qiaxel DNA Screening Kit (Qiagen, Hiden, Germany). Preparation of single-stranded DNA was done using PyroMark Q24 vacuum workstation (Qiagen, Hiden, Germany) according to the manufacturer instructions. The pyrosequencing reaction was analyzed on the Pyro Mark Q24 (Qiagen, Hiden, Germany)

Results: The frequency of EGFR mutations found is presented on Table 1. All mutations together represent only 27% of the samples.

Conclusion: The results are consistent with previous studies and reports. The single-point mutation L858R (CTG> CGG) on exon 21 and the frame deletions on exon 19 represents the majority mutations found in Brazilian lung adenocarcinoma samples, although most samples showed no mutation at the target regions.

Table 1. Frequency of EGFR mutations found in lung adenocarcinoma samples.

Results	**Frequency**
Wild type	73%
2235del15 (exon 19)	3,3%
2236del15 (exon 19)	3,3%
2237_2255>T (exon 19)	3,3%
2239_2248>C (exon 19)	3,3%
CTG>CAG (L861Q)	3,3%
CTG>CGG (L858R)	10%

B-065

Soluble CD14-subtype, a possible new biomarker increases in septic patients' plasma from pediatric department.

H. Yamaguchi, S. Fukuoka, H. Oto, T. Soga, M. Inoue, S. Kitazawa, Y. Umeda, S. C. Kimura. *Showa University Northern Yokohama Hospital, Yokohama City, Japan*

Background: Soluble CD14-subtype, named presepsin (P-SEP) is a fragment of CD14 peptide produced through phagocytosis of microorganisms by neutrophils. Increased serum concentration of P-SEP was reported in adult patients with severe bacterial sepsis (Shozushima T, et al. J Infect Chemother 2011;17:764-9), however, there have been limited reports on pediatric patients. In order to clarify the significance of P-SEP as a marker of septic disease in children, we conducted a study of serum P-SEP concentration in pediatric patients with febrile diseases.

Methods: Forty-eight children (29 males, 19 females, 0.6 to 152 months after birth, mean age 2.43 years old) admitted to our hospital were enrolled. Plasma was obtained within 24 hours after blood withdrawal. P-SEP was assayed using PATHFASTTM chemiluminescent enzyme linked immunoassay system (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). This automatic analyzer enables to get results within 20minutes. Procalcitonin, white blood cells and C-reactive protein concentration were assayed simultaneously. The ethic committee of Showa University Northern Yokohama Hospital approved this study.

Results: P-SEP concentration was 442 plus minus 301 ng/L (mean and SD) in patients whose blood culture was positive on admission (n=4). For example, staphylococci were detected with blood culture from a 30 months-old female patient. Her P-SEP concentration was 866 ng/L on admission, then decreased after antimicrobial treatment to 235 ng/L when she was discharged. P-SEP concentrations were 191 plus minus 47 in viral infections (n=9), 313 plus minus 90 ng/L in Kawasaki's disease (n=6). On the other hand, cases with blood culture negative but urine and/or sputum culture positive showed 349 plus minus 202 ng/L (n=9). Other culture negative patients (n=20) showed 267 plus minus 132 ng/L.

Discussion: P-SEP has been reported to be an indicator of prognosis in adult septic patients (Masson S, et al. Crit Care 2014;18:R6), and critically ill preterm newborns (Mussap M et al. J Matern Fetal Neonatal Med 2012;25:51-3). Though statistically not significant, plasma P-SEP was higher in septic children compared to those without bacterial infections. Reference interval of plasma P-SEP concentration in adults under the age of 70 is ranged 201 to 457 ng/L (Chenevier-Gobeaux C, et al. Clin Chim Acta 2014;427:34-6). Our study suggests reference interval in children is likely to be lower than that in adults. More study is required to confirm the results.

Conclusion: Increased plasma concentration of P-SEP was observed in pediatric patients with bacterial sepsis. P-SEP could be a possible biomarker of sepsis in pediatric patients.

B-066

Analysis of blaKPC gene from Hodge Test screening confirming KPC enzyme resistance

S. S. Michelotto. *LANAC, Curitiba, Brazil*

Background:The Klebsiella Pneuminiaie Carbapenemase (KPC) is responsible for human infections, especially in hospital environment. It is an enzyme produced by Gram-Negative bacilli and its detection in bacterial isolates confers resistance to the carbapenem antibiotics and furthermore inactivates penicillins, cephalosporin and monobactams.The transmission, in hospital environment, occurs through the contact between secretion from infected patients. The objective of this study was to evaluate the correlation of positive results on the Hodge Test with detection of the blaKPC gene confirmed by Molecular Biology (Life Technologies - Real Time PCR System 7300).

Methods: 105 cultures from samples of LANAC Laboratory of different materials were selected and the resistance profile was observed to multiple antibiotics (especially carbapenems) using the method of disk diffusion and automated system MicroScan WalkAway - Siemens Healthcare Diagnostic during 2011 and 2012.

Results:Multidrug resistant strains are not new or specific for Klebsiella species. In 100 of 105 analyzed cultures *K. pneumoniae* was isolated (95.2%). 98% of these isolates showed concordance between Hodge Test and the results obtained by Life Technologies - Real Time PCR System 7300 (blaKPC gene detectable). 2% of the isolates were indeterminate by Hodge Test. The others microorganisms isolates on the analyzed cultures were *E. cloaceae* (2,8%); *Enterobacter sp* (0.95%) and *Proteus vulgaris* (0,95%). In only two samples the correlation between the tests were not confirmed.

Conclusion: In this study was observed 98% of correlation between Hodge Test and the detection of blaKPC gene by Molecular Biology (Life Technologies - Real Time PCR System 7300). The determination of the Minimum Inhibitory Concentration (MIC) using the equipment MicroScan WalkAway - Siemens obtained an excellent performance in the correlation between positive Hodge Test and detectable blaKPC gene by Molecular Biology (Life Technologies - Real Time PCR System 7300). Hodge Test and microbiology system automation seems to be an excellent alternative for clinical laboratory routine with high sensitivity and lower cost.

B-067

Ziehl-Neelsen staining as an aid in screening for diagnostic of systemic fungal infection.

C. F. A. Pereira[1], D. P. Silva[1], L. G. S. Carvalho[1], A. M. Cervo[2]. *[1]DASA - Group, Cascavel, Brazil, [2]UNIPAR, Cascavel, Brazil*

Background: Mycology is still an area that has little clinical importance, although the number of susceptible patients to fungal infection arises through the years. Every single day, in laboratory cytology routines, sputum samples are collected for Acid-fast stain (Ziehl-Neelsen method) for the differential staining procedure to members of the genera mycobacteria (M. tuberculosis, M. leprae), bacteria (Nocardia) and fungus (Cryptosporidium,). It's known that many systemic fungal infections are similar to other common pulmonary diseases, and the differential diagnostic is difficult. We have tried, through a visual screening of compatible fungal structure, to identify medically significant fungi, to an additional specific Mycosel agar culturing step. Although Ziehl-Neelsen is not intended to staining of fungal genera, we thought if it could also be used for the primary identification of fungal pathogens.

Methods: The test was performed with routine sputum samples from Laboratório Alvaro (DASA group), collected by spontaneous or induced expectoration and kept under refrigeration of 2 - 8°C. These samples were primary intended to Ziehl-Neelsen staining procedure for identification of M. Tuberculosis. After staining and visual observation of fungus structure, the cytologist is capable of reporting if a fungal infection is or is not present in the sample. After visual inspection, 150 potential positive samples were selected. Mycosel Merck culture medium was prepared by dilution as described in technical data sheet. This medium is specific for isolation of pathogenic fungi. After inoculation, samples remained in an incubator set to 35 ° C for approximately 30 days. After the incubation time, each grown fungal structure was identified by slide morphological observation, in which Cotton blue staining (specific for examination of fungal colonies) was applied.

Results: From 150 samples, we had no growth in 14% (21/150), 86% (129/150) were positive, where 55% (37/150) corresponding to C. albicans yeast, 9% (6/150) to C tropicalis, 5% (3/150) C. glabrata, 5% (3/150) of yeast and hyphae C. albicans, 1% (1/150) C. parapsilosis, 1%(1/150) Nigrospora and 1% (1/150) of Candida Krusei. Although there is significant positivity for Candida genera, it can't be easily implicated in systemic fungal infection, as opposed to 9 % (6/150) of fungal, normally associated to pulmonary disease. Our final finding was 4% of Histoplasma sp, 3% Aspergillus sp, 2% of Paracoccidioides sp, fungus that are morphologically classified as positive for severe pulmonary disease.

Conclusion: This study provides evidence of the presence of etiologic agents of severe pulmonary fungal disease in sputum of patients originally submitted to Acid-Fast staining. The simple screening to fungal structure in Ziehl-Neelsen stained slides, have shown to be applicable, simple and effective to directing potential positive samples to further culturing in selective medium for isolation of pathogenic fungi. This new procedure can be meaningful in evaluating TB like suspect patients not just on the basis of symptoms, clinical signs, but providing another reliable screening tool.

B-068

Development of a Point-of-Care Diagnostic for Ebola and Sudan Virus Detection

D. Oottamasathien[1], A. Jones[1], M. Boisen[1], P. Kulakosky[2], R. Wilson[2], L. Branco[2], E. Ollman Saphire[3], R. Garry[4], K. R. Pitts[1]. *[1]Corgenix, Inc., Broomfield, CO, [2]Autoimmune Technologies, New Orleans, LA, [3]The Scripps Research Institute, La Jolla, CA, [4]Tulane University, New Orleans, LA*

Background: Viral hemorrhagic fevers are serious, often fatal illnesses characterized by high fever, damage to the vascular system, and multi-organ failure. Because of their rapid progression, the ability to detect and distinguish hemorrhagic fevers is paramount to treatment and survival. To this end, we report on the characterization of rapid point-of-care diagnostic tests for Ebola (EBOV) and Sudan (SUDV) virus detection.

Methods: Using recombinantly produced proteins, we generated a library of polyclonal and monoclonal antibodies recognizing EBOV and SUDV GP, NP, and VP40. Antibodies were initially tested using a multivariate approach with each antibody being tested for use as both capture and detection capability. Testing was performed over a range of plate coating concentrations, nitrocellulose dot blots and stripings, HRP and gold conjugation conditions, and sample dilution ratios. The pairings were further optimized by testing the EBOV and SUDV plate coating concentrations and HRP-conjugate dilutions against various sample dilution titrations to determine the conditions that favored sensitivity and signal. Testing was performed using chosen antibody pairings for the EBOV and SUDV ELISA to confirm that the pairings are optimal by running dose-response curves of both purified EBOV VP40 or SUDV VP40 antigen spiked into a normal human serum control matrix, determining the signal to noise ratio, linear range, LOD, LOQ, and LOB. Candidate antibody pairings identified during the antibody screening process for use on the lateral flow immunoassay (LFI) format were conjugated to gold nanoparticles and striped onto nitrocellulose using the Biodot XYZ dispenser. Testing was performed using purified antigen spiked into normal human serum control matrix.

Results: As demonstrated by ELISA, we found polyclonal antibody pairings against EBOV and SUDV VP40 to be the most reliable in detecting purified recombinant protein in spiked samples. Pairings exhibited limits of detection as low as 10ng/mL, and limits of quantitation ranging from 10-100ng/mL, suggesting that these critical reagents possess the ability to detect low amounts of EBOV and SUDV protein. Pairings migrated to the LFI test strips showed the ability to detect both EBOV and SUDV VP40 recombinant protein in a dose-dependent manner down to 100ng/mL within 10 minutes. Importantly, this dose-dependency was distinguishable with the naked eye, indicating the utility of this rapid test in environments lacking conditioned power and/or significant medical training.

Conclusions: We have developed and characterized prototype ELISA and LFI tests capable of detecting EBOV and SUDV proteins in sample matrix. In the ELISA format, multiple pairings were able to detect EBOV and SUDV VP40 antigens in spiked matrix with acceptable sensitivity, suggesting that with further optimization and ELISA test for the detection of EBOV and SUDV is within reach. In the LFI platform, two pairings showed the ability to detect EBOV and SUDV antigens in a concentration-dependent manner. Importantly, this concentration dependency was discernible without the aid of any instrumentation, suggesting a path forward for the optimization of a rapid, point-of-care test that can be used in austere environments.

B-069

Heparin Binding Protein for Discrimination of Infected and Non-infected Critical Ill Patients from Cardiovascular Conditions - Results of a Pilot Evaluation

E. Spanuth[1], M. Preusch[2], A. Vasishta[3], E. Giannitsis[4]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]Department of Internal Medicine III, Intensive Care Unit, University Hospital Heidelberg, Heidelberg, Germany, [3]Axis-Shield Diagnostic Ltd., Dundee, United Kingdom, [4]Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany*

Background Heparin binding protein (HBP) is an inflammatory mediator released into the circulation during neutrophil activation. HBP has been shown to contribute diagnostic information to the differentiation of viral and bacterial infections. Bacterial infection is the most important trigger for the development of sepsis. Especially in critical ill patients the early detection of infection is necessary for appropriate treatment. We thought to investigate whether HBP is able to detect bacterial infection in critical patients from cardiovascular conditions admitted at the intensive care unit (ICU).

Methods 20 patients admitted at the ICU with severe cardiovascular conditions were included. 12 patients developed additional infectious diseases of whom 4 patients developed sepsis. Serum HBP concentrations were measured using the Heparin Binding Protein EIA (Axis-Shield Diagnostics Ltd. Dundee). C-reactive protein (CRP) was determined using the cobas assay (Roche Diagnostics).

Results The discrimination of HBP and CRP concentrations between patients with (n=12) and without infection (n=8) was examined by Mann-Whitney independent sample test. The results are displayed in the table.

Tab. 1: HBP and CRP values in ICU patients with cardiovascular conditions with and without additional infectious diseases

	Without infection, n=8 Median (IQR)	With infection, n=12 Median (IQ)	p value
HBP, µg/L	60 (39-95)	145 (121-238)	0.0087
CRP, mg/L	72 (34-124)	159 (99-206	0.0136

The determination of HBP in serum provided a higher significance level for differentiation between patients with and without infectious diseases compared to CRP. These results could be confirmed by ROC analysis yielding area under the curve (AUC) values of 0.854 and 0.833 for HBP and CRP, respectively. Logistic regression analysis with HBP and CRP as independent variables revealed an AUC value of 0.906 demonstrating that the simultaneous determination of HBP and CRP provided additional diagnostic information.
Conclusion HBP allows highly significant discrimination between infected and non-

infected critical ill patients with cardiovascular complications admitted at the ICU which was superior compared to CRP. Additionally, simultaneous determination of HBP and CRP showed higher diagnostic efficacy than both markers alone.

B-070

Diagnostic Evaluation of Focus Diagnostics Simplexa™ Dengue real-time polymerase chain reaction (RT-PCR) detection and typing of dengue virus

I. C. Ibraim, G. J. Cruz, E. C. C. Mateo, A. C. S. Ferreira. *Instituto Hermes Pardini, Vespasiano, Brazil*

Dengue is the most important arthropod-borne viral infection of humans and the incidence of dengue has grown dramatically. Dengue virus (DENV) infection affects over 40% of the world's population. Worldwide, an estimated 2.5 billion peopleare at risk of infection.

Dengue viruses belong to the genus flavivirus within the *Flaviviridae* family. The virus group consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that manifest with similar symptoms. DENVs produce several syndromes that are conditioned by age and immunological status.Laboratory confirmation of dengue infection is crucial as the broad spectrum of clinical presentations can make accurate diagnosis difficult.

Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods.Seroconversion of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. Viral antigens also provides evidence of infection and virus isolation provides the most specific test result.The RT-PCR and other PCR-based techniques have become a primary tool to detect virus in the early course of illness. In addition, molecular testing allows the monitoring of outbreaks by detecting the emergence of new serotypes, thus permitting the implementation of control measures.

Therefore, the aim of the project was to evaluatethe diagnostic accuracy of the commercially available Focus Diagnostics Simplexa™ Dengue real-time polymerase chain reaction (RT-PCR) assay for the in vitro detection and typing of dengue virus serotypes 1, 2, 3 and 4 and compare with results obtained from serology.

The RNA of 37 IgM and/or IgG positive samples were extracted using the QIAamp RNA Viral Kit (Qiagen, Germany) according to the manufacturer's recommendations. The amplification of the extracted RNA used bi-functional fluorescent probe-primers and reverse primers. The assay amplifies four serotype specific regions: dengue 1 (NS5 gene), dengue 2 (NS3 gene), dengue 3 (NS5 gene) and dengue 4 (capsid gene). An RNA internal control was used to monitor the extraction process and to detect RT-PCR inhibition. A positive control for all four serotypes was added during the extraction and RT-PCR reaction.

We tested 37 serologically positive samples. In order to compare serologywith RT-PCR, any samples positive for IgM and/or IgG were considered a 'positive' diagnosis of dengue. Of the 37 samples tested from patients with positive serology, 8 (21.62%) were found positive by RT-PCR, with Ct values ranged between 30.3 to 39.6. All positive RT-PCR samples were IgM positive and were negative for IgG. The assay did not detected viral RNA in positive IgG sample (32.43%).

Serological assays are commonly used for diagnosis of dengue infection, as they are relatively inexpensive and easy to perform. However, the detection of antibodies in a dengue-infected person is only possible after 4-5 days of disease onset. One advantage of the RT-PCR assay is the ability to detect and serotype viral RNA early in dengue illness, which is important to diagnosing acute infection and provides the opportunity to impact patient management.

B-071

Analytical Reactivity and Preliminary Performance Results of the BD MAX™ QS Vaginal Panel*

N. Paquette[1], V. Brochu[1], J. Cormier[1], S. Tremblay[1], C. Lehouillier[1], S. Morasse[1], F. Harel[1], M. Tremblay[1], D. Cantin[1], L. Belley-Montfort[1], M. Farell[2], S. Sajo Beqaj[3], C. Roger-Dalbert[1]. *[1]BD Diagnostics, Québec, QC, Canada, [2]Planned Parenthood, Houston, TX, [3]Pathology Inc, Torrance, CA*

***The BD MAX™ QS Vaginal Panel is not available for sale or use in the U.S**

Background: The BD MAX™ QS Vaginal Panel is an automated qualitative *in vitro* diagnostic test for the direct detection of *Candida* species associated with vulvovaginal candidiasis (VVC), trichomoniasis, and bacterial vaginosis (BV) from vaginal swabs in women with clinical symptoms of vaginitis/vaginosis. The test utilizes real time PCR for the detection and identification of organisms. This study aimed to (1) challenge the assay with a wide range of strains in an analytical reactivity (inclusivity) study, (2) evaluate the capacity of the assay to detect targets during a co-infection and potentially support the rationale for patient treatment decisions and (3) collect preliminary results from clinical specimens tested with the BD MAX™ QS Vaginal Panel on the BD MAX™ System.

Methods: An analytical reactivity study was performed in the presence of simulated vaginal matrix, on a minimum of 5 strains for each cultivable organism (58 strains total), originating from 12 countries. The capacity to detect co-infection was tested using two combinations i.e. low load of *Trichomonas vaginalis* (TV), *Candida glabrata* and *Candida krusei* in the presence of a high load of *Candida albicans* and low load of TV in presence of high load of *C. glabrata*. Vaginal swabs collected from women with vaginal symptoms were characterized using various reference methods and were then tested with the BD MAX™ QS Vaginal Panel. In Pouch™ TV test was used as the reference method for TV while culture followed by BD Phoenix™ identification was used for *Candida* species and the Nugent Score was used as the reference method for BV. Amsel's Criteria were used to provide a final result for specimens with intermediate Nugent Score. The preliminary performance of the assay for detection of trichomoniasis, *Candida* species associated with VVC and BV was established using a Receiver Operating Characteristic (ROC) curve analysis. The diagnosis of BV was determined using an algorithm based on PCR parameters for the detection of five BV associated markers (*Lactobacillus* species, *Gardnerella vaginalis*, *Atopobium vaginae*, BVAB-2 and *Megasphaera-1*).

Results: The assay identified all strains tested for each analyte in the inclusivity study. Simulated co-infection studies demonstrated the capacity of the assay to detect low loads of a specific organism in the presence of high load of another organism. The preliminary assay performance results (sensitivity/specificity) based on analysis of 771 characterized clinical samples were as follows: TV (94.4%/100%), Candida species (86.8%/94.8%), Bacterial Vaginosis (91.9%/86.2%).

Conclusion: The BD MAX™ QS Vaginal Panel demonstrated high levels of detection for BV, trichomoniasis and *Candida* species associated with VVC simultaneously from vaginal specimens.

B-072

A fast and sensitive (1→3)-β-D-glucan microfluidic assay for the diagnosis and treatment monitoring of invasive fungal infections

R. Kapoor, I. Yamaguchi, W. Chang. *Wako Life Sciences, Inc., Mountain View, CA*

Incidence of invasive fungal infections (IFIs) is on the rise in recent decades with increasing morbidity and mortality rate in many critically ill patients. Diagnosis is often difficult with conventional methods, including biopsies (risk of complications), imaging (nonspecific and limited use for early detection), and culture (slow, high rate of false negative). Without proper diagnosis and treatment, IFI patients may die within weeks.

(1→3)-β-D-glucan (BDG) is a major cell wall component of pathogenic fungi (e.g. Candida, Aspergillus, Fusarium, Acremonium and Pneumocystis), and the increase of BDG concentration in blood has been correlated to fungal infection in patients. Currently, BDG assays are based on the recognition of BDG by coagulation factor G from horseshoe crab amebocyte lysate. We have used a recombinant β-glucan recognition protein (rBGRP) that contains the binding domain of factor G to develop a liquid-phase binding electrokinetic analyte transport assay (LBA-EATA) for BDG in serum.

Our LBA-EATA assay takes advantage of some inherent features of a micro total analysis system (μTAS), including shorter reaction time, low reagent consumption, and minimal reagent and sample handling. In a microfluidic chip channel, the complex of BDG bound by DNA-rBGRP to speed complex migration and fluorescent dye conjugated rBGRP for detection is concentrated by isotachophoresis (ITP) to enhance detection sensitivity. The concentrated complex is subsequently separated from noise signals in another part of the chip channel by capillary zone electrophoresis (CZE) and detected by laser induced fluorescence (LIF). The ITP-CE process is completed within 3 minutes, and the assay can detect BDG in clinical specimens in the low picogram per milliliter range (~ 10pg/ml). This sensitivity is sufficient to differentiate BDG in healthy human population (10-40 pg/mL) and should be capable of detecting the early onset of fungal infections when used in conjunction with other diagnostic methods.

B-073

Multicenter Evaluation of Mindray Fourth-generation CL-2000i HIV Ag/Ab Combination Assay

S. Xu[1], X. Han[2], L. Li[3], H. Wang[4], L. Zhu[5], L. Wu[5], F. Xia[5]. *[1]Department of Cell Biology, National Institutes of Food and Drug Control, Beijing, China, [2]Key Laboratory of AIDS Immunology of Ministry of Health, Department of Laboratory Medicine, The First Hospital of China Medical University, Shenyang, China, [3]Department of Laboratory Medicine, The Sixth People's Hospital of Shenyang, Shenyang, China, [4]Key Laboratory of Infectious Diseases, The Third People's Hospital of Shenzhen, Shenzhen, China, [5]Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China*

Background: The Centers for Disease Control and Prevention (CDC) recently proposed to use fourth-generation HIV immunoassays for screening. Mindray CL-2000i HIV Ag/Ab Combination assay (CL-2000i) is a newly developed fourth-generation HIV assay that simultaneously detects HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2. The objective of this study is to evaluate the performance of CL-2000i via a multi-center study in three clinical trial centers and National Institutes for Food and Drug Control (NIFD) of China on well-characterized specimens.

Methods: The evaluation was performed on 635 HIV-infected and 1793 HIV-uninfected specimens at three clinical trial centers and NIFD. HIV-infected specimens were either confirmed with nucleic acid amplification testing (NAT), or repeatedly reactive by other chemiluminescence immunoassays and clinical diagnostics. Positive samples of antibodies to HIV-2, HIV-1/O, p24 antigens, and seroconversion panels are obtained commercially. All samples were tested by CL-2000i in comparison with ARCHITECT.

Results: The sensitivity of CL-2000i was 100% for antibodies of HIV-1 (635/635; 95% confidence interval: 99.40 - 100.00%). All the positive samples of the following analytes were reactive: antibodies to HIV-1 Group O (5/5) and HIV-2 (30/30), and HIV p24 antigen (23/23). The specificity of the assay was 99.83% (1790/1793; 95% confidence interval: 99.51 - 99.94%). Testing of 13 HIV-1 seroconversion panels indicates a comparable power of detecting acute HIV infection between CL-2000i and ACHITECT. In each of 3 seroconversion panels, CL-2000i can detect one more positive sample than ACHITECT, equivalent to at least 2 days earlier detection. This was attributed to the high sensitivity of HIV-1 p24 antigen (< 0.25 IU/mL, the most sensitive p24 assay in the market). One HIV antibodies negative sample determined by third-generation HIV EIA assays are strongly reactive with both CL-2000i and ACHITECT, indicating the power of detecting HIV p24 antigen by the fourth-generation HIV Ag/Ab combination assays.

Conclusion: CL-2000i exhibits high sensitivity and specificity, and the ability of early HIV detection. It can detect all the available known antibody positive samples for HIV-1/M, HIV-1/O, HIV-2, and HIV p24 antigen. It is well suited for screening of early HIV infection.

B-074

Use of an Integrated Molecular Diagnostic Platform with a Diverse Array of Specimen Types To Address Laboratory Automation Needs

P. Rodriguez[1], S. L. Moseley[1], J. Osiecki[2], M. Lewinski[2]. *[1]Roche Diagnostics Corporation, Inc., Indianapolis, IN, [2]Roche Molecular Systems, Inc., Pleasanton, CA*

Introduction: Molecular methods have revolutionized the way clinical labs identify the presence of microorganisms in patient samples, monitor viral responses to therapy, and characterize genetic disorders. With the demand on test menu expansion, and common limitations in lab space, sample flexibility, budget for new instruments and time for operator training, molecular laboratories require greater platform consolidation. In addition to this, automated systems for PCR reduce tech time, deliver faster and reliable results, minimize handling and processing errors, and help improve confidence in reporting patient results. For these reasons, the cobas® 4800 System was developed.

Objective: To review the key design features and testing solutions of an innovative molecular diagnostic platform which address the automation and integration challenges faced by molecular diagnostic laboratories.

Methodology: The cobas® 4800 System is an automated PCR system, which offers consolidation of Women's Health, Oncology and Microbiology* testing on a single platform. The system is configured with two units - the cobas x 480 instrument for sample preparation/PCR set up and the cobas z 480 analyzer for amplification and detection. On the cobas x 480 instrument, multiple primary and secondary sample types can be loaded and automatically scanned to allow for positive sample ID tracking. Pipetting channels have built-in liquid-level detection and monitoring of all pipetting steps to ensure quality sample processing. A robotic hand transfers specimens to multiple incubation positions to assist with lysis, washing and elution of nucleic acid. The cobas® 4800 system has built-in enzymatic and engineered contamination controls to prevent sample-to-sample or run-to-run carryover contamination. All processes are controlled by intuitive software, which guides the operator through initiating a run, monitoring of the instrument and run status. To increase efficiency a new run can be started in parallel with the amplification and detection of a previous run. User defined workflow software provides open-mode capabilities on the cobas z 480 allowing the laboratory to design customized applications that fit their needs, offering the potential for further platform consolidation.

Results and Conclusions: The cobas® 4800 System test offerings cover a broad range of disease state biomarkers and a diverse array of specimen types. For instance, testing for high-risk HPV and HPV 16/18 genotyping can be done on cervical specimens collected in PreserCyt either before or after cytology processing in order to accommodate sample workflow. *C. trachomatis* and *N. gonorrhoeae* infection can be assessed from male and female urine specimens, endocervical swabs, self-collected or clinician-collected vaginal swabs and cervical specimens collected in PreservCyt solution, which supports CDC recommendations for testing with a wide range of specimen types. BRAF and EGFR mutation testing requires formalin-fixed paraffin embedded tissue sections, including those already mounted on a glass slide. Other tests currently in development include KRAS*, MRSA/SA*, HSV-1/2* and *C. difficile**. Collectively, the cobas® 4800 System is an innovative molecular diagnostic solution that addresses the increasing demand for test integration and sample flexibility.

*The cobas® MRSA/SA Test, cobas® HSV 1 and 2 Test, and the cobas® Cdiff Test are currently in development and not available for sale in the US.

B-075

Metabolomics approach to predict disease severity in influenza virus infection

C. Lin[1], S. Lin[2]. *[1]Department of Laboratory Medicine, Chang-Gung Memorial Hospital, Tao-Yuan, Taiwan, [2]Research Center for Emerging Viral Infections, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan*

Background: Influenza A virus spread on a worldwide scale and infects a large proportion of the human population. Early diagnosis and treatment can have an important role in preventing the development of long-term complications or in interrupting transmission of the infectious agent. A promising approach for the predicting disease progression of influenza infection is targeting host factors that affect disease outcome. Measuring metabolites represents the dynamic metabolomics status of living system. Therefore metabolites levels can be regarded as the ultimate response of biological systems to virus infection. This proposal is using metabolomics strategy to decipher the disease progression after pathogenic influenza A infection.

Methods: Three different strains of influenza A viruses were used to investigate how these different pathogenicity of influenza viral strains affect the metabolism of the host. The three different influenza A H1N1 strains are A/Taiwan/141/2002 (141), A/Taiwan/126/2009 (swine-origin influenza virus, SOIV) and A/PR8/34 (PR8). These three strains have the same antigenicity in their hemaglutinin and neuraminidase (H1N1). PR8 is a high-pathogenicity, SOIV is defined as moderate-pathogenic strain and 141 is defined as mild-pathogenic strain. Female C57Bl/6 animal (6-12 weeks) were anesthetized with Isoflurane and then infected by intranasal application of 200 PFU of viruses. Mice were monitored and weighted daily. Infected and naïve mice (3 mice per group) were sacrificed on day 7 after infection. Bronchoalveolar lavage fluid (BALF) samples were collected and apply to liquid chromatography MS/MS assay based metabolomic analysis (AbsoluteIDQTM180 kit). Dissected mouse lung were fixed and stained with hematoxylin and eosin for pathologic evaluation.

Results: Animals infected with PR8 had 23% weight loss and heavy leukocyte infiltration was observed in lung. Principal component analysis was used to analyze the correlations between the metabolites concentration in samples obtained from naïve mice and mice after different strains of influenza infection. It shows significant difference in these four groups of BALF samples. All the amino acid concentrations were dramatically elevated in PR8 infected mice reflected the extremely active immune response. Long chain acylcarnitine were accumulated when the mice was infected with PR8. Short chain acylcarnitines help the body produce energy and help increase circulation. Acetylcarnitine (C2) was thought to be a more bioavailable form for cells and can induce weight loss. It was significant elevated in PR8 infected BALF. Sphingomyelins were significantly increased in 141 infected BALF; there was no significant difference between the naïve and the SOIV and PR8 infected groups.

Conclusion: Amino acids concentrations in mice BALF are correlated with the

severity of influenza infection. Combination of multiple markers of amino acids, acylcarnitines, sphingomyelines can help to predict the severity of the infection. The metabolic profiling could be a useful method applied to diagnose patients with H1N1infection and can predict the disease severity.

B-076

Evaluation of the Dynex M² MMRV Multiplex Immunoassay Panel vs. Three Commercial Test Kits

S. Higgins, A. Karmali, M. Poncheri, C. Randall, A. Fusellier. *Dynex Technologies, Inc., Chantilly, VA*

Background: Multiplex analysis of clinical samples offers significant advantages in terms of sample usage and processing time but acceptance has been hindered by high initial costs, lack of full automation, or both. We have developed M²®, a robust, fully automated and cost effective chemiluminescent multiplex immunoassay system to address these shortcomings. Here we present performance comparisons of the M² multiplex panel vs. two qualitative singleplex and one multiplex immunoassay system for antibodies against Measles, Mumps, Rubella and Varicella-Zoster Virus (MMRV).

Methods: Polystyrene beads separately coated with antigen to MMRV immobilized within 96-well M² assay strips. Positive and negative control beads were coated with goat anti-human IgG and combined MRC-5 and E6 cell lysate. Samples were 32 previously characterized human plasma and 7-point 4-fold dilution series of a 5-donor pool of normal serum and run on a modified Dynex DS2® automated ELISA processing system. Identical samples were processed using conventional ELISA kits and a commercially available multiplex assay designed for the Luminex® Model 200.

Results: Sensitivity and specificity of the M² system was calculated independently for each kit using the 32-member reference panel. For each commercial system any sample that tested above the negative cut off was considered as a True Positive (TP), and any sample that fell below was considered a True Negative (TN). Sensitivity was calculated as TP/(TP+FN). Specificity was calculated as TN/(TN+FP). Dilution series of pooled positive serum shows M² to possess greater assay-assay reproducibility than any of the three kits that were examined.

Conclusion: The Dynex M² multiplex immunoassay system shows excellent correlation in both sensitivity and specificity vs. commercial ELISA and multiplex kits across all analytes in an MMRV panel.

Sensitivity and Specificity of Dynex M² vs. Commercial Kits

	Predicate System	Measles	Mumps	Rubella	Varicella
Sensitivity, %	SinglePlex 1	100.0	89.1	100.0	100.0
	SinglePlex 2	85.0	89.1	90.0	100.0
	Luminex	87.5	89.1	82.0	95.5
Specificity, %	SinglePlex 1	100.0	100.0	100.0	100.0
	SinglePlex 2	100.0	100.0	100.0	92.3
	Luminex	100.0	100.0	100.0	100.0

B-077

Workflow Efficiency Through the Use of Mixed Batch Testing for Microbiology Applications on the cobas® 4800 system

M. Lewinski[1], P. Rodriguez[2], S. L. Moseley[2], J. Osiecki[1]. *[1]Roche Molecular Systems, Inc., Pleasanton, CA, [2]Roche Diagnostics Corporation, Inc., Indianapolis, IN*

Introduction: System flexibility for molecular diagnostic testing is becoming increasingly important for clinical laboratories as space constraints and staffing shortages impact efficiency. The ability to simultaneously run multiple assays on a single instrument can reduce turn-around time, improve workflow, and have a positive impact on job satisfaction for laboratory technologists. Mixed batch testing streamlines laboratory processing through the use of automated sample extraction and amplification with identical parameters optimized for multiple applications, allowing users to mix samples and tests being included in a single run on the same instrument system. The purpose of this study was to determine the impact of mixed batch testing for *Clostridium difficile* (Cdiff), Methicillin-resistant *Staphylococcus aureus* (MRSA), and Herpes simplex virus (HSV) when evaluated on the cobas® 4800 system compared with 3 other configurations of commercially available systems.

Methods: Batches of specimens for MRSA, Cdiff, and HSV were tested with molecular diagnostic systems in the most efficient possible configuration; system A - BDmax™ Cdiff and MRSA and BD Viper HSV, system B - GeneXpert® XVI Cdiff and MRSA, and BD Viper HSV, system C - GeneXpert® Infinity 48 Cdiff and MRSA, and BD Viper HSV. Batch sizes of 46, 22, and 6 (including controls) MRSA, Cdiff, and HSVspecimens, respectively, were assessed with the cobas® MRSA/SA Test*, cobas® Cdiff Test*, and cobas® HSV 1 and 2 Test* to reflect sample numbers that would be processed on a typical day in a medium sized clinical laboratory. Hands-on time is defined as the labor elements associated with each system/process required to start and finish a testing run that require a manual interaction. Automation time is defined as the time during testing where the operator has no manual interactions with the samples. Turn-around time is the actual clock time from start to finish to complete a testing cycle.

Results: Mixed batch testing on the cobas® 4800 system showed improvement for hands on time of 3, 5, and 6-fold less than comparator systems A, B, and C, respectively, when processing the same number of specimens. Automation time for comparator systems A, B, and C was 2.5, 2.4, and 2.2 fold higher than what was observed with the cobas® 4800 system running mixed batch testing. Evaluation of each configuration showed mixed batch testing improved workflow by reducing turn-around time by 70%, 39%, and 33% over method A, B, and C, respectively.

Conclusions: The system flexibility the cobas® 4800 system allows for mixed batch sample testing for MRSA*, C.diff* and HSV* on a single system which can provide superior workflow efficiency for the increasing demands of the clinical laboratory.

* The cobas® MRSA/SA Test, cobas® HSV 1 and 2 Test, cobas® Cdiff Test and the cobas® KRAS Test are currently in development and not available for sale in the US

B-078

Next-Generation Sequencing for Hepatitis B Genotype and Resistance Testing in a Clinical Microbiology Laboratory

C. F. Lowe[1], L. Merrick[1], T. Mazzulli[2], C. H. Sherlock[1], G. Ritchie[1]. *[1]Providence Health Care, Vancouver, BC, Canada, [2]Mount Sinai Hospital, Toronto, ON, Canada*

Objective: Comparison of hepatitis B (HBV) genotyping and resistance testing utilizing an in-house developed PCR assay and next-generation sequencing with a line-probe assay.

Introduction: HBV is one of the most common causes of cirrhosis and hepatocellular carcinoma worldwide. Antiviral therapy has been associated with a delay in disease progression. In addition, HBV genotypes have been associated with different rates of development to advanced liver disease and responses to interferon based therapy. Newer diagnostic modalities such as next-generation sequencing (NGS) can provide both genotype and resistance testing in one assay, and have the potential for increased sensitivity and detection of resistant HBV subpopulations.

Methods: 80 clinical patient samples (plasma) were retrospectively studied. Genotype (n=50) and resistance (n=80) were previously characterized by line-probe assay (INNO-LiPA HBV DR Assay, Version 2/3 and INNO-LiPA HBV Genotyping Assay; Innogenetics, Gent, Belgium). An in-house developed assay for hepatitis B genotype and resistance testing was studied using the GS Junior (454 Life Sciences, Branford, Connecticut). DNA was extracted using the MagNA Pure LC 2.0 (Roche Diagnostics, Mannheim, Germany). PCR amplified a 418bp amplicon of the polymerase region (codons 143 - 281). Amplicons were then sequenced on the GS Junior following the manufacturer's protocols. A third party bioinformatics software company (ABL TherapyEdge, Luxembourg) provided support in the interpretation of genotype and resistance profile of the HBV based on EASL Clinical Practice Guidelines. Sanger sequencing of the PCR amplicons was performed using the 3730 DNA Analyzer (Applied Biosystems, Foster City, USA) for discrepant genotype results between the line-probe assay and NGS.

Results: 50 samples were compared to the INNO-LiPA HBV Genotyping. There was concordance in 47/50 samples (A=3,B=24,C=14,D=5,E=1). Sanger sequencing for the 3 discrepant samples (NGS = B,C,C vs. INNO-LiPA=E,D,B, respectively) confirmed the results of the next-generation sequencing assay. Resistance testing for 80 samples included mutations at the following loci: M204V/I, L180M, A181T/V, N236T, V173L, T184G and S202I/G. No resistance mutations were detected by line-probe assay in 61 samples. Five of these samples had a mutant subpopulation (% of the virus population with a base pair mutation at known resistant loci) detected by NGS: 2 samples with M204I (1.6%; 2.5%), 1 sample with M204I(100%)/L180M(2.5%), 1 sample with V173L (2.5%) and 1 sample with A181T (3.9%). 19 samples with resistance mutations detected by line-probe were also confirmed by NGS.

Conclusions: Utilizing an in-house developed assay with a novel PCR targeting the polymerase region of HBV, genotyping and resistance testing for the most significant mutations can be performed with 1 PCR and 1 NGS reaction. NGS can potentially provide clinicians with increased sensitivity, earlier detection and detailed analysis of resistance profiles, as well as accurate detection of genotype. As a result, next-generation sequencing may become more accessible to incorporate into clinical microbiology laboratories for hepatitis B genotyping and resistance testing.

B-079

An Immunoturbidimetric Assay for Hyaluronic Acid

J. A. Chapo, B. Hurley, I. Muncy, K. R. Pitts. *Corgenix, Inc., Broomfield, CO*

Background: Hyaluronic acid (HA), also known as hyaluronate or hyaluronan, is a linear glycosaminoglycan - a high molecular weight polysaccharide with an unbranched backbone composed of alternating sequences of β-(1-4)-*D*-glucoronic acid and β -(1- 3)-*N*-*D*-acetylglucosamine moieties. Each dimer is referred to as one unit and has a molecular weight of approximately 450 Da. The HA molecule can vary in length from less than 10 to more than 1,000 units. HA is mainly produced by fibroblasts and other specialized connective tissue cells. It is a major constituent of connective tissue matrix (proteoglycan) and participates in various cell-to-cell interactions. HA is widely distributed throughout the body and can be found as a free molecule in plasma and synovial fluid. In plasma, the half-life of the HA molecule has been estimated to be about 5-6 minutes. HA is found in synovial fluid in high concentrations and is responsible for normal water retention and articular lubricant. Synovial HA may pass into plasma via the lymphatic system. In circulation, HA levels are maintained by an efficient receptor-dependent removal mechanism present in sinusoidal endothelial cells (SEC) of the liver and by the enzymatic action of hyaluronidase. Because the liver plays a central role in maintaining HA homeostasis, increased plasma levels of HA may serve as a sentinel for hepatic inflammation, fibrosis and cirrhosis. We report here the development of an immunoturbidimetric method for detecting HA in a blood sample.

Methods: An R1 reagent/reaction buffer was developed and optimized to augment the specific agglutination reaction of the coated microparticles with hyaluronic acid in the serum samples. An R2 reagent/coated polystyrene microparticles was developed using functionalized polystyrene microparticles covalently coated with HA binding proteins using standard conjugation techniques. Iterative combinations of R1 and R2 reagents were systematically tested to achieve consistent linearity and precision. Numerous iterations of coating and blocking buffers were assayed to further enhance assay manufacturability and consistency. Finally, in-process testing of linearity and precision was conducted to ensure robust performance to the end-user.

Results: The assay's limit of detection (LOD) was determined to be 11.36ng/mL; limit of blank (LOB) was found to be 6.68ng/mL; limit of quantitation (LOQ) was determined to be 20.00ng.mL. Rigorous precision testing demonstrated the assay's consistency of the course of 20 operating days with a 5.3% overall percent coefficient of variation. Assay linearity was between 25ng/mL to 750ng/mL for samples tested. The overall average percent recovery was 103.5% and lot to lot values showed no statistical difference ($p= 0.736$) across both the medical decision range and the range above. Real time stability concluded the assay can reliably and consistently measure samples over the course of at least 12 months, with a deviation of less then 10% of the mean for each group.

Conclusions: The data presented herein highlight the robust performance of this immunoturbidimetric assay. In summary, these data demonstrate the overall performance of the assay was consistent with a predicate HA-ELISA and that values obtained will be consistent both over time and from lot to lot.

B-080

PCR-Reverse Blot Hybridization Assay for Identification of Pathogens causing Sepsis from Positive Blood Cultures

H. Kim[1], J. Kim[1], H. Wang[2], S. Kim[3], Y. Kim[1], S. Park[4], E. Choi[4], G. Kim[1], S. Ahn[1], S. Park[1], S. Park[5], H. Jin[6], Y. Kim[7], Y. Uh[4], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [3]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of, [6]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [7]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of*

Background : Sepsis is a lethal medical condition leading to the systemic inflammatory response to infection. Sepsis is the 10th leading cause of death in the United States, accounting for 6% of all deaths and an estimated 135,000 patients die each year of sepsis-associated complications in Europe. Early detection of pathogens responsible for septicemia and antimicrobial resistance are significantly important for appropriate antimicrobial therapy. This study aimed to evaluate the PCR-reverse blot hybridization assay (PCR-REBA) capable of the identification of pathogens and antimicrobial susceptibility test (AST) in blood culture specimens.

Methods : The PCR- REBA, REBA Sepsis-ID® (M&D, Wonju, Republic of Korea) was designed to contain a total of 25 probes including 6 Gram-positive bacteria (GP) specific probes, 8 Gram-negative bacteria (GN) specific probes and 5 *Candida* species specific probes with a pan-bacteria, a pan-GP and a pan-GN probes. In addition, it includes *mec*A ,*van*A and *van*B probes for detection of antibiotic-resistant bacteria. For evaluation of the REBA Sepsis-ID®, a total of 300 positive blood culture bottles from BACTEC™ FX (Becton Dickinson Diagnostic System, Spark, MD, USA) or BacT/ALERT 3D (bioMérieux, Marcy, France) were used. The identification of organisms and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux).

Results : The correct agreement rates between conventional identification and AST methods and PCR-REBA for GP, GN, *Candida* and polymicrobials were 94.5%, 97.3%, 100% and 91.7%, respectively. Of 92 methicillin-resistant *Staphylococcus* species, *mec*A gene was detected in 90 (97.8%) samples and *van*A gene was correctly detected in one (100%) sample which was identified to vancomycin-resistant *Enterococcus* (VRE) by phenotypic examination.

Conclusion : Newly developed REBA Sepsis® was a rapid and accurate molecular-based method for simultaneous rapid detection of causative agents and antimicrobial resistant genes in positive blood cultures even though there was a limitation for evaluation with negative cultures such as nonviable after exposure to antibiotics or small amount bacteria.

B-081

Assessment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the cobas® CT/NG v2.0 Test on the cobas® 4800 system: Infection prevalence in pregnant women enrolled in a large multicenter clinical trial

J. Osiecki[1], P. Rodriguez[2], S. L. Moseley[2], J. Duncan[1], M. Lewinski[1]. *[1]Roche Molecular Systems, Inc., Pleasanton, CA, [2]Roche Diagnostics Corporation, Inc., Indianapolis, IN*

Background: Miscarriage, pre-term delivery, low birth weight, and morbidity in the neonate are potential consequences when pregnant women become infected with sexually transmitted diseases. In an effort to identify women with infection, treatment guidelines recommend screening pregnant women for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhea* (NG) on the first prenatal visit. This study was performed to determine the frequency of CT and NG infection observed in pregnant women enrolled in a large clinical trial study population.

Methods: This multicenter retrospective cohort analysis was performed with archived specimens collected during the VENUS clinical trial and prospective specimens collected during the VENUS II clinical trial, to characterize the clinical performance of the cobas® CT/NG v2.0 Test on the cobas® 4800 system. As recommended by the FDA, Patient Infected Status (PIS) was determined for each enrolled participant using two FDA-cleared nucleic acid amplification tests (NAATs) as comparator assays. PIS was defined as positive when results from NAATs with different target regions generated positive results with collected samples. Diverse settings in the United States served as specimen collection sites and included obstetrics-gynecology practices, family planning clinics, and STD clinics.

Results: Of 6,035 eligible participants, 6,004 subjects were evaluated for CT and/or NG for primary anlaysis. PIS determined 365 women and 92 men were infected with CT, and 122 women and 67 men were infected with NG. Of the female patients evaluated, 6.93% (365/5265) were found to be positive for CT infection and 1.75% (92/5265)were positive for NG according to PIS outcomes. Alternatively, 8.4% (17/202) of eligible pregnant women were positive for CT, where 1.48% (3/202) of pregnant women were considered positive for NG by PIS.

Conclusion: Screening of pregnant women for CT and NG with the cobas® CT/NG v2.0 Test on the cobas® 4800 system compared to two additional NAATs during the VENUS clinical trial revealed comparable rates of infection for CT and NG between pregnant women and non-pregnant women in the general female population.

B-082

Performance evaluation of the Access HCV Ab PLUS assay on the UniCel DxI 800 system

H. LE GUILLOU-GUILLEMETTE[1], D. NOGUES[2], A. DAVID[2], R. FALCOU-BRIATTE[3], F. LUNEL-FABIANI[1]. *[1]Laboratoire de virologie, Département des Agents infectieux et Pharmaco-toxicologie CHU Angers, ANGERS, France, [2]BIO-RAD, STEENVOORDE, France, [3]BIO-RAD, MARNES LA COQUETTE, France*

Background: The Access® HCV Ab PLUS assay (Bio-Rad) is a chemiluminescent microparticle immunoassay (CLIA) for the qualitative detection of antibodies to the hepatitis C virus in human serum or plasma. The purpose of these studies was to evaluate diagnostic performance.

Four serological automated assays were compared: Access HCV Ab PLUS assay on UniCel® DxI 800 Immunoassay system (Beckman Coulter Inc.), Architect® anti-HCV assay on Architect I2000SR analyser connected to the APS system (Abbott Diagnostics), Elecsys® Anti-HCV II assay on MODULAR® ANALYTICS E170 system or Cobas® e601 system (Roche Diagnostics) and ADVIA Centaur® HCV assay on ADVIA Centaur XP system (Siemens).

Methods: The Access HCV Ab PLUS assay is a two-step indirect antibody detection format.

First study: 659 fresh samples tested for HCV diagnosis in the routine virology laboratory at the University hospital of Angers were prospectively tested, 199 frozen positive samples from a retrospective data collection of patients' sera and 2 commercial panels were tested on Access HCV Ab PLUS and Architect anti-HCV assays. Two other commercial panels were tested only on Access assay. For Architect assay, data from the supplier were used.

Second study: the specificity was estimated by testing 500 non-selected fresh serum samples from a routine laboratory, 3 commercial panels plus one anti-HCV low titer performance panel were tested on Access HCV Ab PLUS, Elecsys anti-HCV II and ADVIA Centaur HCV assays. Another panel was used on Access and Elecsys assays. For ADVIA Centaur assay, data from the supplier were used.

Results: First study: On unselected routine samples, the agreement between the two assays was equal to 99.5%. The clinical specificity was 98.9% (95% CI: 97.8-99.6%) and 99.7% (95% CI: 98.9-100%) for Access HCV Ab PLUS and Architect anti-HCV assays, respectively. The clinical sensitivity for all positive samples was 100% for both assays. The sensitivity on seroconversion samples showed performance in accordance with the state-of-the-art for Access and Architect assays.

Second study: 488 of the 500 non-selected samples were true negative. The clinical specificity was 99.6% (95% CI: 98.53-99.95%) for Access HCV Ab PLUS, Elecsys anti-HCV II and ADVIA Centaur HCV assays. The concordance between the 3 assays was 99.20%. The clinical sensitivity from 12 positive samples was 100% for all assays. Using 4 commercial seroconversion panels and one anti-HCV low titer performance panel, Access HCV Ab PLUS and Elecsys anti-HCV II assays showed equivalent performance and detected earlier than ADVIA Centaur HCV assay.

Conclusion: The performance of the Access HCV Ab PLUS assay on the UniCel DxI 800 immunoassay system was excellent in terms of specificity and sensitivity. The clinical specificity was slightly better with Architect anti-HCV assay as compared to the other assays. The clinical sensitivity on true positive samples was 100% for all assays. The seroconversion sensitivity was better on UniCel DxI 800, Architect and Modular than on ADVIA Centaur. Adapted for high throughput routine testing, the Access HCV Ab PLUS assay performed on UniCel DxI 800 immunoassay system is fully suited for the screening of HCV infection in diagnostic laboratories.

B-084

Carbapenem-Resistant Enterbacteriaceae (CRE) in Geriatric Population:

R. Khoury, P. Patel, B. P. Salmon, A. Gandhi, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

Background: CRE refers to Carbapenem-Resistant and/or Carbapenemase-producing Enterobacteriaceae. These are families of bacteria that are resistant to several classes of antibiotics including one of the carbapenen group. Carbapenem antibiotics are used to treat infection caused by gram negative bacteria as the last line of treatment. Multidrug-resistant gram negative bacteria especially CRE are becoming the new super bug in the Long-Term Care Facilities where most of the residents are elderly, frail and are on multiple medications. The most common enterobacteriacae are *Klebsiella* species and *Escherichia col.;* over 40% mortality has been reported with invasive infection with CRE. Infection with CRE has limited therapeutic and high morbidity and mortality.

Methodology: 35,330 specimens were collected for culture from residents in Long-Term Care Facilities over a period of 6 months. All positive cultures were subcultured and then identified using MicroScan Walkaway 96 conventional panels, the isolate was considered CRE if it was resistant to one or more of the carbapenem, with Ertapenem nonsusceptibility being the most sensitive indicator of carbapenemase production. Statistical analysis were done using Analyse-it.

Results: 18,569 (52.6%) specimens were positive, 320 patients had CRE positive culture, the most common source was urine 250 cases (78.1%) followed by wound 42 cases (13.1%), respiratory 16 cases (5.0%)), rectal swab 5 cases (1.6%) and blood 4 cases (1.3%). Majority of the cases were reported in August (23.8 % of all cases) and the lowest was in November (11.3 % of all cases); we noticed an increase in the cases in January which was due to respiratory infections. The most common organism was *Klebsiella Pneumoniae (ESBL or MDR),* followed by *E. CLOACAE MDR, E. Coli,* and *SERRATIA MARCESCENS.*

Conclusion: CRE incidence is high in the long-term care facilities, facilities should follow the CDC recommendation to implement the "detect and protect" strategies. Prompt implementation of infection prevention and control measures requires close collaboration between clinical laboratory, infection prevention staff, physicians, and nurses. Early detection and implementing infection control and prevention will help reducing the transmission to other residents in addition to identifying the risk factors for CRE. Cautious and appropriate use of antimicrobial therapy for the treatment of suspected infections in residents of long-term care facilities are very important to prevent the occurrences.

B-086

Reduced Methicillin-resistant *Staphylococcus aureus* infections rate after the three-year implementation of a Rapid Molecular Screening in Intensive Care Unit

P. Stano, M. Avolio, R. De Rosa, M. L. Modolo, A. Camporese. *"S. Maria degli Angeli" Hospital, Pordenone, Italy*

Background: Previous studies have suggested that Methicillin-resistant *Staphylococcus aureus* (MRSA)-colonized patients are at higher risk for acquiring MRSA infections compared with non-colonized patients, while in the hospital, thus, MRSA-carriers should be monitored closely. Molecular screening methods have the advantage of high sensitivity and rapid turnaround times (TATs), assuring a rapid delivery of results and, consequently, improving infection control procedures and the clinical impact. Here, we assess the effects of implementation of a specific MRSA bundle, based on rapid molecular screening for MRSA and decolonization, on the prevalence of MRSA infection at our ICU, over a study period of three years.

Methods: The study was conducted over two time periods, before and after implementation of a specific MRSA bundle. A total of 431 and 886 nasal screening swabs were obtained from ICU patients, respectively before and after the bundle implementation and analyzed by the molecular test Xpert® MRSA (Cepheid). The pre-bundle period (from April 2009 through December 2010), has been used to assess the rate of ICU colonization and to evaluate the more appropriate measures to be applied in MRSA-carriers, thus screening results did not activated any preventive measures in patients colonized. Later, an MRSA bundle was implemented from January 2011 through December 2013 at our ICU (post-bundle period). The bundle consisted of rapid molecular screening for MRSA nasal carriage, at the ICU admission, contact precautions and nasal decolonization (mupirocin 2% ointment three times-a-day for five days) for patients colonized with MRSA. Clinical samples from patients suspected as being infected with MRSA were tested by standard laboratory culture procedures. The results of rapid nasal screening were available to physicians within 2 hours from specimen receipt.

Results: In the pre-bundle period, 9 patients (2%) developed a generalized MRSA infection, but during the three years that followed the bundle implementation (post-bundle period), MRSA infection rates declined from 2 % to 0.2% (2 patients) with a total MRSA infection decrease of 100% in the third year post-intervention. On the contrary, MRSA colonization rates at admission increased from 7,1% in the pre-bundle period to 8,6 % in the post-bundle period. Overall, during the three years post-intervention, the relative risk reduction, absolute risk reduction and relative risk were as follows: 0.9 (95% confidence interval: 0.58-0.98), 0.26 (95% confidence interval: 0.1-0.33) and 0.09 (95% confidence interval: 0.014-0.4), respectively. Moreover, the risk of MRSA infections among colonized patients, already reduced in 2011 (Relative Risk 0.18, 95% confidence interval: 0.008-1.1) and 2012 (Relative Risk 0.12, 95% confidence interval: 0.006-0.8), compared with the pre-bundle period, dropped

dramatically in 2013 (Relative Risk 0.000, 95% confidence interval: 0.000-0.6), with no case of MRSA infection reported.

Conclusion: The present study showed that a strategy of active surveillance based on rapid molecular screening for MRSA, immediately after admission, rapid reporting and prompt nasal decolonization, resulted in a significant decline in MRSA infections rate in our ICU over the three years post-bundle period. Real time PCR demonstrated a superior sensitivity to culture and rapid TATs, allowing a better management of MRSA-carriers who will more likely develop MRSA infections.

Wednesday, July 30, 2014

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

Lipids/Lipoproteins

B-087

A study of the difference in the Request of laboratory lipid metabolism tests in Primary Care in Spain

C. Hernando de Larramendi[1], M. Lopez-Garrigos[2], L. Maiz[3], L. Rabadan[4], M. D. Calvo[5], A. Rodriguez-Rodriguez[6], C. Gallego[7], M. Herranz-Puebla[8], V. Poncela[9], M. J. Baz[10], M. J. Martinez-Llopis[11], T. Avello[12], I. Llovet[13], C. Lorenzo[14], M. Lopez-Hoyos[15], M. J. Zaro[16], L. Lopez-Yepes[17], M. Salinas[2]. *[1]Hospital Universitario Severo Ochoa, Leganes, Spain, [2]Hospital Universitario San Juan, San Juan de Alicante, Spain, [3]Hospital Universitario Lucus Augusti, HULA, Lugo, Spain, [4]Complejo Asistencial de Soria, Soria, Spain, [5]Hsopital Clinico de Valladolid, Valladolid, Spain, [6]Complejo Asistencial de Palencia, Palencia, Spain, [7]Hospital Rafael Mendez, Lorca, Spain, [8]Hospital Universitario de Getafe, Getafe, Spain, [9]Hospital Universitario de Burgos, Burgos, Spain, [10]Hospital de Llerena, Badajoz, Spain, [11]Hospital de Denia, Denia, Spain, [12]Hospital San Agustín, Aviles, Spain, [13]Hospital Verge de la Cinta, Tortosa, Spain, [14]Hospital Santa Barbara, Puertollano, Spain, [15]Hospital Universitario Marques de Valdecillas, Santander, Spain, [16]Hospital Don Benito, Villanueva, Spain, [17]Hospital Virgen del Castillo, Yecla, Spain*

BACKGROUND: To compare the inter-practice variability in lipid metabolism laboratory tests requested by General Practitioners (GPs) in Spain, according geographic and hospital characteristics, using appropriateness indicators, to try to ascertain the degree in requesting appropriateness.

METHODS: We obtained the number of serum cholesterol (Chol), HDL-cholesterol (HDL-chol) and tryglicerides (Tryg) requested by GPs for the year 2012 from 76 laboratories at different hospitals from diverse regions across Spain. Every patient seen in any primary care center (PCC) of any of these 76 health departments, regardless of the reason for consultation, gender or age, was included in the study.

Two types of appropriateness indicators were calculated: every test requests per 1000 inhabitants and ratio of related tests requests (HDL-chol/Chol, Tryg/Chol). The indicators results obtained in different location and for type of management were compared.

RESULTS: In total GPs requested 16013622 laboratory lipid metabolism tests in year 2012 in a Spanish population (17679195 inhabitants) that is almost half of the whole country population. Chol, HDL-chol and Tryg per 1000 inhabitants indicators results ranged from 106.3 to 550.7; 20.4 to 417.5 and from 94.0 to 439.2 respectively. The variability of HDL-chol/Chol, Tryg/Chol indicators results was also considerable, and ranged from 0.19 to 1.00 and from 0.54 to 1.00 respectively.

There were significant differences according to hospital setting in tests requests per 1000 inhabitants. In rural location Chol, HDL-chol and Tryg were higher. However, no significant differences according to hospital setting in related tests requests indicator results were detected.

In relation to institution management, no significant differences were obtained.

DISCUSSION: The high variability observed is difficult to explain by differences in patient case mix between regions.

CONCLUSION: There is a need to design and establish strategies from laboratory in consensus with requesting clinicians to improve lipid metabolism tests appropriate use and hence clinical decision making.

B-089

Lipoprotein A Levels in Individuals with Type 2 Diabetes Mellitus Attending Tribhuvan University Teaching Hospital ,Nepal

L. Shrestha. *IOM, Nepal, kathmandu, Nepal*

Background: Type-2 diabetes mellitus is a common disease, affecting a large proportion of individuals worldwide. It is also recognized as an independent risk factor for cardiovascular diseases. Various markers for assessing risk for cardiovascular diseases are used in clinical laboratory. One of the emerging marker in this regard is lipoprotein a, elevated level of which is regarded to be associated with increased risk of atherosclerosis and thrombotic diseases. This study intends to assess the level of lipoprotein a in diabetic patients.

Methods: The study included 204 patients with type 2 diabetes mellitus and 204 age and sex matched controls. Lipoprotein a levels were measured and comparison was done between the lp(a) levels in diabetic patients and control.

Result: Mean serum Lp(a) levels in diabetes mellitus patients was 44.2±35.8 mg/dl, which was significantly higher when compared to control group (mean 21.1±11.2 mg/dl, $p < 0.05$).

Conclusion: The result of present study indicates that levels of Lp(a) are increased in patients with type 2 diabetes mellitus.

B-091

High-fat diet and lipid profile.

P. D. Perris, I. Fernandez, M. C. Sanahuja, M. C. Mambrin, N. H. Slobodianik, M. S. Feliu. *School of Pharmacy and Biochemistry, Buenos Aires, Argentina*

The importance of diet in maintaining health is widely accepted and recognized. Diet lipid profile is important to prevent chronic diseases and improve the quality of life of individuals. The objective is to analyze the effect of high-lipid diet from different sources, on triglycerides (TG), total cholesterol (TC), noHDL cholesterol and fatty acid profile in serum of growing rats. Weanling Wistar rats were fed during 10 days with 40% dietary fat provided: by butter (B group); by olive oil (O group) and by high oleic oil (AO group). Control group (C) received normocaloric diet according to AIN´93. Diets fatty acid profiles were determined by gas chromatography (GC); ω6/ω3 and unsaturated/saturated (PUFA/SFA) ratios of diets were calculated. Serum levels of TG and TC were determined by enzymatic-colorimetric method and fatty acid profile was determined by GC. The statistical analysis used Bartlett's test, followed by one-way analysis of variance (ANOVA) and Dunnett as post test (*p<0.01). RESULTS: diets: ω6/ω3 ratio: B=5,6/1; O=49,6/1; AO=86/1; C=9/1; PUFA/SFA, B=0.06; O=1,36; AO=0,72; C=3,89. Serum (mean±SD mg/dL) TG B=113.0±31.2*; O=77.6±12.1; AO=67.0±15.9 C=59,1±14,8; TC B=89.2±10.1*; O=73.1±7.3; AO=71.0±10.6 C=62.1±13.6 Fatty acids profile expressed as area%±SD were:

	B	O	AO	C
Palmitic	21,2±2,5	15.7±1,7	13.5±0.7	17.3±1,4
Oleic	19.1±5.2 *	22.0±5.1 *	33.0±4.8 *	10,6 ±2,0
Linoleic	8.9±1.8 *	11.8±2.8 *	8.9±1.0 *	19.0±3,5
α-Linolenic	0.4±0.1*	0.5±0.2*	0.3±0.1*	1.2±0.3
Araquidonic	6.36±1.45	8.15±1.97	9.48±1.73	8.59±2.15
EPA	0.93±0.82	0.67±0.30	0.88±0.19	0.83±0.44
DHA	1.22±0.30	0.74±0.20*	0.83±0.18*	1.25±0.24

TG and TC levels in B group were statistically higher compared to C. Experimental groups showed higher serum oleic acid levels with lower α-linolenic and linoleic acids levels compared to C. This fact would exacerbate the route of the ω9 family and decreases essential fatty acids. It seems that the sources of dietary lipids provoked changes in serum fatty acid profile levels, but not in response to the high fat percentage.

Supported by UBACyT 20020120200068

B-093

Comparison of a Direct Enzymatic Assay and Polyacrylamide Tube Gel Electrophoresis for Measurement of Small Dense Low-Density Lipoprotein Cholesterol

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

Background: Small-dense low density lipoprotein cholesterol (sdLDL-C) has been linked to the progression of cardiovascular disease. We compared two methods for determination of sdLDL-C: a direct enzymatic method (sdLDL-EX) and polyacrylamide tube gel electrophoresis (sdLDL-PGE). In addition, we evaluated the associations of these lipid measures with other atherosclerosis-related markers.

Methods: A total of 242 outpatients (age more than or equal to 19 years old) were recruited. All blood samples (excluding those with triglycerides over 400 mg/dL) were analyzed for lipid profile with sdLDL-PGE (Quantimetrix Lipoprint™, CA) and sdLDL-EX assay (Denka Seiken, Japan) an enzymatic-surfactant-based assay. We also evaluated the following atherosclerosis-related markers: apolipoprotein A-I (apoA-I), apoB, glucose, hemoglobin A1c (HbA1C), high-sensitivity C-reactive protein (hsCRP), creatinine, cystatin C, and vitamin D. The sdLDL-PGE method

separates the intermediate density lipoprotein (IDL) particles into three midbands (MID-A to C) and the LDL particles into seven subfractions (LDL-1 to 7); the sdLDL-PGE result is calculated as the sum of cholesterol concentrations from LDL3 to LDL7.

Results: The mean age of the patients (58 males and 184 females) was 54.5 years. The regression equation between the sdLDL-PGE (x) and sdLDL-EX assay (y) was $y_{mg/dL} = 0.748x + 26.14$, $r = 0.713$. The sdLDL-EX assay yielded higher measured sdLDL-C concentrations than the sdLDL-PGE assay (33.06±12.79 *vs*. 9.3±12.19 mg/dL, P <0.001); however, the absolute difference between two methods did not significantly correlate with the average sdLDL-C concentration ($R^2 = 0.005$, P = 0.290). sdLDL-C as measured with the sdLDL-EX assay exhibited significant positive correlations with VLDL, MIDC, MIDB, and LDL2 (all P <0.001), which have been suggested as atherogenic lipoproteins, but did not correlate with the less atherogenic lipoproteins MIDA (P = 0.891) and LDL1 (P = 0.604). The sdLDL-EX and sdLDL-PGE methods yielded similar patterns of correlation between sdLDL-C and atherosclerosis-related markers: positive correlations with TG, TC, LDL-C, apoB, glucose, and HbA1C but inverse correlations with HDL-C and vitamin D. However, there was no significant correlation between sdLDL-C and apoA-I, hsCRP, or creatinine levels with either method.

Conclusion: The direct enzymatic assay for sdLDL-C correlated well with the assay based on polyacrylamide gel electrophoresis. The enzymatic method appears to measure cholesterol in a boarder range of atherogenic lipoprotein particles than PGE, and thus contribute in directing specific interventions of cardiovascular prevention. Because the direct enzymatic assay can be automated, it can be used as a routine method to assess small dense LDL cholesterol.

B-094

Performance Evaluation of a New Ready-to-use Liquid Triglycerides Assay on the High-Throughput ADVIA Chemistry Systems

P. Datta[1], J. Dai[2]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Siemens Healthcare Diagnostics, Glasgow, DE*

Background: Measurement of serum triglycerides is an important component to determine lipid status of a patient. Increased serum triglycerides are risk factors for cardio vascular disease. Serum triglycerides are also used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders. The automated ADVIA® Clinical Chemistry Systems currently have a Siemens serum triglycerides assay that requires manual mixing of two reagent components before use on the system. An improved assay, Triglycerides_2 (TRIG_2), using ready-to-use liquid reagents, is under development. Furthermore a new concentrated reagent (TRIG_c) was developed to be automatically diluted on-system to provide larger number of tests per kit for high-volume users. The objective of this study was to evaluate the performance of both new assays on the ADVIA Chemistry Systems.

Methods: In the ADVIA Chemistry TRIG_2 and TRIG_c assays, sample is diluted and reacted with a single reagent for 5 minutes. The lipase in the reagent hydrolyzes triglycerides into glycerol, which is then converted into glycerol-3-phosphate by glycerol kinase, the latter then being oxidized to H_2O_2 by glycerol oxidase. The H_2O_2 is colorimetrically (at 505 nm) detected by a Trinder's reaction. The triglyceride concentration in a sample is determined from a linear calibration curve using Siemens ADVIA Chemistry Calibrator. The performance evaluation in this study included precision, interference, linearity, and correlation with a commercially available triglyceride (TGL) assay run on the Dimension® XPAND system. Data were collected for all ADVIA Chemistry Systems (ADVIA 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400), which use the same ADVIA Chemistry TRIG_2 or TRIG_c reagent packs, calibrators, and commercial controls.

Results: The imprecision (total %CV) of the new ADVIA Chemistry assays with two-level commercial controls and two serum pools ranging from ~90 to ~500 mg/dL (n = 80) on all ADVIA Chemistry Systems (1200/1650/1800/2400) was ≤2.1% (for both TRIG_2 and TRIG_c). The analytical ranges of the new assays are from 10 - 550 mg/dL (extendable to 1100 mg/dL by auto-dilution). The assays correlated well with the Dimension TGL assay: TRIG_2 = 0.94 [TGL] + 4.4 and TRIG_c = 0.93 [TGL] + 4.3 (r = 0.99, n = 101; sample range: 20-540 mg/dL for both). The new assays demonstrated no interference at a triglycerides level of ~150 mg/dL with unconjugated or conjugated bilirubin (up to 15 mg/dL), hemoglobin (up to 500 mg/dL), and ascorbic acid (up to 3 mg/dL). Minimum on-system stability for both was 60 days (with reagent blanking every 14 days).

Conclusion: The data demonstrates good performance of the TRIG_2 and TRIG_c assays on the high-throughput ADVIA Chemistry Systems from Siemens Healthcare Diagnostics.* Under development. Not available for sale in the USA.

B-095

Comparison of equations for the calculation of LDL-C in hospitalized patients

T. S. Pillay[1], J. Martins[1], L. M. Murray[1], S. A. S. Olorunju[2]. *[1]University of Pretoria/NHLS, Pretoria, South Africa, [2]Medical Research Council, Pretoria, South Africa*

Background: Prediction of cardiovascular disease (CVD) mortality is dependent on the calculation of low density lipoprotein-cholesterol (LDL-C). The Friedewald equation is the most widely used formula to calculate LDL-C but is less accurate in patients with comorbidities and extreme lipid values. Several novel formulae have been reported to outperform the Friedewald formula over a wide-range of lipid levels.
Methods: This study was a retrospective evaluation of lipid profiles in 14219 patients in South Africa, from 1 January 2013 to 30 June 2013. We evaluated four formulae (Friedewald, Chen, de Cardova, Hattori) and compared these to our direct measurement of LDL-C, (total cholesterol) TC, (HDL-cholesterol) HDL-C using Beckman reagents and instruments (Beckman Coulter). Linear regression and ROC analysis were performed.

Results: Average age of the population was 52 years (39% male, 61% female, mean LDL-C 2.9 mmol/l±1.15 SD). Directly measured LDL-C highly correlated with non-HDL (r=0.93; 95% CI 0.926-0.933). The de Cardova formula showed a high correlation with directly measured LDL-C (r=0.90 p<0.001), comparable to Friedewald calculated values for directly measured LDL-C (r=0.95 p<0.001). The de Cardova formula was favorable in some ranges of HDL, TC and the lowest TG range (r=0.97 p<0.001) but performed least well compared with three other LDL calculations (AUC=0.8331). The Chen formula performed better than Friedewald (AUC=0.9049). The Hattori formula outperformed all formulae including Friedewald over various ranges of lipid values (AUC=0.9097, Figure 1).
Conclusions: We confirm that the de Cardova formula could replace directly measured LDL-C if validated in the laboratory. In contrast to several recent findings, we show favorable correlations of this formula with Friedewald at extreme TG values. However, the Hattori formula appears to be the best for application in hospitalized patients, even at extreme lipid value **Figure 1 ROC analysis of Friedewald, Chen, de Cardova(nwLDL-C), Hattori calculations**

B-097

Comparison of Lipoprotein (a) methods using two commercially available immunoturbidimetric methods on an automated chemistry analyzer.

E. L. Ryan, J. C. Ritchie. *Emory Univerity School of Medicine, Atlanta, GA*

Background: Lipoprotein(a) is an LDL-like particle that can vary in size and components based upon the variable number of kringle IV repeats present in apolipoprotein(a). Plasma Lipoprotein (a) levels are static in an individual and can help with assessing an individual's risk of developing atherosclerotic plaques and coronary artery disease. Previously we ran the Diasorin SPQ II immunoturbidimetric method on a Roche Cobas Fara.

Objective: To compare two commercially available immunoturbidimetric user-defined methods for measuring Lipoprotein (a) on an automated chemistry analyzer to the method used by our reference laboratory.

Methods: Method#1 (Kayama Biomedical Company K-Assay Lp(a) immunoturbidimetric assay) and Method #2 (Pointe Scientific, Inc. Lp(a) immunoturbidimetric assay) were evaluated sequentially on the same Beckman UniCel DxC 800 Synchron chemistry analyzer. Each method was performed per manufacturer's instructions. The methods were evaluated for accuracy, precision, linearity and patient sample comparisons were performed. The medical decision point of 30mg/dL was used to assess patient sample results between the assays. We used 37 frozen Lithium Heparin plasma samples ranging from below the reportable range for either assay up to above the reportable range for the current method.

Results: Both assays were linear from across the reportable range with the Method#2 showing a negative bias at values above 90mg/dL (ranging from 2.4-9 mg/dL). Accuracy was within the allowable error limit of 10mg/dL for both methods. Assay variation was assessed using the same material and similar between methods, %CV were 2-5% for Method#1, and 3-12% for Method #2. Recovery of QC material was lower in Method#2 than in Method#1. The patient samples results were similar with a mean biases of 4.97mg/dL for Method #1 and -7.02mg/dL for Method #2. In method #1 two samples had a bias > 10mg/dL, both were above 60mg/dL. Method #2 had seven samples with a bias> 10mg/dL, four between 30 and 60 mg/dL and three above 60 mg/dL.

Conclusion: Both assays were comparable to the reference method. Method #1 yielded results for the QC material and samples more similar to historical values.

B-098

Lipid hydroperoxides in apolipoprotein E-containing high-density lipoprotein

S. Mamiya, A. Yoshimoto, M. Sato, R. Ohkawa, M. Tozuka. *Tokyo Medical and Dental University Analytical Laboratory Chemistry, Graduate School of Health Care Sciences, Tokyo, Japan*

Background: Higher levels of high-density lipoprotein (HDL)-cholesterol have been associated with lower risk of coronary heart disease. It is noteworthy that the low level of HDL-cholesterol remains predictive risk of cardiovascular disease (CVD) even when low-density lipoprotein (LDL)-cholesterol concentration has been kept to low level by the treatment. Some cholesteryl ester transfer protein (CETP) inhibitors are currently undergoing clinical evaluation. In fact, CETP inhibitors increase HDL-cholesterol levels; however, the mortality rate of CVD has not largely changed. It indicates that the HDL levels do not necessarily reflect its functions. So it is important to estimate the quality of HDL together with its quantity. It is well known that CETP inhibitors raise apolipoprotein (apo) E-containing HDL, a minor subpopulation of HDL. Therefore we investigated the qualitative evaluation of apoE-containing HDL, especially antioxidant ability estimated by containing lipid hydroperoxides (LOOH) level.

Methods: HDL (1.063<d<1.210 g/mL), isolated from serum obtained from 10 healthy volunteers by ultracentrifugation, was separated into apoE-containing and apoE-deficient HDL by Heparin-Sepharose choromatography. The compositions of HDL were determined by Lowry's Method (protein) and enzymatic test kits (cholesterol, phospholipids, and triglyceride). Before and after the oxidation by $CuSO_4$, the concentration of LOOH was measured by the ferrous oxidation in Xylenol Orange (FOX) assay using triphenylphosphine to get higher specificity for LOOH. Non-denaturing gel electrophoresis was performed for analyzing distributions of apoE and particle sizes. Surface charge was characterized by lipoprotein electrophoresis using agarose-gel.

Results: The particle size of apoE-containing HDL, developed by CBB-R250 staining and immunoblotting using anti-apoE antibody, was larger than apoE-deficient HDL. The relative electrophoretic mobilities were obviously small in apoE-containing HDL on agarose gel electrophoresis pattern. Concentrations of LOOH in apoE-containing HDL and apoE-deficient HDL were 22.3 and 1.2 nmol/mg protein, respectively. After the oxidation by $CuSO_4$, LOOH levels were increased to 153.8 and 294.0 nmol/mg protein in apoE-containing HDL and apoE-deficient HDL, respectively.

Conclusion: ApoE-containing HDL which is known to increase by CETP inhibitors is different in the particle size and surface charge from apoE-deficient HDL. ApoE-containing HDL has extremely higher level of LOOH than apoE-deficient HDL. This suggests that apoE-containing HDL might protect LDL from early and weak oxidation due to its higher susceptibility to oxidation or its rapid ability to accept LOOH from oxidized LDL. However, the total capacity of antioxidant ability per unit protein mass could be smaller than apoE-deficient HDL, suggesting that the increase of apoE-containing HDL did not simply reflect the increase of antioxidant ability.

B-099

LDL Subfractions Analysis in Pro-atherogenic Dyslipidemia

N. Muniz, E. Nunez. *Quantimetrix Corp., Redondo Beach, CA*

Background: Early recognition of pro atherogenic risk factors is important for prevention and treatment of coronary artery disease (CAD). The NCEP ATP III guidelines identified LDL cholesterol (LDL-C) as the primary target for CAD therapy and risk assessment. New ACC/AHA guidelines replaced traditional lipid risk factors with a 10 year ASCVD risk calculator weighing heavily on non-lipid risk factors, ignoring a large body of evidence clearly recognizing specific dyslipidemic profiles with increased CAD risk. Numerous studies clearly demonstrate that small dense LDL, VLDL remnants and IDL are independently atherogenic while large buoyant LDL, HDL and possibly large VLDL may not be. Exclusion of such evidence could result in patient misclassification possibly leading to under or overtreatment of individuals. In this study, pro atherogenic lipoprotein subfractions were measured using the Quantimetrix Lipoprint LDL system, *(Quantimetrix Corporation, Redondo Beach, CA)*. The test yields critical information for early detection of individuals at risk or with existing CAD and allowing for a more individualized implementation of treatment.

Objective: Demonstrate the benefit of measuring the atherogenic LDL subfractions with the comprehensive analysis on the Lipoprint LDL system and assist clinicians in identifying, stratifying and customizing treatment for those at risk.

Methods: Lipid profiles for a total of 273 recruited subjects were determined by testing their total cholesterol, triglycerides, LDL-C and HDL-C using standard clinical methods. Subjects were segregated into two groups, "normolipedemic" and " dyslipidemic" based on ATP III desirable lipids status. Cholesterol levels in the lipoproteins subfractions, large VLDL, Mid-C (VLDL remnants), Mid-B (large IDL), Mid-A (small IDL), LDL-1 and LDL-2 (large buoyant LDL), LDL-3 to LDL-7 (small dense LDL) and HDL were also measured in both groups using the Quantimetrix Lipoprint LDL system, a linear polyacrylamide gel electrophoresis method. Results from the traditional lipid profile were compared to the lipoprotein subfraction profiles obtained by Lipoprint.

Results: The lipid test results, mean and range, for the 273 study subjects were: total cholesterol 196 (104 - 319) mg/dL, triglycerides 96 (25 - 345) mg/dL, LDL-C 117 (58 - 215) mg/dL and HDL-C 55 (26 - 137) mg/dL. Out of the 273 total subjects, 133 (49%) were classified normolipidemic according to the ATP III lipid guidelines while140 (51%) had at least one parameter outside the recommendations. LDL subfractions analysis by the Lipoprint system revealed that 17 (13%) out of the 133 previously classified "normal" subjects had cholesterol levels outside the 95 % confidence interval range for a given LDL subfraction. Of the 141 "dyslipedemic subjects," 69 (49%) had a normal LDL subfraction distribution. Lower levels of large buoyant LDL-1 were observed in many of the dyslipidemic subjects.

Conclusions: Clinical studies identify small dense LDL, VLDL remnants and IDL subfractions independently associated with increased CAD risk above other lipid factors. Measurement of these highly atherogenic lipoprotein subfractions as demonstrated by the Lipoprint system could be a better predictor of CAD risk than measurement of other traditional lipid risk factors.

B-100

Cell Surface Re-Engineering by Lipid Anchoring Approach

P. Vabbilisetty, X. Sun. *Cleveland State University, Cleveland, OH*

Background: Many of the biological processes such as cell-cell adhesions, extracellular/ intracellular communications occurring on the cell surface are governed and guided by the cell surface receptors. Cell surface is a platform for introduction of various biomolecules such as proteins, carbohydrates, etc., that may further improve the potentiality of the cells. Lipidation of cell membranes is one such approach which plays an important role in many biological applications such as drug/gene delivery and serves as biomimicking models. Introduction of chemoselective functional groups via bio-orthogonal copper-free click chemistry at the cell surface further facilitates for many cellular modifications and enables for rapid and efficient cell surface labeling. Lipids can be introduced on to the cell membrane in different forms such as liposomes, micelles for efficient delivery of drug/gene or other bioactive molecules of interest.

Objective: To study and evaluate the potential anchoring effects of phospholipid (DSPE-PEG2000-DBCO) and cholesterol (CHOL-PEG2000-DBCO) based lipids on cell surface for cell surface re-engineering.

Methods: To investigate the lipid anchor incorporation effects on cell membranes, different concentrations of biotin conjugated anchor lipids were prepared by reacting N3-Biotin with anchor lipids namely DSPE-PEG2000-DBCO and CHOL-PEG2000 -DBCO via Copper free click chemistry for 1hr at RT, PBS buffer pH 7.4. The obtained conjugated anchor lipids at varying concentrations were incubated with raw 267.4 cells for different incubation times ranging from 5- 20 mins at 37°C. The lipid conjugated cells were further labeled with Streptavidin-FITC for 5 mins and the effects were examined using Confocal microscopy and Flow cytometry.

Results: Confocal microscopy and flow cytometry data suggests that 5 mins of incubation of the anchor lipid (CHOL-PEG2000 -DBCO, 5 μM) with cells is enough to see its incorporation into the cells; however a higher fluorescent intensity signal was observed at 20 mins indicating more lipid incorporation. The confocal microscopy data clearly depicts the intact incorporation of CHOL-PEG2000 -DBCO -biotin conjugate into the cell membrane without any internalization. In comparison at 20 mins, for (DSPE- PEG2000 -DBCO, 5 μM) there was decreased fluorescent signal and seen from both the cell membrane and cytoplasm indicating internalization of this conjugated lipid. This data shows the different effects of phospholipid and cholesterol based anchor lipids on cell membrane and thus can be used for introduction or transport of various drug/gene/carbohydrates/biomolecules for cell surface re-engineering purposes.

Conclusion: CHOL-PEG2000-DBCO was shown to rapidly incorporate into the cell membrane within 5 mins (20 mins being the optimum incubation time) and it was visibly evident that there is no internalization of the lipid into the cytoplasm,

unlike the DSPE-PEG2000-DBCO. Moreover the fluorescent signal intensity for DSPE based anchor lipid was very weak when compared to CHOL anchor lipid. This comparative study of lipid anchoring via copper free Click Chemistry suggests that they can serve for potential in vivo cell surface re-engineering applications.

B-101

CORRELATION BETWEEN GLYCATED HEMOGLOBIN AND SERUM LIPIDS IN TYPE 2 DIABETICS IN EASTERN LIBYA.

N. Naseb[1], A. R. Said[2], S. Shakila[2], S. D. Kolla[3], L. T. Peela[4], S. J. Dhoipode[2], J. R. Peela[2]. *[1]Department of Biochemistry, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [2]Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [3]Department of Biochemistry,Rangaraya Medical College,, Kakinada, India, [4]Great Eastern Medical School, Srikakulam, India*

Background: Diabetes mellitus with its accompanying vascular complications is on a rise globally and a similar trend has been observed in Libya too. Early detection, effective monitoring and timely management is important to control this growing problem. Various studies have shown dyslipidemia as a significant risk factor of atherosclerosis leading to vascular complications in diabetes mellitus. HbA1c as a marker of long term glycemic control in diabetics is an established fact. The present study is aimed at correlating HbA1c with lipid parameters in blood to understand its role as a marker of dyslipidemia.

Materials and methods: Sixty subjects in the age group ranging from 40 to 70 years have been recruited from Seventeenth February Teaching Hospital, Al- Baida for the study, twenty controls with no history of diabetes, twenty recently diagnosed diabetics under treatment and twenty old cases of diabetes mellitus who have suffered an episode of coronary artery disease (CAD) or cerebrovascular accident (CVA). Venous samples were drawn after an overnight fast for glucose, glycated hemoglobin, total cholesterol, triacylglycerol and HDL cholesterol and these tests were performed using authenticated kits and Cobas integra 400 analyzer. LDL cholesterol was calculated using Friedwald's formula.

Results: Slightly high levels of fasting blood glucose ($p=0.03$), Glycated hemoglobin ($p=0.02$), high levels of triglycerides ($p<0.001$) and low levels of HDL cholesterol ($p<0.001$) were observed in diabetics under treatment when compared with controls, but total cholesterol and LDL cholesterol showed no difference. However, diabetics with complications showed higher levels of fasting blood glucose ($p<0.0001$), glycated hemoglobin ($p<0.0001$), total cholesterol ($p<0.001$), triglycerides ($p<0.001$), low density lipoprotein cholesterol ($p<0.001$), and low HDL cholesterol ($p<0.0001$). There is positive correlation between glycated hemoglobin and serum triglycerides and inverse correlation with HDL cholesterol in both the diabetic groups, though not strong in recently diagnosed diabetic patients under treatment. A strong correlation was observed between glycated hemoglobin and total cholesterol and LDL cholesterol only in the diabetic group with coronary artery and cerebrovascular complications.

Conclusion: The present study has shown a significant correlation between glycated hemoglobin and serum total cholesterol and LDL cholesterol; the dyslipidemia risk factors causing atherosclerosis in diabetic mellitus not under control. Hence measurement of glycated hemoglobin is significant as a dual marker not only to monitor long term glycemic control but also in predicting dyslipidemia in type 2 diabetes mellitus.

B-102

CARDIOVASCULAR RİSK FACTORS İN PATIENTS WITH HEMODIALYSIS:PARAOXONASE AND HYPERHOMOCYSTEINEMIA

M. DEMIR[1], K. KÖSE[2], C. YAZICI[2], A. ÖZŞAHİN[2], B. TOKGÖZ[2], C. UTAŞ[2]. *[1]MEDİCALPARK HOSPİTAL, ANTALYA, Turkey, [2]ERCİYES ÜNİVERSİTY MEDİCAL FAKULTY, KAYSERİ, Turkey*

Increased risk of cardiovascular disease (CVD) has been recognized as an important cause of morbidity and mortality in chronic renal failure (CRF). Hyperhomocysteinemia has also been accepted as an independent risk factor for CVD. Paraoxonase (PON1) is an enzyme with antioxidant activity, which circulates in plasma attached to HDL.

The aim of this study was to investigate the risk of CVD in CRF by depending on lipid/lipoprotein profiles, homocysteine and PON1 activity in plasma of hemodialyzed patients.

MATERIAL and METHODS

Subjects: Total of 42 patients undergoing hemodialysis (HD), and 43 healthy volunteers were included the study. The clinical data of patients and the controls are summarized in Table 1.

Table 1:Clinical features of the study groups

	Control	Hemodialysis
Number of participants	43	42
Sex (M/F)	21/22	21/21
Age (years)	24-61	16-64
Mean age(months)	34,7±10,7	36,9±13,3
Dialysis duration (months)	-	8-108
Mean dialysis duration (months)	-	36,6±22,2

Methods: Fasting blood samples obtained from study groups were drawn into anticoagulant-free tubes and centrifuged at 2,000 g for 10 min. Serum samples were used for the measurements of triglyceride (TG), total cholesterol (TC), LDL-C, HDL-C, and homocysteine levels,arylesterase activity and also paraoxonase activity.
RESULTS

There was no significant difference in age and sex distribution between the study groups.When compared to controls increased plasma TG but decreased TC,HDL-c and LDL-C levels were observed HD patients, even though all lipid/lipoprotein levels were in normal laboratory range.Significantly decreased PON1 and arylesterase activities, but increased homocysteine values were found in HD patients than those of control

DISCUSSION Patients with CRF, especially HD patients, experience excessively high cardiovascular morbidity and mortality.increased TG, but decreased TC, HDL-C and LDL-C levels , and also decreased PON1 and arylesterase activities observed in HD patients in the present study were in agreement with many previous reports. That increased homocysteine levels in the present study have supported the previous studies , and homocysteine may be suggested as an independent risk factor for CVD in HD patients.İn conclusion, as reflected by decreased PON1 and increased homocysteine levels, CVD risk might be increased in HD pat ents.

B-103

The usefulness of non-HDL cholesterol in less resourced countries

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

Background: Common characteristic features of diabetic dyslipidemia are the elevation of plasma triglycerides and triglyceride-rich VLDL cholesterol, reduced HDL cholesterol, and an increased number of small dense LDL cholesterol particles (1). Although LDL cholesterol is not typically elevated in patients with diabetes, the changes in LDL cholesterol composition that can accompany the disease make the LDL cholesterol exceptionally atherogenic (2,3).

Aim: This study primarily aims to determine and compare the power and influence of non-HDL cholesterol and LDL cholesterol, in predicting coronary heart disease among diabetic versus non-diabetic adult Ghanaians.

Methods: A cross-sectional study was performed on 302 subjects who consisted of 154 previously diagnosed diabetes patients and 148 non- diabetics. BMI, WC, blood pressure (BP), total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), fasting glucose, high-sensitivity C-reactive protein (hs-CRP), adiponectin and resistin were measured.

Results: The mean age was 52.2 (±8.7). There was a higher (negative) correlation between adiponectin and non-HDL cholesterol ($r=-0.5756$; $p<0.0001$) than adiponectin and LDL cholesterol ($r=-0.5152$; $p<0.0001$). Higher positive correlation was observed with resistin and non-HDL cholesterol ($r=0.5756$; $p<0.0001$) than resistin and LDL cholesterol ($r=0.5494$; $p<0.0001$). Similar patterns of correlation were observed with hs-CRP, blood pressure and 10 year cardiovascular disease risk.

Conclusion: This study contributes to the existing body of literature by suggesting that easily calculated non-HDL cholesterol is superior to LDL cholesterol in cardiovascular disease risk assessment. The use of non-HDL would also be more practical and reliable target for lipid lowering therapy in less resourced country like Ghana

References: 1. Sniderman AD, Scantlebury T, Cianflone K: Hypertriglyceridemic hyperapoB: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med* 135:447-459, 2001

2. Turner RC, Millns H, Neil HAW, Stratton IM, Manley SE, Matthews DR, Holman RR, for the United Kingdom Prospective Diabetes Study Group: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus (UKPDS 23). *BMJ* 316:823-828, 1998

3. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, Robbins DC, Howard BV: Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the Strong Heart Study. *Diabetes Care* 26:16-23, 2003

B-104

Comparison of the Vantera NMR Analyzer Tests for Lipid and Lipoproteins to Conventional Enzymatic Assays on the Siemens Dimension Vista Chemistry Analyzer

M. L. Sampson, V. Gounden, R. Shamburek, A. T. Remaley. *National Institutes of Health, Bethesda, MD*

Objective: Traditional lipid cardiovascular biomarkers are largely based on the cholesterol content of the major lipoprotein fractions. Lipoproteins, however, have a polydisperse size distribution and their association with cardiovascular disease has been shown to vary not only based on their cholesterol content but also on their size and particle count. The Vantera analyzer uses NMR technology to quantify not only lipid concentrations but also size and particle counts of lipoproteins. In this study, we evaluated several lipid and lipoprotein assays on the Vantera and compared the results to conventional enzymatic assays for lipids and lipoproteins, as measured on the Siemens Vista. **Methods:** The Vantera has 3 FDA approved tests: Triglycerides (TG), HDLc and LDL particle number (LDLp). We measured these tests, as well as several other lipid and lipoprotein test parameters, in 450 patients with a wide variety of lipid disorders by the Vantera and Vista. LDLc was calculated by the Friedewald equation, using total cholesterol (TC) from either the Vantera or the Vista as indicated. **Results:** The results from the two assay systems show good correspondence (refer to Table below). Similar calculated LDLc results were also obtained, using either Vantera TG and HDLc plus Vista TC (LDLc1) or all Vantera parameters, including TC (LDLc2), when compared to LDLc calculated with Vista parameters. In addition, we compared the measured LDLc on Vantera (mLDLc) with the calculated LDLc from Vista.

	TC	TG	HDLc	LDLc1	LDLc2	mLDLc
Deming Slope	0.96	0.89	0.88	1.13	1.09	1.17
Intercept	2.71	1.69	6.42	-0.23	3.30	3.59
R^2	0.93	0.92	0.93	0.93	0.85	0.87

We also validated the lipoprotein particle count and size measurements for the major lipoprotein classes by showing that the calculated lipoprotein core volumes matched the measured core lipids, namely cholesteryl esters and TG ($R^2 = 0.91$). **Conclusions:** The Vantera NMR analyzer generates comparable lipid and lipoprotein results to traditional methods, while at the same time yielding additional cardiovascular risk information related to lipoprotein particle count and size.

B-105

Establishment of Reference Range for HDL Subfractions in Japanese Population with a New Automated Assay

Y. Ito, N. Satoh, T. Ishii, J. Kumakura. *Denka Seiken Co.,Ltd., Tokyo, Japan*

Background: Measurement of high-density lipoprotein (HDL) 2 and HDL3 subfractions might be more useful for evaluating coronary risk than total HDL-cholesterol (C). However, methods of measuring HDL2 and HDL3 are quite laborious for general clinical use and thus so far it has been difficult to establish reference ranges. Recently, we have succeeded in establishing a fully automated homogeneous assay for HDL3-C. We carried out a study to establish a reference range for HDL subfractions in Japanese population using our novel homogeneous assay for HDL subfractions.

Methods: HDL-C and HDL3-C were measured by our homogeneous assays on a Hitachi 917 automated clinical chemistry analyzer (Hitachi). HDL2-C was calculated as the difference between total HDL-C and HDL3-C. Subjects were recruited in Japan. 670 volunteers who did not have a history of CAD/CHD were invited to participate. Subjects were partitioned according to the following four parameters: male, female, young (male: equal or younger than 45 y, female: equal or younger than 55 y) and old (male: older than 45 y, female: older than 55 y). Data from the stratified subjects were analyzed for their distribution characteristics with the Shapiro-Wilk test, and estimated values were computerized. Based on distribution characteristics and independency, an appropriate test was chosen to compare the distribution of the variables among sub-groups.

Results: The Shapiro-Wilk test revealed that all sub-groups were nonparametric. Therefore, the Wilcoxon rank-sum test was used to compare the distribution of variables among sub-groups for HDL3-C, HDL2-C, total HDL-C, and HDL2-C/HDL3-C ratio. Females had significantly higher HDL3-C ($p < 0.01$), HDL2-C ($p < 0.001$), total HDL-C ($p < 0.001$), and HDL2-C/HDL3-C ratios ($p< 0.001$) compared to males. Regarding age, HDL2-C ($p = 0.72$) and total HDL-C ($p = 0.62$) did not show any significant differences, whereas older group showed higher HDL3-C ($p < 0.001$) and lower HDL2-C/HDL3-C ratios ($p < 0.05$) than younger group. Consequently, only sex difference was found in HDL2-C, whereas both sex and age differences were identified in HDL3-C.

Conclusion: Our analysis suggests that HDL subclasses can identify the CHD risk more accurately than total HDL-C.

B-106

Revising the Lipid Profile (LP): Evaluation of the Apo-A1 and Apo-B Assays on the Vitros-5600 [V-5600] Analyzer

R. Pillappa[1], C. Hoang[2], D. Moore[2], E. S. Pearlman[2]. *[1]University of Tennessee Health Sciences Center, Memphis, TN, [2]Veterans Affairs Medical Center, Memphis, TN*

Backgroundt: In view of recent literature we are considering revision of the VAMC LP to consist only of apo-B, apo A1 and a calculated ratio, The initial step was to assess the properties of the apo-A1 and apo-B assays on our V-5600 analyzers (OCD: Parsippany, NJ).

Methods: On day 1 apo-A1 and apo-B were assayed once on each of 3 V-5600 analyzers in 36 specimens on which an LP had been requested. On the same day aliquots were sent to a referral laboratory [RL] (ARUP; Salt Lake City, UT) for assay of apo-A1 and apo-B using immuno-nephelometry and after 24 hour refrigerated storage the apo-A1 and apo-B assays were re-run on one of our V-5600 analyzers. All in-house assays were done according to manufacturer's instructions.

Results: The TC and TG concentration ranges were [99, 298 mg/dL] and [56, 746 mg/dL.] respectively. Data suggested that median within machine, between day CVs for apo-A1 (1%) and apo-B (0.65%) were significantly less than between machine, within day CVs [2.17 and 1.36%; p<.002 for both analytes, Mann-Whitney test]. Between-machine within day CVs were an increasing function of concentration for apo-A1 and fit by the equation: **CV (%) = 0.78 + 0.000089 [apo-A1]^2** while for apo-B the corresponding CV has a minimum at a concentration of 100 mg/dL. Assuming RL apo-A1 and apo-B assays to be done the following day we compared the second day V-5600 assays (Y) to the RL assay (X) using Deming regression with results: **Y = 24.42** (14, 34.8) **+ 0.716** (0.64, 0.79) **X** and **Y = 7.38** (1.78, 13) **+ 0.994** (0.935, 1.05) **X for apo-A1 and apo-B respectively.** With respect to classification into accepted risk categories [<80, 80-119 and 120 mg/dL.] the V-5600 and RL apo-B assays place 32/36 (89%) patients into the same risk category and the remaining 4 are one category apart.

Conclusions: The current data indicates acceptable precision parameters for the V-5600 apo-A1 and apo-B assays. Significant proportional and constant bias in the V-5600 apo-A1 assay relative to the RL assay indicates that results for this assay remain method dependent. This appears less problematic for the apo-B assay where only constant bias is noted. V-5600 and RL Apo-B assays are in satisfactory agreement with respect to risk classification..

B-108

Determination of reference intervals for LDL and HDL cholesterol subclasses and their clinical relevance in acute coronary syndrome

H. Ookura, H. Takahashi, R. Suzuki, Y. Kadosaka, M. Yoshika. *Kansai Medical University, Hirakata, Osaka, Japan*

Background: To evaluate the performance of a newly developed assay system in quantifying small-dense LDL cholesterol (sdLDL-C) and HDL3 cholesterol (HDL3-C), we collected blood from 2041 control subjects during an annual health check-up conducted at the Kansai Medical University.

Methods: Exclusion criteria included hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg), a high body mass index (BMI ≥ 30 kg/m2), or a decrease in the estimated glomerular filtration rate (≤60 ml/min), HbA1c (≥6.6%), LDL (40-160 mg/dL), AST (≥ 60 U/L), ALT (≥ 70 U/L), or triglyceride (TG) (>250 mg/dL) levels. As a result, samples from 1412 subjects (average age 34 ± 10 years; 293 men and 1119 women) were used for further analysis. Twenty samples collected from acute coronary syndrome (ACS) patients during their first visit to our emergency department were used to examine the clinical utility of the novel assay.

LDL-C, HDL-C, sdLDL-C, and HDL3-C were simultaneously measured using a homogenous assay system (Denka-Seiken Co., Ltd.). Large buoyant (lb) LDL-C levels were calculated by subtracting sdLDL-C values from LDL-C values. Similarly, HDL2-C levels were calculated by subtracting HDL3-C from HDL-C. Samples were centrifuged within an hour of collection, and the serum was stored at 4°C until measurement, which was done within 8 hours of collection. Reference intervals were

determined for 4 groups (based on gender and age). This was determined using the normalized data generated from the Box-Cox power transformation model, and the 95% confidence interval was calculated using a parametric method.

Results: Coefficient of variance values for the intra- and inter-assay variation in LDL-C, HDL-C, sdLDL-C and HDL3-C were less than 6.13%. LDL-C, sdLDL-C, lbLDL-C, HDL-C, HDL2-C, and HDL3-C values positively correlated with age in both men and women. LDL-C and lbLDL-C in men, and LDL-C, sdLDL-C, and lb LDL-C in women positively correlated with BMI, but HDL-C and HDL2-C in both groups negatively correlated with this parameter. sdLDL-C and HDL3-C in men, and HDL-C, HDL2-C, and HDL3-C in women positively correlated with alcohol consumption, while this negatively correlated with lbLDL-C in men.

The sdLDL-C reference interval was the lowest in younger women (13-22 mg/dL) and the highest in older men (16-53 mg/dL). The lb-LDL-C interval was the highest in older women (49-119 mg/dL), and the lowest in younger women (43-111 mg/dL). Reference intervals for HDL2-C and HDL3-C were the highest in older women (29-77 mg/dL and 19-36 mg/dL, respectively), and the lowest in young men (22-66 mg/dL and 17-30 mg/dL).

In ACS patients, both sdLDL-C and lb-LDL-C values were higher while HDL2-C and HDL3-C values were significantly lower than those in the control group. In relation to ACS, a multiple logistic regression analysis revealed significant odds ratios in age (1.3), lbLDL-C/sdLDL ratio (26.4) and sdLDL-C/HDL2-C ratio (269.7).

Conclusion: These findings suggest that the LDL-C and HDL-C subclasses may be useful biomarkers in predicting cardiovascular complications in patients with atherosclerosis.

B-109

Overexpression Of Del-1, An Oxidized LDL Blocking Protein, Suppressed Atherogenesis In Mice Without Lowering Oxidized LDL Concentration, But with Reducing LOX-1 Ligand Containing ApoB Activity (LAB).

A. Kakino, A. Nakano, Y. Fujita, T. Sawamura. *National Cerebral and Cardiovascular Center, Suita, Japan*

Background: Oxidized LDL (oxLDL) is implicated in the pathogenesis of atherosclerosis. However, measurement of circulating oxLDL concentration often fail to provide the accurate state of atherosclerosis or the risk of atherothrombotic diseases such as myocardial infarction and ischemic stroke. To solve this problem, we have devised a novel ELISA assay system based on the binding of modified LDL to an oxidized LDL receptor LOX-1, rather than determining the concentration of a specific epitope of anti-oxLDL antibody. LOX-1 binding activity of apoB-containing lipoprotein was designated as LAB. LAB well predicted the risk of coronary artery disease and ischemic stroke (Inoue, Clin Chem 2010), and reflected the intimal thickening of carotid artery (Okamura, Atherosclerosis 2013).

Aim: To understand the reason why the receptor-based assay has been superior to the antibody-based assay in evaluating the progression of atherosclerosis and the risk of atherosclerosis-related diseases.

Methods and Results: We found that Del-1 selectively bound to oxLDL but not to native LDL, leading to the inhibition of the uptake of DiI-labeled oxLDL (DiI-oxLDL) via oxLDL receptors including LOX-1, SR-A, CD36, and SR-B; but not to the inhibition of DiI-labeled native LDL uptake via LDL receptor expressed in COS-7 cells. We also found that Del-1 inhibited DiI-oxLDL uptake by cultured human umbilical vein endothelial cells (HUVEC) and THP-1-deribed macrophages. Furthermore, Del-1 suppressed oxLDL-dependent signal transduction in LOX-1 expressing CHO cells and in HUVEC. Del-1 also suppressed oxLDL-induced secretion of endothelin-1 in HUVEC.

To examine in vivo effects of Del-1 on atherogenesis, we established Del-1 transgenic mice (Del-1Tg), and fed their males high-fat diet along with control wild-type mice (WT) (n=6 each) for 20 weeks from the age of 24 weeks. Oil red O-positive atheromatous area at aortic roots dramatically decreased in Del-l Tg compared with WT (3.1±1.4 vs. 17.7±2.0 % of aortic roots area, P<0.001). Reflecting the antiatherogenic effects, plasma LAB activity was significantly decreased in Del-1Tg compared with WT mice (13.3±4.3 vs. 106.2±20.1 ng/ml, P<0.05), while oxidized LDL concentration determined by conventional antibody-based assay did not differ between Del-1Tg and WT (698.9±34.4 vs. 741.9±46.0 nmol/ml). Other lipid parameters except triglycerides were not different. Plasma tryglyceride concentration in Del-1Tg was slightly lower than that in WT.

Conclusion: In the presence of Del-1, an oxLDL blocking protein, the oxLDL concentration determined by conventional anti-oxLDL antibody-based assay dissociates from atherogenicity, while LAB well associated with it. This might be a reason why circulating LAB activity better reflects the state and the risk of atherosclerotic diseases. This might be a reason for LAB activity reflects the progression and the risk of atherosclerotic disease better than oxLDL concentration determined by anti-oxLDL antibody.

B-110

Hypertriglyceridemia is a major contributor of high small dense LDL level in patient with metabolic syndrome

S. Lee, Y. Cho, J. Kim. *Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: Patients with metabolic syndrome (MetS) have shown higher small dense low density lipoprotein cholesterol (sdLDL-C) level than healthy controls. However, which component of MetS made the largest contribution to an increase in sdLDL-C has not fully determined. We aimed to determine major contributing component of MetS to high sdLDL-C concentration and sdLDL-C/LDL-C ratio.

Methods: Four hundred and forty seven subjects (225 men; 222 women) with MetS were randomly selected from the Korean Metabolic Syndrome Research Initiatives-Seoul cohort study. Age and sex-matched 360 healthy controls (181 males and 179 females) were also randomly selected from the same cohort.

Results: When we compared means of sdLDL-C concentration between subgroups divided according to whether subjects met or not met each MetS component in patients with MetS (Table 1), significant difference in sdLDL-C concentration was found only between subgroups divided according to whether subjects met or not met triglyceride (TG) criteria. For healthy control, there were significant differences in sdLDL-C concentration according to the presence or absence of TG and waist circumference components. We observed similar pattern for sdLDL-C/LDL-C ratio. Pearson correlation analysis showed total cholesterol, LDL-C, and TG showed relatively strong correlations with sdLDL-C concentration (r = 0.730, 0.508 and 0.543 respectively for men; 0.748, 0.692 and 0.653 respectively for women), whereas only TG maintained a strong correlation with sdLDL-C/LDL-C ratio (r = 0.789 for men and 0.745 for women). In multiple regression analysis, we found TG level was a significant determinant of sdLDL-C concentration and sdLDL-C/LDL-C ratio.

Conclusion: Among five MetS components, only the abnormal TG level worked as a differing factor of sdLDL-C concentration and sdLDL-C/LDL-C ratio, and which results were reproducible in both genders with or without MetS. Our results also supported a hypothesis that atherogenic effect of hypertriglyceridemia could be partially mediated by elevated sdLDL related to high TG.

Table 1. Differences of sdLDL-C between subgroups divided by whether met or not met each component

Components	Male (n = 406)			Female (n = 401)		
	Component -	Component +	*p* value	Component -	Component +	*p* value
Patients with metabolic syndrome						
Waist circumference	48.66 ± 18.9	48.77 ± 18.93	0.9691	43.08 ± 12.55	44.88 ± 18.06	0.5891
Triglyceride	30.44 ± 11.22	51.78 ± 18.18	< 0.0001	33.06 ± 11.93	47.72 ± 17.3	< 0.0001
High density lipoprotein cholesterol	49.95 ± 20.26	47.69 ± 17.6	0.3709	46.75 ± 20.86	43.71 ± 15.64	0.2344
Blood pressure	50.44 ± 19.09	48.39 ± 18.87	0.5400	46.76 ± 16.3	43.77 ± 17.74	0.2473
Fasting blood sugar	49.78 ± 17.65	46.95 ± 20.85	0.2796	44.34 ± 16.49	45.05 ± 18.72	0.7673
Healthy controls						
Waist circumference	35.55 ± 15.66	42.72 ± 18.33	0.0463	26.81 ± 12.58	33.24 ± 17.17	0.0147
Triglyceride	29.7 ± 12.01	48.23 ± 15.72	< 0.0001	24.54 ± 9.63	47.44 ± 16.96	< 0.0001
High density lipoprotein cholesterol	35.71 ± 16.46	41.13 ± 13.39	0.1195	27.21 ± 13.33	31.35 ± 15.05	0.1137
Blood pressure	36.09 ± 15.96	38.65 ± 17.36	0.4559	28.1 ± 13.76	26.86 ± 13.72	0.7384
Fasting blood sugar	36.53 ± 16.14	32.17 ± 19.11	0.6437	28.00 ± 13.72	-	-

B-111

Comparison of Lipoprotein Profile Analysis by Nuclear Magnetic Resonance (NMR) and Agarose Gel Electrophoresis.

V. Gounden, M. L. Sampson, R. Shamburek, A. T. Remaley. *National Institutes of Health, Bethesda, MD*

Objective: Lipoprotein analysis by agarose gel electrophoresis is useful for identifying rare genetic lipid disorders and confirming the level of the major lipoprotein fractions. The NMR Vantera method can also be used for quantifying lipoprotein subfractions, size and particle counts but the two methods have not been compared. **Methods:** Serum samples from 250 patients with a wide variety of lipid disorders were analyzed on the Sebia lipoprotein gel electrophoresis system and the Vantera NMR system. The major lipoprotein fractions (alpha-HDL, pre-beta-VLDL and beta-LDL) were quantified by densitometric scanning of the electrophoresis gel; these values were divided into tertiles and compared to the NMR particle count (HDL-P, VLDL-P and LDL-P). The speed of migration of each fraction on electrophoresis was characterized

and catergorized into tertiles (fast, medium, slow), then compared to lipoprotein particle size (HDL-Z, VLDL-Z and LDL-Z). Data was analyzed by ANOVA to identify differences between the methods.

Results: Results from the particle count by NMR analysis showed the same trend as did quantification of the major bands by lipoprotein electrophoresis.

		Electrophoresis Grouping Tertiles		
NMR particle count (mean)		low	medium	high
HDLp (μmol/L)	p<0.001	27.8±0.9	33.1±0.9	36.2±0.9
VLDLp (nmol/L)	p<0.001	26.9±4.4	36.4±4.4	84.8±4.4
LDLp (nmol/L)	p<0.001	1056±62.1	1154±60.7	1490±62.6

Although ANOVA showed a significant correlation (p<0.006) between size of lipoproteins with migration speed by electrophoresis, the results were not always consistent between the three fractions, particularly for VLDL. Using a novel method for displaying NMR results, we also show that the pattern of NMR results typically overlap with the interpretation of the lipoprotein phenotype based on electrophoresis for disorders, such as Type I, IIa, III hyperlipidemia and LCAT Deficiency. **Conclusions:** Because the analysis by NMR and electrophoresis depend on different physical properties of lipoproteins, there was not always a complete concordance of the results. However, in general NMR analysis of lipoproteins usually leads to the same clinical interpretation of the lipoprotein phenotype as electrophoresis and provides additional information that may be potentially useful for assessing cardiovascular risk.

B-112

A serum oxidized high-density lipoprotein marker and its association with the smoking status in males

K. Kotani[1], S. Mashiba[2], M. Ueda[2], N. Taniguchi[1], T. Yamada[1]. *[1]Jichi Medical University, Shimotsuke-City, Tochigi, Japan, [2]Ikagaku Co. Ltd., Kyoto, Japan*

Background: High-density lipoprotein (HDL) particle, whose major protein is apolipoprotein A-I (apoA-I), protects against atherosclerosis via its anti-oxidant properties. The oxidative modification of apoA-I is associated with dysfunctional HDL. Cigarette smoking, a major atherosclerotic risk factor and a precipitating condition leading to oxidative stress, is often accompanied by low HDL-cholesterol levels in the circulation. However, the adverse effects of smoking on atherosclerosis remain incompletely understood with regard to dysfunctional HDL, and easy biomarkers for smoking-related HDL modifications are needed. A new assay we developed for oxidized apoA-I, oxHDL, may be a suitable marker, since we have found high oxHDL levels under some oxidative stress conditions. The aim of this study was to investigate the association between the oxHDL levels and the smoking status in males.

Methods: A total of 260 Japanese males (mean age, 61 years) were consecutively recruited from general health check-ups. The subjects who had a history of cardiovascular disease, had been diagnosed with metabolic syndrome or received lipid-modulating drugs were excluded. The smoking status was self-reported. Clinical data, including serum lipid levels, were obtained from subjects in a fasted state. The serum oxHDL levels were quantified using a sandwich ELISA system, which utilizes monoclonal antibodies prepared by immunization with H_2O_2-oxidized human apoA-I.

Results: The mean/median levels of the relevant variables were as follows: low-density lipoprotein cholesterol, 3.0 mmol/L; triglycerides, 1.0 mmol/L; HDL-cholesterol, 1.5 mmol/L; oxHDL, 221 U/mL and oxHDL/HDL-cholesterol ratio, 3.9. Compared to non-smokers (n = 188), current smokers (n = 71) tended to exhibit higher oxHDL levels (217 versus 239 U/mL) and lower HDL-cholesterol levels (1.6 versus 1.5 mmol/L), while current smokers showed significantly higher oxHDL/HDL-cholesterol levels (3.9 versus 4.1, $p < 0.05$). The difference in the oxHDL/HDL-cholesterol levels remain significant after adjusting for age, body mass index, blood pressure, other lipids and glucose levels. Moreover, a significant inverse correlation ($r = 0.3$, $p < 0.05$) was found between the oxHDL/HDL-cholesterol levels and the Brinkman index (number of cigarettes smoked per day × number of years of the habit) in current smokers.

Conclusion: The present findings suggest that smoking may independently and oxidatively modify HDL particles, thus leading to dysfunctional HDL in males. The oxHDL/HDL-cholesterol ratio may therefore be useful for assessing the atherosclerotic burden in relation to the smoking status.

B-113

Effects of body weight on low-density lipoprotein and high-density lipoprotein subclasses assessed by homogenous small-dense low-density lipoprotein and high-density lipoprotein 3 cholesterol assays

H. Takahashi, H. Ookura, R. Suzuki, Y. Kadosaka, M. Yoshika. *Kansai Medical University, Hirakata, Osaka, Japan*

Background: The incidence of overweight has been increasing in epidemic proportions, and it adversely increases the prevalence of most cardiovascular (CV) diseases. Obesity results in diverse changes in laboratory parameters such as triglyceride (TG) levels, high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) levels, and glucose metabolism due to insulin resistance. Of these, HDL-C and LDL-C levels play a key role in the development of CV diseases; as such, they are very valuable biomarkers for the prediction of future CV events. Both lipoproteins have subclasses: small dense LDL (sdLDL) and large buoyant LDL (lbLDL) and HDL2 and HDL3, which may be superior biomarkers to LDL and HDL, respectively. However, assaying these subclasses using ultracentrifugation, electrophoresis, or high-performance liquid chromatography is not easy, requires significant time, and commonly produces inaccurate, quantitative results.

Methods: A homogenous assay system for sdLDL-C and HDL3-C has recently become available, and it is reported to have favorable performance (Clinical Chemistry 57:57-65, 2011; Clin Chim Acta. 427:86-93, 2014). In the present study, using these homogenous assay systems, we explored the relationship between body weight and LDL and HDL subclass concentrations. The levels of lbLDL-C and HDL2-C were calculated by subtracting those of sdLDL-C and HDL3-C from the levels of LDL-C and HDL-C, respectively. Data are expressed as means ± standard deviation.

The enrolled subjects were women aged 34 ± 10.5 years (20-64 years) (n = 1,276) working in our hospital, and informed consent was obtained at the annual health checkup during blood sampling.

Results: Body mass index (BMI) was significantly correlated with both lbLDL-C ($r = 0.2691$, $p < 0.01$) and sdLDL-C ($r = 0.3223$, $p < 0.01$). Similarly, waist circumference (cm) was significantly correlated with lbLDL-C ($r = 0.2638$) and sdLDL-C ($r = 0.3315$). In contrast, BMI was negatively correlated with HDL2-C ($r = -0.2715$, $p < 0.01$) but not HDL3-C ($r = -0.0173$). Waist circumference was significantly correlated with HDL2-C ($r = -0.2771$) but not HDL3-C ($r = -0.0686$). Multiple regression analysis with age, systolic blood pressure, alanine aminotransferase, TG, cystatin-C, C-reactive protein, and HDL-C and LDL-C subclasses as independent variables revealed that these parameters were independently correlated with BMI. The lbLDL-C and sdLDL-C values were similarly independently correlated with BMI. Both HDL2 and HDL3 were independently correlated with BMI, but the t-value of HDL2 was much greater than that of HDL3. When waist circumference was used as the dependent variable, it was also independently correlated with the above parameters. The t-value of HDL2-C (8.231) was the highest, followed by those of lbLDL-C (4.549), sdLDL-C (2.931), and HDL3-C (2.361).

Conclusion: The present findings are in contrast to those of earlier studies in which sdLDL-C increased and HDL3-C decreased with weight gain and changes in both lbLDL-C and HDL2-C were more closely related to body weight differences. These findings also indicate that these LDL-C and HDL-C subclasses assessed using the homogenous method may be novel predictive markers for atherosclerosis.

B-114

Effect of SAA on the structure and measurement method of HDL

M. Sato[1], R. Ohkawa[1], T. Kameda[1], A. Yoshimoto[1], S. Okubo[2], Y. Yatomi[2], M. Tozuka[1]. *[1]Analytical Laboratory Chemistry, Graduate School of Health Care Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Clinical Laboratory, The University of Tokyo Hospital, Tokyo, Japan*

Background: Serum amyloid A (SAA), which is one of the acute phase proteins, is commonly found in high-density lipoprotein (HDL) in the circulation. SAA is known to become a major HDL protein component in the acute phase due to a displacement of apolipoprotein AI (apoAI). Consequently, this remodeling could affect HDL metabolism, however the actual influences have not been fully elucidated. In this study, we focused on the structural differences between SAA containing HDL and normal HDL. In addition, the effect of attached SAA on the values of HDL-cholesterol (HDL-C) measurement was estimated.

Methods: HDL (d=1.063-1.210 g/mL) isolated from the patients with or without inflammation was characterized by agarose gel electrophoresis for analyzing the surface charge and by nondenaturing gel electrophoresis for analyzing particle size

and distribution of apoAI and SAA. HDL-C concentrations of the patients with various SAA levels were analyzed by two methods. One is the homogeneous method using α-cyclodextrin sulfate which is commonly used in clinical laboratories, and the other is the ultracentrifugation method as a reference method. SAA was measured by latex agglutination-turbidimetric immunoassay method.

Results: The increase of serum SAA levels induced the decrease of HDL mobility on agarose gel electrophoresis patterns. In the nondenaturing gel electrophoresis, HDLs obtained from the patients with low serum SAA levels were separated to two distinct particles, HDL_2 and HDL_3. On the other hand, HDLs obtained from the patients with high serum SAA levels indicated two kinds of typical patterns; one was characterized as the additional band at the intermediate particle size between HDL_2 and HDL_3, and the other was characterized as two bands extremely larger size than HDL_2 and smaller size than HDL_3. SAA was identified in the additional band for the former and in the larger band for the latter. HDL-C concentrations measured by the homogeneous method were highly correlated with those by the ultracentrifugation method in both the patients with low (SAA≤8 μg/mL, n=94) and high (8<SAA≤4762 μg/mL, n=154) SAA levels. Although no significant difference was observed in the regression lines of both groups, the ratios of HDL-C concentrations obtained by the ultracentrifugation method to those by the homogeneous method showed a tendency to be higher in the patients with acute inflammation.

Conclusion: Our data indicated that the large amount of SAA attached to HDL during inflammation and changed in the surface charge and the particle size of HDL. However, no definite relevance between serum SAA level and HDL particle size was observed. A good correlation between the homogeneous method and the ultracentrifugation method could be explained by the assay principle of the homogeneous method used, in which total cholesterol is measured after inhibition of enzymatic reaction against lipoproteins (mainly VLDL and LDL) except HDL by α-cyclodextrin sulfate. It suggests that the values obtained by the homogeneous method used here are probably not affected by a change in the structure of HDL.

B-115

Accuracy based proficiency test for triglyceride in South Korea

Y. Park[1], G. Kwon[1], J. Lim[1], J. Kim[1], S. Koo[1], H. Park[2], C. Cho[2], K. Lee[2]. *[1]Chungnam National University Hospital, Daejeon, Korea, Republic of, [2]Korea Centers for Disease Control & Prevention, Chungcheongbuk-do, Korea, Republic of*

Background: When laboratory test results are not standardized, a different result may be obtained for the same clinical sample. In this study we performed two trials of accuracy based proficiency test for triglyceride measurement among 53 candidate laboratories and assessed current performance of the routine measurement for triglyceride in Korea. .

Methods : A total of 6 levels of commutable frozen serum pools were prepared as secondary reference materials for triglyceride measurement according to CLSI 37-A. Test results from ReCCS were regarded as target reference values for group using glycerol blanking method, and values from CDC for the other group using method without glycerol blanking. For each trial, 3 levels of pooled serum were sent to participating laboratories and imprecision, bias and total error for each trial were calculated.

Results: The bias of 18 laboratories (34%) using enzymatic method with glycerol blank ranged from -7.62% to -1.47%, and that of 35 laboratories (66%) using enzymatic method without glycerol blanking ranged from -9.09% to 1.67%. Coefficient variations (CVs) ranged from 3 to 5% for each level of reference materials but did not show significant difference between the two groups. When total error (≤15%) was used for acceptability criteria, all of the results from 53 laboratories were acceptable. However, when inaccuracy criteria (≤±5%) is used, unacceptable rates for the 1st and 2nd trial were 33% and 50% in the group using glycerol blank method, 15% and 63% in the group using without glycerol blanking method respectively.

Conclusions : Through accuracy based proficiency test, comparison to the target value determined by a reference measurement procedure allows both an absolute and relative performance yardstick for laboratories using different measurement procedures. And data obtained from this proficiency test made a footstep for further national laboratory standardization for triglyceride.

Table 1. Accuracy based proficiency test for triglyceride among 53 participating laboratories

	TG(mg/dL)	CFS 11302	CFS 11303	CFS 21301	CFS 12-1-1	CFS 12-2-3	CFS 12-2-4
Reference	CDC (total glyceride)	186.38	139.55	231.19	154.73	90.01	239.43
	ReCCS (triglyceride)	175.20	128.50	221.90	144.40	87.10	236.60
Without glycerol blanking (N=35)	Mean(n=35)	181.37	133.28	227.78	148.03	83.15	231.46
	Max	197.17	143.83	248.17	164.17	93.00	255.67
	Min	170.83	121.83	214.83	140.83	78.17	220.00
	Range (Max-Min)	26.33	22.00	33.33	23.33	14.83	35.67
	SD	6.02	4.51	7.46	4.86	3.09	7.63
	%CV	3.3	3.4	3.3	3.3	3.7	3.3
With glycerol blanking (N=18)	Mean(n=18)	177.63	128.42	225.60	147.65	79.18	233.48
	Max	187.67	138.83	246.00	159.33	85.50	251.17
	Min	161.00	117.00	205.00	135.67	74.17	211.33
	Range (Max-Min)	26.67	21.83	41.00	23.67	11.33	39.83
	SD	7.80	5.77	11.01	6.01	2.73	9.35
	%CV	4.4	4.5	4.9	4.1	3.4	4.0

B-117

The association between lipoprotein(a) levels and coronary heart disease risk in different ethnic groups: results from the Multi-Ethnic Study of Atherosclerosis

J. Cao[1], B. T. Steffen[1], G. Warnick[2], J. P. McConnell[2], W. Guan[1], J. H. Stein[3], J. D. Kaufman[4], W. S. Post[5], M. Y. Tsai[1]. *[1]University of Minnesota, Minneapolis, MN, [2]Health Diagnostic Laboratory, Richmond, VA, [3]University of Wisconsin, Madison, WI, [4]University of Washington, Seattle, WA, [5]John Hopkins University, Baltimore, MD*

Background Elevated plasma lipoprotein (a) (Lp(a)) levels are established as a risk factor of coronary heart disease (CHD) in populations mainly of European and African ancestry. The objective of this study was to examine the association of lipoprotein (a) (Lp(a)) levels with coronary heart disease (CHD) events in 4 racial/ethnic groups in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods The MESA consists of 6,814 individuals without clinical evidence of CHD (aged 45-84 years) at the initial recruitment. Individuals taking lipid-lowering medication at baseline or with unavailable samples were excluded from this analysis resulting in a remaining sample of 4,387 who were followed for 8.5 years. Incident CHD was defined as the first occurrence of myocardial infarction, resuscitated cardiac arrest, CHD death, or definite angina. Lp(a) mass concentration was measured with an isoform-insensitive turbidimetric immunoassay (Denka Seiken, Japan) at the Health Diagnostic Laboratory Inc. (Richmond, VA) with *inter-assay* coefficients of *variation* less than 5%. Statistical analyses were conducted using Stata (version 12.1, Stata Corp, College Station, TX) and R. Tukey-Kramer HSD was used to test differences between groups. Since residuals analyses suggested a non-linear relationship between CHD risk and Lp(a) as a continuous measure, we dichotomized Lp(a) into < and > median groups. Cox regression was used to test for association between Lp(a) and CHD events, adjusting for age, gender, race, diabetes, hypertension, smoking status, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and log-triglycerides.

Results The study population was composed of 28.7% African American (AA, n=1,257), 12.4% Chinese American (CA, n=546), 22.7% Hispanic (HS, n=996), and 36.2% Caucasian (CU, n=1,588) participants. The distributions of Lp(a) levels in all ethnic groups were left-skewed, and AA had a significantly higher level of Lp(a) compared to the 3 other ethnic groups ($p < 0.05$). The median levels of Lp(a) in the 4 ethnic groups were: AA 35.1 mg/dL, CA 12.9 mg/dL, HS 13.1 mg/dL, and CU 13.0 mg/dL. The numbers of CHD events were: AA 66, CA 18, HS 49, and CU 105. Using the Lp(a) median level of the entire cohort (17.8 mg/dL), Lp(a) > median was associated with a

significantly higher CHD event rate than Lp(a) < median (*hazard ratio* (HR) = 1.42, $p = 0.0095$) following adjustment for age, gender, race, diabetes, hypertension, smoking status, HDL-C, LDL-C and log-triglycerides. In analyses stratified by ethnic groups, Lp(a) level above the group-specific median was associated with a significantly higher incidence of CHD in CU (HR = 1.55, $p = 0.03$), but not in the 3 other ethnic groups ($p = 0.08$, 0.63 and 0.74 for AA, CA and HS, respectively).

Conclusion In MESA participants not on lipid-lowering medications at recruitment, we found that elevated Lp(a) levels were associated with increased risk of CHD independent of traditional CHD risk factors. When stratified by race and using race-specific Lp(a) median levels as the cutoff, the association was only significant in CU. However, the power of subgroup analysis may be limited by the number of events.

B-118

New Enzymatic Method for Sphingomyelin Measurement verified by Mass Spectrometry

H. Kuwata[1], T. Kimura[2], K. Miyauchi[3], Y. Katayama[1], N. Kayahara[2], T. Honda[4], H. Hidaka[5]. *[1]Research Laboratories, Kyowa Medex Co., Ltd., Shizuoka, Japan, [2]Research and Development Division, Kyowa Medex Co., Ltd., Tokyo, Japan, [3]Customer Support Group, Quality Assurance and Regulatory Department, Kyowa Medex Co., Ltd., Shizuoka, Japan, [4]Department of Laboratory Medicine, Shinshu University, School of Medicine, Nagano, Japan, [5]Department of Biomedical Laboratory Sciences, Shinshu University, School of Medicine, Nagano, Japan*

Background: Serum sphingomyelin (SM) can help to predict the development of coronary arterial diseases. However, no convenient and specific assay for measuring SM in serum is available for routine laboratory practice. We previously developed the new assay for SM using an enzymatic method in combination with a monoglycerolipase and two types of phospholipase D. In this assay, phosphatidylcholine (PC) and lysophosphatidylcholine are eliminated in the first step and the remaining SM is measured in the second step. To validate this assay, we correlated the measurement values with the data by mass spectrometric analysis.

Methods: We prepared 47 sera at Shinshu University Hospital and measured their SM content using an enzymatic method on a Hitachi-7170 autoanalyzer. We also analyzed the lipid extract of the same sera using a TripleTOF 4600 mass spectrometer (AB SCIEX). N-heptadecanoyl-sphingosylphosphorylcholine (sphingomyelin with C17:0 fatty acid; SM 17:0, Matreya) was used as an internal standard. Lipid extraction was performed according to Folch's method. The amount of each identified SM species was determined by the difference of their fatty acid forms. We correlated results of the enzymatic method with that determined by mass spectrometry.

Results: The total amount of SM in the 47 samples ranged from 0.249 to 0.945 mmol/L (mean : 0.497 mmol/L) in the enzymatic method and 0.241 to 0.870 mmol/L (mean : 0.417 mmol/L) in the mass spectrometric method. Identified SM species by the mass spectrometry were SM 16:0, 16:1, 18:0, 18:1, 20:0, 22:0, 22:1, 24:0, 24:1, 24:2. SM 16:0 was the most abundant (0.191±0.101 mmol/L, 46.2±8.3 %) and 24:0 was the second abundant (0.0632±0.0434 mmol/L, 15.1±4.0 %) among the SM species in the 47 samples. The other species each represented less than 10% of the total SM. Within-run coefficients of variation (CVs) at 0.422 and 0.756 mmol/L in pooled sera were 1.55% and 1.45% for the enzymatic method and 13.5% and 5.26% for the mass spectrometry, respectively. We found a high correlation between values of each SM species measured with the enzymatic method (X) and that determined by the mass spectrometry (Y). Correlation coefficients and regression equations were as follows; SM 16:0, r=0.897, Y=0.367X+0.649; SM 16:0+SM 24:1, r=0.912, Y=0.520X−0.249; all identified SM species, r=0.950, Y=0.894X−1.95. Conversely, the correlation coefficient for PC was low (PC 16:0/18:2 + PC 18:0/18:2, r=0.593). These results suggest that the proposed enzymatic method can measure most of SM species in serum with high specificity and accuracy.

Conclusion: Our new enzymatic method can measure SM in serum with high specificity and accuracy, and therefore is very useful in clinical practice.

B-119

Effects of myeloperoxidase-modified HDL on reverse cholesterol transport and monocytic migration

T. Kameda, Y. Usami, N. Sagae, R. Ohkawa, M. Tozuka. *Tokyo Medical and Dental University, Tokyo, Japan*

Background: Myeloperoxidase (MPO) is one of the biomarkers for acute coronary syndromes. In the advanced atherosclerotic lesions, MPO, mainly secreted from macrophages, is known to induce the oxidized apolipoprotein AI (apoAI), such as 2-chloro- or 2-nitro-tyrosyl apoAI and apoAI-AII heterodimer. These products would be expected to give us the different informations from MPO activities in plasma, likely more specific to cardiac disease. We previously revealed that the plasma levels of apoAI-AII heterodimer in patients urgently hospitalized for the treatment of acute myocardial infarction were significantly higher than those of the healthy subjects. In the present study, we investigated the effect of MPO oxidation on the antiatherogenic properties of HDL, such as the cholesterol efflux capacity and the inhibition activity of monocytic migration.

Methods: 1) Oxidation of HDL by MPO; HDL (1.063<d<1.210 g/mL) was incubated with phosphate buffer (pH 7.4) containing hydrogen peroxide, diethylenetriamine pentaacid, L-tyrosine, and MPO for 24 h at 37 °C. 2) Evaluation of cholesterol efflux; THP-1 cells were differentiated into macrophages by addition of phorbol 12-myristrate 13-acetate. Then macrophages were loaded with acetylated LDL and [^{3}H]-cholesterol for 24 h. After 18 hours equilibration, cholesterol efflux was assessed in the media in the presence of HDL, MPO treated HDL, or no acceptor for 4 h. The radioactivity in the medium and the total cell-associated radioactivity were determined by scintillation counting. The cholesterol efflux was calculated as a percentage: [^{3}H]-cholesterol in medium/ ([^{3}H]-cholesterol in medium + [^{3}H]-cholesterol in the cells) x 100. 3) Evaluation of THP-1 cell migration; THP-1 cell migration assays were performed with 8 μm pore size inserts on the PET membranes. HUVEC (human umbilical vein endothelial cell) was stimulated by LPS at 37 °C for 16 h in the presence of HDL or MPO treated HDL. The lower compartments of chemotaxis chamber were filled with supernatant of HUVEC cultured medium. THP-1 cells were placed in upper chamber and incubated for more than 24 h at 37 °C. The THP-1 cells migrated in the lower chamber were counted using an inverted microscope. The THP-1 migration ability is defined by the percentage of migrated THP-1 number against the original number.

Results: ApoAI-AII heterodimer in HDL was apparently increased by the incubation with MPO, which was confirmed by SDS-PAGE under reducing condition followed by CBB R250 staining and immunoblotting using anti-apoAI and anti-apoAII antibodies. No difference in the cholesterol efflux capacity was observed between HDL and MPO treated HDL. In the THP-1 migration assay, the presence of HDL indicated the significant reductive effect (44%) against LPS stimulation of HUVEC. This effect was reduced to 34% by the treatment of HDL by MPO.

Conclusion: MPO oxidation did not largely affect the cholesterol efflux capacity of HDL. However, MPO oxidation partially impaired HDL property to inhibit the monocytic migration, suggesting that the oxidation of HDL by MPO would affect the progression of atherosclerotic plaque. It means that the products, such as apoAI-AII heterodimer, induced by MPO oxidation might be available as a biomarker to reflect a progression of atherosclerosis.

B-121

Increases in Large HDL Particles are associated with Improved Cardiopulmonary Fitness by Exercise-based Cardiac Rehabilitation in Patients with Acute Coronary

S. Koba[1], Y. Ito[2], Y. Yokota[1], T. Hirano[1], M. Shoji[1], Y. Kobayashi[1]. *[1]Showa University School of Medicine, Tokyo, Japan, [2]Denka Seiken Co.,Ltd., Tokyo, Japan*

Background: Exercised-based cardiac rehabilitation (CR) can increase HDL-cholesterol (HDL-C). However it remains unclear how elevated HDL-C and changes of HDL subfractions are correlated with the improvement of exercise tolerance in acute coronary syndrome (ACS) patients participated with CR.

Methods: Concentrations of cholesterol and apolipoproteins (Apo) in HDL subfractions separated by heparin-Mn precipitation method were measured at the onset of ACS and at the end of the 6-month CR program in patients (45 men and 6 women) aged of 64.3 ± 11.8 years. Cardiopulmonary exercise tests were performed at the beginning and the end of the CR program. All patients received successful percutaneous coronary intervention on admission, and then started to take statins.

Results: Serum levels of HDL-C and ApoA1, and concentrations of cholesterol and ApoA1 in large HDL fraction (HDL2) were significantly increased by CR (42.7 mg/dl ± 14.1 mg/dl to 47.4 mg/dl ± 14.2 mg/dl, 128.8 mg/dl ± 23.4 mg/dl to 139.2 mg/dl ± 26.0 mg/dl, 25.3 mg/dl ± 12.4 mg/dl to 30.3 mg/dl ± 13.6 mg/dl, 67.8 mg/dl ± 20.3 mg/dl to 79.4 mg/dl ± 24.5 mg/dl, respectively), while cholesterol and ApoA1 in small HDL fraction (HDL3) were not changed. Moreover HDL2-C / HDL-C ratio, and HDL2-ApoA1 / ApoA1ratio were significantly increased by CR. In addition, Spearman's rank correlation coefficient analysis revealed that only % increases of HDL2-C were significantly associated with % increased of peak oxygen consumption (VO2) and % increases of VO2 at anaerobic threshold ($\rho = 0.439$, $p = 0.007$, $\rho = 0.382$, $p = 0.020$), while neither HDL-C nor HDL3-C were associated with them.

Conclusion: CR can markedly increase the number of HDL2 particles, which is significantly associated with the improvement of cardiopulmonary fitness. These results suggest that CR is very useful therapy for the reverse cholesterol transport, and the secondary prevention.

B-122

A Novel Method Using Cation-exchange and Heparin Affinity Columns Arranged Tandemly to Determine ApoE-containing HDL-cholesterol in Un-pretreated Whole Serum

S. Usui[1], T. Ikeda[1], R. Shinohata[1], M. Okazaki[2], S. Ikeda[1], S. Kusachi[1]. *[1]Okayama University Graduate School of Health Sciences, Okayama, Japan, [2]Professor Emeritus of Tokyo Medical and Dental University, Skylight Biotech Inc., Akita, Japan*

Background: Measurement of HDL-related subclasses has been suggested to be more useful for evaluating coronary artery disease risk than total HDL. Clinical significance of apolipoprotein E-containing HDL (apoE-HDL) has not been clarified. Development of a reliable and rapid assay system to measure serum apoE-HDL levels is thus essential.

Aims: We developed a high-performance liquid chromatography (HPLC) equipped with cation-exchange and heparin affinity columns to measure apoE-HDL-cholesterol (apoE-HDLc) levels in untreated whole serum, and studied the analytical performance for serum apoE-HDLc determination. Separation characteristics of the system and their isolated lipoprotein fractions were also studied.

Methods: An un-pretreated whole serum sample was injected into two tandemly connected columns and eluted by a step-wise gradient manner, as shown in Fig.1. Non-HDL lipoproteins are bound to the cation-exchange column, and unbound HDLs next enter the heparin column. The heparin column retains apoE-HDL but no other HDLs (apoE-deficient HDL).

Results: Our developed HPLC system completed the assay within 16 mins and separated lipoproteins in un-pretreated serum into 3 peaks on the cholesterol pattern. Each peak corresponded specifically to apoE-deficient HDL (first peak), apoE-HDL (second peak), and non-HDL (third peak). The present HPLC system provided acceptable small within-day imprecision values; 1.3% CV (n=8) and good acceptable linearity with the serially diluted pooled serum, up to 18 mg/dL for apoE-HDLc. The system was not affected by triglycerides at concentrations up to 450 mg/dL in apoE-HDLc measurement. The apoE-HDLc levels of healthy volunteers determined by the present HPLC system were 5.3±1.6 mg/dL (n=26), which accounted for approximately 6-11% of total HDLc.

Conclusion: Our developed HPLC system with cation-exchange and heparin affinity columns showed a rapid and precise determination of apoE-HDLc levels in un-pretreated serum. The present system is thus useful in both clinical settings as well as for lipid research.

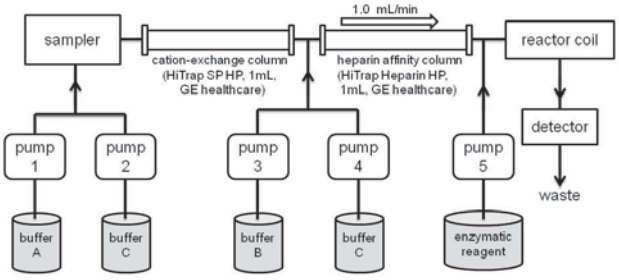


Fig.1 HPLC system
Buffers A (10 mM MOPS, 5 mM magnesium acetate, pH 7.2), B (10 mM MOPS, 0.1 M magnesium acetate, pH 7.2), and C (10 mM MOPS, 1.0 M sodium acetate, 0.01% nonionic detergent, pH 7.2) were used for eluting lipoproteins. The column effluent was mixed with an enzymatic reagent for cholesterol. Enzymatic reactions proceeded at 37°C in a reactor coil, and developed color was detected at 555 nm. Arrows indicate the flow direction.

B-123

Small Dense LDL Ratio Associates with the Metabolic Syndrome

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

Background: Low-density lipoprotein (LDL) cholesterol is often not an effective predictor of cardiovascular risk because of the variability of the cholesterol content within lipid particles. We investigated the association of lipoprotein subclasses, classified with a polyacrylamide tube gel electrophoresis (PGE) method, with scoring for metabolic syndrome (MetS).

Methods: A total of 242 outpatients were scored into six groups, based on their number of MetS components (from 0 to 5 variables) defined by the NCEP ATP III criteria, modified for the Asian cutoff for waist circumference. Blood samples were analyzed for lipid profile, LDL subclass (Quantimetrix Lipoprint™, CA), and atherosclerosis-related markers: apolipoprotein A-I (apoA-I), apoB, glucose, hemoglobin A1c (HbA1C), high sensitive C-reactive protein (hsCRP), creatinine, cystatin C, and vitamin D. The PGE method separates the intermediate density lipoprotein (IDL) particles into three midbands (MID-A to C) and the LDL particles into larger-buoyant LDL (lbLDL; LDL1 and LDL2), small-dense LDL (sdLDL; LDL3 to LDL7), and HDL; sdLDL was calculated as the sum of LDL3 to LDL7.

Results: The mean levels of triglycerides, glucose, and HbA1C rose with increasing MetS score, whereas those of HDL-cholesterol decreased. However, the concentration of total cholesterol, LDL-cholesterol, non HDL-cholesterol, apoAI, hsCRP, and vitamin D did not trend with increasing MetS score (all P >0.170). Using PGE, the mean concentrations of VLDL, MIDC, MIDB, LDL2, and sdLDL positively correlated with increasing MetS score, but those of MIDA and LDL1 inversely correlated, similar to the pattern observed for HDL. Using backward stepwise logistic regression, MIDC, MIDB, MIDA, LDL1, LDL2, and sdLDL were considered the independent variables. LDL1 and sdLDL [regression coefficient = -0.033 and 0.054, odds ratio = 0.968 (95% CI, 0.943-0.994) and 1.055 (95% CI, 1.023-1.089), respectively] were identified as being significantly associated with MetS (P <0.02). In the logistic model, the sdLDL/LDL1 ratio showed the strongest association with MetS and demonstrated an odds ratio of 5.544 (2.030-14.542, 95% CI). For predicting MetS, the area under the ROC curve of the sdLDL/LDL1 ratio had the greatest diagnostic value (0.700), followed by VLDL (0.694), sdLDL (0.689), HDL (0.669), LDL1 (0.648), MIDC (0.605), and MIDB (0.596), which showed good discrimination power for MetS (P ≤0.010), whereas the value of MIDA (0.572) indicated a poor power (P = 0.055).

Conclusion: Respective subpopulations of IDL and LDL particles can vary in their ability to identify MetS. These variations may partially explain why a quantitative assessment using absolute LDL-cholesterol concentrations, as typically measured in conventional practice, is poorly associated with MetS. We show that the ratio of sdLDL/lbLDL is strongly associated with the metabolic syndrome (high odds ratio and highest area-under the curve). It may be a potentially important tool to maximize the effectiveness of risk assessment for cardiovascular disease.

Wednesday, July 30, 2014

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

Management

B-124

Critical Values Reporting: The Search For an Effective Solution.

R. Khoury, P. Gudaitis, B. P. Salmon, A. Gandhi, J. Camoia, D. Pasquale, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

Background: Critical laboratory result according to Dr. George Lundeberg is: "A laboratory test result that represents a pathophysiologic state at such variance with normal as to be life-threatening unless something is done promptly and for which some corrective action could be taken." Although his description of critical was more than 30 years ago, critical vales received more attention when the Clinical Laboratory Improvement Amendments Clinical Laboratory implemented the importance and the concept of critical values. CLIA, CAP, State agencies and JCAHO require laboratories to have written procedure for reporting critical value, defining critical results, documentation and read back, and turnaround time for reporting critical results to the care giver. The determination of critical cutoff depends on the population, setting, and the feedback from the caregivers.

Design: data from 79,328 tests ordered on 13,579 Specimens that were collected from residents in Long-Term Care facilities were collected and included results from: chemistry, hematology, coagulation, therapeutic drug monitoring, and cardiac markers. Critical result calls and any problem with reporting the results to the caregiver (because the patients are resident in Long Term Care Facilities the results are given to the nurse in charge of that patient) were documented for every critical test. Statistical analysis was done using Analyse-it.

Results: 1,784 (2.8%) critical results were documented; the majority of the critical samples were for chemistry followed by hematology and coagulation. 616 (34.5%) values were reported immediately to the caregiver by the technologist who performed the test due to the severity of the results; 1168 values were reported by our call-in department, 22% of the calls were unsuccessful due to either no one answering the calls, nurses are busy/unable to take the calls, or nurses refuse to provide her/his name to be documented in addition to read back the result. All unsuccessful calls were followed by another call until the results were given to the appropriate caregiver. Most of the unsuccessful calls were between 10-11 AM, followed by 5-6 PM, and 7-8 PM. which coincide with morning meetings, passing medication, lunch/dinner break.

Conclusion: the majority critical values are reported to the caregiver without any delay; more than one fifth of the results took longer time to relay the result due to inability to reach the care giver. Implementing reliable communication systems will help improving the critical values reporting, such as immediate electronic notification via fax/online, automated notification system, default phone number or alternate caregiver in case of inability to reach the facility. Auditing record will help identifying any inadequate documentation, weakness, and the facilities with the most unsuccessful call to work with them to solve the problem.

B-126

Six Sigma Reagent Performance Quality Metrics for Beckman Coulter AU Clinical Chemistry Instruments

D. D. Koch[1], D. N. Greene[2], S. Westgard[3]. *[1]Emory University, Atlanta, GA, [2]Permanente Medical Group, Berkeley, CA, [3]Westgard QC, Inc., Orange, CT*

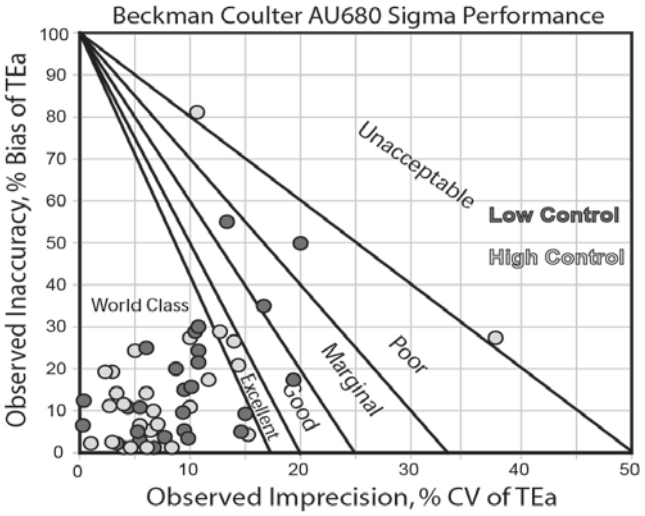
Background: Clinical chemists should be aware of the analytical strengths and limitations of their laboratory methods. Six Sigma reagent performance quality metrics are an excellent way to quantify the precision and accuracy of analytical methods. However, the literature is scant with publications describing these metrics for today's clinical chemistry instruments. Data for 26 clinical chemistry analytes on two AU platforms (AU680 and AU5800) were used to calculate sigma metrics. We display the performance of these methods on Method Decision Charts, a popular tool for portraying Six Sigma metrics.

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 the expected mean).

Results: The AU680 results are summarized on the accompanying Method Decision Chart. The majority of methods evaluated on both the AU680 and AU5800 were classified as "Good" or better with many achieving Six Sigma performance. The latter group should be easy to control with cost-effective QC practices. In contrast, a few analytes do not achieve desired status and therefore may require more rigorous QC practices.

Conclusions: Most clinical chemistry methods function at world-class specifications using the Beckman Coulter AU680 and 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 choose which analytes to focus on for method improvement. Additionally, optimal QC practices for laboratory production are implied.



B-127

The Empower project - integrated tool to evaluate the quality and effectiveness of laboratory testing.

L. A. C. De Grande[1], D. Stöckl[2], K. Van Uytfanghe[1], L. M. Thienpont[1]. *[1]Laboratory for Analytical Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Gent, Belgium, [2]STT-Consulting, Horebeke, Belgium*

Background Classical external quality assessment (EQA) is well established, however, the need for integrated EQA-services recently emerged, among others to empower clinical laboratories for future tasks, e.g., contribution to the development and implementation of global health-care policies. Also, ISO 15189 accreditation requires laboratories to identify and monitor quality indicators. From this perspective, we developed the Empower project.

Methods The project comprises 4 pillars: (i) master comparisons with panels of frozen single donation sera, (ii) virtual EQA-1 and (iii) -EQA-2 based on data readily available in the laboratory, i.e., patient- and internal quality control (IQC) results, and (iv) conceptual/statistical education about analytical quality. The pillars (i) to (iii) are conducted across laboratories and manufacturers. The master comparisons aim at participation of 20 laboratories per manufacturer, and encourages the latter to include also their in-house laboratories. It is essential that the participants use homogeneous systems, i.e., instrument, calibrator and reagent from the same manufacturer. Virtual EQA-1 requires the laboratories to calculate and send the daily medians of the results for outpatients. We plot the moving median of the collected data in time. The participants can consult a graphical user-interface to monitor the mid- to long-term stability of their performance in comparison to other peer group laboratories. Virtual EQ-2 part (not operational yet, except in a few laboratories) plans a similar approach, but on the basis of the daily IQC means.

Results The value of the master comparisons is in showing the intrinsic quality of assays as performed under 'field' conditions, the performance of the individual laboratory within its peer group and the calibration fix-point and interchangeability of results between laboratories/manufacturers. Monitoring of patient- and IQC-data gives evidence about the mid- to long-term analytical variation of testing in the individual

laboratory, backed-up by information on peer group performance. It also enables to uncover biases between different instruments in a laboratory, as well as occurrence of shifts/drifts. If the cause of the aberration can be identified, the problem can be solved either by the laboratory or the manufacturer. For example, an unacceptable lot-to-lot variation may require factorizing by the laboratory or fundamental improvement of the lot stability by the manufacturer. The observation of biases may also help laboratories to understand the sometimes fluctuating flagging frequency of results.

Conclusions The Empower project reflects on the mid- to long-term analytical stability of laboratory/assay performance and enables to uncover all major bias components/sources. From this perspective, we believe it is a new integrated tool for modern quality management. Its major asset is that it works with data generated from commutable samples, and linked to observations in daily IQC practice. It strengthens the position of laboratories in their claims from manufacturers, facilitates the dialogue at the laboratory-clinician interface, and is a tool for the discipline to derive realistic quality specifications. On longer term, it might establish a constructive relationship between laboratories, manufacturers, clinicians and health policy makers, so that together they can build towards a common understanding about manageable quality of performance to the benefit of the patient.

B-128

The relevance of a computerized system for temperature and humidity control in a large Brazilian laboratory

R. C. Ferreira, F. T. C. Sakamoto, L. G. Pereira, C. F. d. Pereira, D. Q. Moraes. *Diagnosticos da America, Barueri, Brazil*

Background: The controls and records of temperatures and humidity are very important in many areas. In clinical laboratories, the temperature control of samples and reagents are fundamental for analytical quality. This activity can be complex and difficult when involves several areas and many instruments as refrigerators, freezers, ultra freezers, climate chambers and acclimatized rooms, resulting in 60 points of control in our DASA SP Central Lab. This Lab is currently processing around 4.5 million tests / month, with huge sample flow and local reagents stock representing a large monetary amount. This reality demanded a technical solution. The team of equipment management (SELAB) opted for a system based on thermo transmitter technology, using radio frequency, software for data analysis and creating an emergency flow.

Objectives To enumerate the main features and benefits of a computerized system for temperature and humidity control as: Capability to monitor sixty points of temperature or humidity; Standardization of our monitoring procedure - change from manually to computerized Registration and safety in storage of data in compliance with FDA title 21, CFR part 11; Analyze equipments performance; Easy submission of records in audit processes as PALC, CAP, ISO.

Methodology The laboratory acquired the DataNet System produced by Fourier Systems. This solution provides an intelligent network sensor system with assurance of reception and no data loss. The ZigBee is a standard-based protocol built around the IEEE *802.15.4 wireless protocol, providing the network infrastructure required for wireless and low power network applications. Basically the system consists of thermo transmitters, receiver, server and an integrated phone. The thermo transmitters were fixed next to the equipments. With a thirty minutes interval the measurements are sent to server. Using the software, the ranges of alarm and pre-alarms are defined for all points to be monitored. Together with The Department of Analytical Quality, different ranges were set up. When the temperature reaches the control limits, the software sends an email containing information about the area and temperatures that are outside the acceptable range.

Results: In February of 2012, the system was on line, monitoring 24 hours these instruments:

• 5 climate chambers;

• 6 ultra freezers (range: -80 to -60°C);

• 15 refrigerators (range: 2 to 8°C);

• 15 freezers (range: -30 to -8°C);

• 7 heated chambers (various intervals);

• 12 acclimatized room (range: 15 to 25°C)

Two year after implementation several risk situations were avoided for samples and reagents through the alerts emailed to areas and staff working in the lab.

Conclusion: The system has shown a good level of reliability and has helped us to avoid problems through tendency of temperature, shown in graphs. The daily registers allow us to identify low performance equipment. During this period there weren't any problems with samples or reagents.

The Company reached a high level of security for the temperature data. Now is easy the submission of records in audit processes. DataNet also keeps the traceability of all users' activities, following the regulation from The Food Drugs Administration, Title 21, Code of Federal Regulations Part 11.

B-129

Managing Good Internal Quality Control by Adopting Risk Analysis Framework

J. J. Jeyaraman, Z. Sakiman, E. B. Tan. *Sunway Medical Centre Berhad, Petaling Jaya, Malaysia*

Background: Internal Quality Control (IQC) is the heart of quality assurance and plays a pivotal role in not only ensuring accurate and reliable patient results but also ensuring high standards of quality in materials, method performance and manpower. SUNMED lab has implemented newly designed Analytical Quality Control (AQC) strategy and it could actually improve overall assays monitoring and performance. However, the question on whether the newly designed AQC strategy alone is it effective enough for managing good analytical quality? Our objectives are to determine whether applying Risk Analysis Framework could actually reduce analytical and the probability of medical error, comply with accreditation standards and further improve customers' outcomes.

Methods: We have adopted Six-Step Risk Analysis Framework and came up with Quality Control Plan (QCP). We did the following:

• Firstly we identified the potential failures in incorrect test results.

• After that we estimated the risk using the probability, severity and detectability.

• The identified risks were evaluated and prioritized using criticality matrix (for example staff competency, IQC and test algorithm)

• In addition to that we identified control plans to reduce the proritized risks

• We further implemented the mitigation plans into QCP (for example staff competency, gap analysis, training strategies, revised test algorithm and AQC strategy)

• We also reviewed the system for effectiveness of QCP

Results: The results showed

• Significant risk reduction from the average criticality rating of 35 (unacceptable) down to 12 (acceptable); hence reducing the probability of medical error.

• Marked improvement in the ISO 15189 audit nonconformance from 17 in the year 2010 to 3 in Jan 2013 assessment.

• Improved overall customer satisfaction rate by 15% in the 2013 against the year 2011.

Conclusion: By adopting Six-Step Risk Analysis Framework and implementing it in QCP enables our SUNMED lab not only to mitigate but also assist in preventing possible hazard or risk that may occur before incorrect results are reported to health care providers and clinical actions being taken.

B-130

Head, Shoulders, Knees and Toes... Baby Steps to Specimen Nomenclature & Informatics

R. Merrick[1], C. Johns[2], P. Banning[3]. *[1]Vernetzt, LLC, Sacramento, CA, [2]LabCorp, Burlington, NC, [3]3M Health Information Systems, West Linn, OR*

Objective: Applying informatics to laboratory reporting involves complex standards and terminologies. Accurate portrayal of the specimen is crucial to appropriate processing at the front end, and correct clinical interpretation by the provider. Adding layers of computerization; secondary use cases of tumor registries, public health reporting, laboratory assay research; US federal Meaningful Use mandates in future years intensifies the needs. Work has been accomplished to date to create a standardized specimen cross-mapping table using the SNOMED CT terminology. The Specimen Cross-Mapping Table (CMT)'s goals are: 1) Give guidance to SNOMED CT ® encoding for Specimen related terms in the context of HL7® v2.x messaging using the Specimen (SPM) segment, 2) Identify gaps in the existing standard nomenclature, 3) Map the existing HL7 specimen terminology to SNOMED CT and 4) Provide references about common collection methods and 5) Indicate specimen preferences for specific laboratory domains.

Methods: Local specimens terms from partner labs were collected and mapped to a single term, called the Public Health Interoperability Project (PHLIP)-preferred term. For each PHLIP-preferred term a definition is provided as well as a link to a description of the collection method, where possible. Each PHLIP-preferred term is then mapped, either one-to-one or one-to-many, to the HL7 defined SPM fields, using

SNOMED CT concepts from the appropriate hierarchies for each applicable SPM field. HL7 terms were also mapped to the PHLIP-preferred term, facilitating a HL7 to SNOMED CT crossmapping. The resulting Specimen-CMT, at this time focused on human sample types, is now being reviewed by professional associations for the accuracy of the definitions as well as to provide validation about preferred specimen types for each laboratory section (microbiology, chemistry, pathology, etc).

Results: The Specimen-CMT has been incorporated into a simple mapping tool for ease of mapping local codes to standards and across standards. Several new terms have been submitted to SNOMED CT and guidance on use of specific vocabulary forwarded to message guide authors.

Conclusion: This work is ongoing at the Laboratory Messaging Community of Practice (LMCoP), comprised of laboratory and standard experts from public health laboratories at the state and federal level, national clinical laboratories, the National Library of Medicine and professional organizations, to improve and expand beyond human samples to animal and environmental domains. The goal is to provide a starter set of specimen related vocabulary for electronic data systems in the health care arena.

B-131

Test Overutilization is an Issue That Should be Solved: Folate Studies Taken From Internal to External Laboratory Reduced the Number of Orders by 76%!

E. Serin, A. Koro, I. Topcu, M. Gokce, T. Aksoy, A. Dogru, T. Kisaarslan. *Istanbul Research and Education Hospital Clinical Biochemistry Lab., Istanbul, Turkey*

Background: The most frequent reason for the heavy load of the routine clinical laboratories is overutilization of tests. Some of the requested parameters are not used by the clinicians in their daily practice. In addition, test turnaround times are gradually shortened by the laboratories to help the clinicians speed up their patient management. This study evaluated whether prolongation of the test turnaround times will affect the ordering frequency of the test by the clinicians.

Methods: The number of folate, vitamin B12 and ferritin test requests were counted in ten-day periods throughout January and February 2014. Between January 21st and February 2nd, folate requests were sent to an external laboratory because of the shortage of folate in the distributor's stocks. The supplier was not able to provide the folate test for about two months and our laboratory consumed its own stocks. Then we took the statistical numbers of requests of folate, vitamin B12 and ferritin for this ten-day period(TP2), before(TP1), and after this ten-day period(TP3). One-way ANOVA and Tukey post-hoc tests were used to compare the numbers of requests of the three parameters between these three ten-day periods. Statistical significance was set at $p<0.05$.

Results: There was a statistically significant difference between the three ten-day periods for folate orders as determined by one-way ANOVA($F(2.27)=173.745$,$p<0.001$). A Tukey post-hoc test revealed that the number of test requests was significantly different for folate orders between TP1(350.40±46.14), TP2(84.30±11.13) and TP3(146.40±33.06)($p<0.001$), whereas there was no statistically significant difference between the ten-day periods for B12($p=0.224$) and ferritin($p=0.155$).

Conclusion: This study indicated that there is an obvious excess of folate test request in our hospital. There was a remarkable difference between the essentially and the routinely ordered numbers of folate tests. In contrast, no such difference was observed for vitamin B12 and ferritin test requests. This remarkable gap between request numbers of parameters of even the same diagnostic panel such as anemia suggests that a limitation given to a test request may result in prevention of overutilization.

B-132

Developing, Implementing, and Validating Auto verification in the Medical University Laboratory in Thailand

S. Vanavanan, P. Srisawasdi, N. Teerakanjana, N. Kumproa, K. Khupusup, P. Kitpoka, S. Wongwaisayawan. *Pathology Department, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Objective: The purpose of this study is to build auto verification rules and evaluate against manual verification using historical data. Rules are optimized to match the expected outcomes from manual review.

Relevance: Instrument Manager provides a flexible middleware platform for implementing auto verification using its sophisticated rules engine, freeing the laboratory staff of other activities.

Methodology: Analysis results over a week were used to evaluate reliability of rules developed for 36 Chemistry and Immunology tests. These rules were developed to cover 3 major areas, Auto verification range, Delta check (%) and Integrity Check. Auto verification ranges were derived based on Reagent Assay Inserts, international guidelines, and laboratory experience. Delta check values were calculated as %RCV. Integrity checks were developed to ensure consistency, e.g. serum indices (HIL), contamination, etc.

Validation: Analysis results were processed through Instrument Manager based on rules developed. The same data set was compared with manual review and release. Adjustments to rule parameters were made to ensure auto verification closely matched manual review by reaching 0 % of false auto released results.

Results:

Metrics	Rules setting at the beginning		After revised rules set	
	Number	%	Number	%
Total Samples	7,676	100%	9,831	100%
Auto released Samples	6,248	81.4%	8,387	85.3%
Manual released samples	1,428	18.6%	1,444	14.7%
Released result correctly	7,259	94.6%	9,821	99.9%
False Auto released	196	2.6%	0	0%
False hold for manual released	221	2.9%	10	0.1%

Conclusion: Adoption of auto verification successfully reduced the tedious manual review process. 85.3% of test results were auto-released, and only on the remaining 14.7% required staff manual review. Of all these samples, almost 99.9% were released correctly compared to our manual review. Only 0.1% was held for review when they could not be auto-released. There is no impact on patient safety as manual check is required and no results were falsely released.

B-133

Improving Workflow in the SGC Phlebotomy Area Utilizing Front Line Co-workers and Lean Tools

J. K. Witt, B. Barksdale. *Mercy, Smith-Glynn-Callaway, Springfield, MO*

Background: The project addressed workflow in the clinic phlebotomy area utilizing lean and six sigma techniques. Workflow consisted of 6 phlebotomists working in 9 phlebotomy rooms. Phlebotomists collected 350 specimens a day with errors in collecting/receiving of the specimens in the LIS of 75 specimens a month. The area design was disjointed and inefficient, without defined roles for the phlebotomists in collecting/receiving and calling patients. No computers were in the phlebotomy rooms for collecting/receiving patient specimens.Two computers were located in the main phlebotomy area. The 6 phlebotomists used these two computers, resulting in bottlenecks, inventories and delays in specimen processing, inefficient movement, lost specimens and chaos.

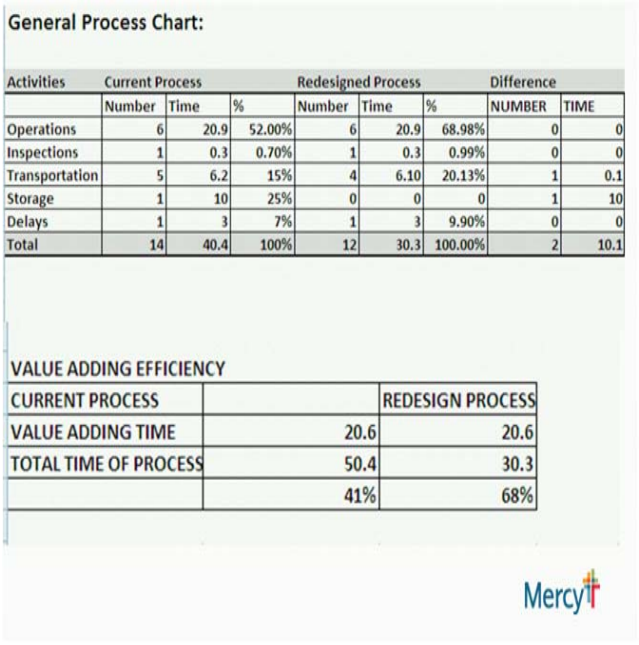
SGC Mercy clinic monitored unreceived specimen errors for 12 months. All errors are a source of patient, physician and co-worker dissatisfaction as well as an increase in cost and inefficiency.

Data was gathered to understand the current process and all phlebotomists were invited to participate. The data was placed in a 5w2h, general process, process activity, spaghetti and flow charts. Suggestions for improvement were also placed in charts for comparison.

Methods: Frontline co-worker focus groups, observation, VOC, VOP and flowcharts..

Results: The phlebotomists identified the need of computers in the main rooms and the need of better defined job roles. Unreceived errors improved by approximately 60%.

Conclusion: Involving front line co-workers to investigate and improve processes led to the 60% improvement in collecting/receiving errors. Value adding efficiency increased 27%. The phlebotomists are encouraged to make suggestions for further workflow improvements as well as debrief on each months issues and problems. Quality improvement is ongoing.



General Process Chart:

Activities	Current Process			Redesigned Process			Difference	
	Number	Time	%	Number	Time	%	NUMBER	TIME
Operations	6	20.9	52.00%	6	20.9	68.98%	0	0
Inspections	1	0.3	0.70%	1	0.3	0.99%	0	0
Transportation	5	6.2	15%	4	6.10	20.13%	1	0.1
Storage	1	10	25%	0	0	0	1	10
Delays	1	3	7%	1	3	9.90%	0	0
Total	14	40.4	100%	12	30.3	100.00%	2	10.1

VALUE ADDING EFFICIENCY

CURRENT PROCESS		REDESIGN PROCESS
VALUE ADDING TIME	20.6	20.6
TOTAL TIME OF PROCESS	50.4	30.3
	41%	68%

Mercy IT

B-134

Differences in laboratory requesting patterns in emergency department in Spain.

A. Andrade-Olivie[1], M. Lopez-Garrigos[2], J. L. Barbera[3], J. L. Quilez-Fernandez[4], J. L. Ribes[5], J. M. Gonzalez-Redondo[6], J. Sastre[7], J. V. Garcia-Lario[8], J. Asensio[9], J. I. Molinos[10], J. Molina[11], J. R. Martinez-Ingles[12], J. Diaz[13], L. Navarro-Casado[14], L. Martin-Martin[15], M. Salinas[2]. *[1]Hospital Xeral-Cies, CHU, Vigo, Spain, [2]Hospital Universitario de San Juan de Alicante, San Juan de Alicante, Spain, [3]Hospital de Manises, Valencia, Spain, [4]Hospital Universitario Reina Sofia de Murcia, Murcia, Spain, [5]Hospital de Manacor, Manacor, Spain, [6]Hospital Santiago Apostol, Miranda de Ebro, Spain, [7]Hospital Virgen de los Lirios, Alcoy, Spain, [8]Hospital Virgen de las Nieves, Granada, Spain, [9]Hospital Universitario Infantil Niño Jesus, Madrid, Spain, [10]Hospital Sierrallana, Torrelavega, Spain, [11]Hospital Comarcal de la Marina, Villajoyosa, Spain, [12]Hospital General Universitario Santa Lucia, Cartagena, Spain, [13]Hospital Francesc de Borja, Gandia, Spain, [14]Complejo Hospitalario Universitario, Albacete, Spain, [15]Hospital General de La Palma, Santa Cruz de Tenerife, Spain*

Background:Compare laboratory requiring patterns in patients admitted to emergency department (ED), in 76 Hospitals in Spain. Methods:20 tests ordered by ED physicians during 2012 were examined in a cross-sectional study. Data were collected from laboratory databases and indicators that measured every test request per 1000 ED admissions and related test requesting ratios, were calculated. Results:Table shows mean, median, range and variability index (Percentil90/Percentil10) of every indicator result. The frequency of ordering the stat tests ranged from 9.8 to 466.2 per 1000 ED patient's admissions. Procalcitonin and NT-proBNP were only measured in 61 and 49 stat laboratories respectively. Total proteins were measured in every ED. Albumin was measured in one. Also, lipase instead of amylase in one. Conclusion:Considerable variability exists in the use of stat laboratory test by physicians in 76 ED. Variability between centers was extremely high, especially in the less requested tests, despite clear indications of such request in emergency setting, indicating that can be often determined as a matter of routine or out of habit in some areas. These large variations included tests that are clearly redundant, as urea/creatinine and AST/ALT. What is really surprising is the high demand for some tests in some ED, such as procalcitonin and NT-proBNP, compared to the absence of measurement in other settings. The high variability of indicator results shows a probable stat abuse and misuse, a dangerous issue in Emergency setting. Requests not justified may lead to delays in testing for patients who have truly life-threatening conditions. Appropriateness indicators can be applied across a spectrum of laboratories, being useful for comparing requesting patterns. There is a need to unify demand by optimizing the use of appropriate tests, through interdepartmental communication to achieve a good use of diagnostic testing, on which many emergency clinical decisions are based.

	Mean	Median	Range	Variability index
Tests requesting per 1000 ED admissions				
Alanine transaminase (ALT)	147,81	127,85	0,00-607,62	50,39
Albumin	16,95	0,65	0,00-415,69	∞
Amilase	97,81	93,85	0,58-228,06	3,45
Aspartate transaminase (AST)	143,40	112,70	0,00-609,60	∞
Brain natriuretic peptide (NT-proBNP or proBNP)	14,52	1,57	0,00-96,31	∞
Calcium	66,03	30,40	0,00-425,80	45,46
Cell Blood Count (CBC)	466,21	400,40	59,15-1053,42	2,63
C-Reactive protein (CRP)	232,11	233,44	0,00-692,84	8,33
Creatine Kinase (CK)	115,61	111,89	2,03-468,67	31,98
Creatinine	438,07	395,71	33,92-999,33	2,62
Glucose	425,38	394,76	36,45-988,32	2,42
Lipase	9,87	0,00	0,00-181,85	∞
Potassium	434,37	399,15	35,44-1004,40	2,43
Procalcitonine (PCT)	18,09	10,88	0,00-145,06	∞
Sodium	427,16	398,76	25,48-1004,39	2,40
Total bilirubin	116,90	100,61	0,00-521,56	20,84
Total protein	66,54	22,21	0,11-429,67	197,05
Troponin	104,92	99,35	0,03-230,16	2,93
Urea	395,26	373,70	1,88-997,29	3,89
Urinalysis	160,49	161,07	9,29-439,74	3,14
Related test requesting ratio				
AST/ALT	8,84	1,00	0,00-537,63	∞
CK/Troponin	2,53	1,07	0,02-68,50	17,91
CRP/CBC	0,54	0,61	0,00-0,95	7,88
PCT/CRP	0,15	0,05	0,00-2,84	∞
Urea/Creatinine	0,91	1,00	0,00-1,43	1,86

B-135

Impact of Technology in Improving the Quality of Pre-analytical Phase of Laboratory Investigations in a Tertiary Care Oncology Center.

C. V. Hingnekar, H. K. V. Narayan. *Tata Memorial Hospital, Mumbai, India*

Background: This is a study to assess the impact of technology in improving the processes in the preanalytical phase of laboratory investigations as compared to the erstwhile manual method. The objective of this study is to assess the impact in terms of turnaround time, elimination of transcription and labeling errors resulting in enhanced patient satisfaction. The technologies such as Laboratory Information System for Requisitioning and Reporting, Smart card for patient identification and payments, Patient Summoning System, Automatic Phlebotomy tube Labeler (APTL) and Pneumatic Tube System have been adopted as part of the Automation.

Methods: A comparative study based on objective and subjective parameters was carried out to find out the efficacy of the automated system over erstwhile manual operations. 29 staff members from different sample collection areas and lab personnel who had experienced both the systems were interviewed and their responses were tabulated.

Results: The comparative data after automation reveals that there is a significant reduction in sample labeling time (75%), reduction in transcriptional errors (81%) and reporting errors (100%) as compared to the manual era. Sample collection per day has increased by 166%. Similarly with the use of pneumatic tube system the time required in delivering the samples has decreased (93%) so has the breakage due to mishandling (10%). The need for repeat sample collection also decreased (66%). By installation of Patient summoning system the average patient waiting time is reduced by 73%.The issues of patient identification and traffic has improved.

Conclusion: The results show that with the introduction of integrated automated systems working in tandem there has been a significant reduction in Turn around Time and elimination of errors. Staff efficiency has improved and so has the quality of care resulting in improved patient satisfaction. It is evident therefore that technology driven management can improve the quality of patient care, however the challenge of perpetual innovation and maintenance of such systems remain.

B-136

Recommendations for New QC Rules Based on Precision from 2012 Data.

d. S. plaut[1], N. Lepage[2], T. Taylor[3], W. McLellan[4]. *[1]unaffiliated, Plano, TX, [2]Children's Hosp. Eastern Ontario, Quebec, ON, Canada, [3]Ingalls Hospital, Chicago, IL, [4]unaffiliated, Hollywood, FL*

Objectives: To show that data based on current precision indicate that new QC rules be seriously considered.

Relevance: Current data indicate a significant increase in precision since the first QC rules were proposed by CAP (1974) and AACC (1981).

Methodology: Two CAP (1988 and 2012) quantified precision. Our survey of 35 analytes from chemistry, hematology and hemostasis indicated a decrease of 58%

in CV (average; range 22-83). We 'translated' the 2012 SD into a new set of rules to detect both systematic and increased random errors. Our translations indicate these rules to be quite effectively: 1 4SD, 2 3SD and R 5 SD with cumulative sum for certain analytes.

Results: The table shows representative changes.

Conclusions:

1) The improvements in precision indicate that QC rules be reevaluated. These changes significantly reduce false rejects while increasing error detection and lower patient risk.

2) Each analyte should be assessed to determine the proper rule(s).

Conclusion:

Representative Data

Analyte	CAP 1988		CAP 2012		
	Mean	%CV	Mean	%CV	% Decrease
Cholesterol	330mg/dL	5.6	217mg/dL	1.0	87
Hemoglobin	19.5gm/dL	3.8	15.1gm/dL	1.7	41
Prothrombin Time	19.0 sec.	6.4	11.5 sec	2.8	56

B-139

Changes in Primary Care Requesting patterns in a two year period in Spain

M. Salinas[1], M. Lopez-Garrigos[1], M. Ortuño[2], M. Graells[3], M. Garcia-Collia[4], M. Yago[5], M. Frutos[6], N. Estañ[7], N. Fernandez-Garcia[8], P. Garcia-Chico[9], A. Rodriguez-Piñero[10], R. Franquelo[11], R. Gonzalez-Tamayo[12], S. Pesudo[13], V. Granizo[14], V. Villamandos[15], V. Perez-Valero[16]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]Hospital Universitario La Ribera, Alzira, Spain, [3]Hospital General Universitario de Alicante, Alicante, Spain, [4]Hospital Ramon y Cajal, Madrid, Spain, [5]Hospital de Requena, Requena, Spain, [6]Hospital Universitario Nuestra Señora de la Candelaria, Tenerife, Spain, [7]Hospital Dr Peset, Valencia, Spain, [8]Hospital Universitario Rio Hortega, Valladolid, Spain, [9]Hospital General Universitario de Ciudad Real, Ciudad Real, Spain, [10]Hospital Universitario de Mostoles, Mostoles, Spain, [11]Hospital Virgen de la Luz, Cuenca, Spain, [12]Hospital de Torrevieja, Torrevieja, Spain, [13]Hospital La Plana, Valencia, Spain, [14]Hospital Universitario de Guadalajara, Guadalajara, Spain, [15]Hospital Santos Reyes, Aranda del Duero, Spain, [16]Hospital Regional de Malaga, Malaga, Spain*

BACKGROUND: To compare Primary Care Requesting patterns between two different years in Spain, using appropriateness indicators, to try to ascertain if better demanding behaviours are achieved along years.

METHODS: 36 and 76 laboratories for the year 2010 and 2012 respectively from diverse regions across Spain filled out the number of 29 tests requested by GPs.

Two types of appropriateness indicators were calculated. Every test requests per 1000 inhabitants of the following: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (CA), cell blood counts (CBC), C-reactive protein (PCR), cholesterol (CHOL), creatinine (CREA)erythrocyte sedimentation rate (ESR), ferritin (FERR), phosphate (FOSF), gammaglutamiltranspeptidase (GGT), glucose (GLUC), HDL-cholesterol (HDL), glycated hemoglobin (HBA1), iron (IRON), lactate dehidrogenase (LDH), prostate specific antigen (PSA), thyrotropin (TSH), total bilirubin (TBIL), triglycerides (TG), urate (URAT), urea (UREA), urinalysis (URIA). And ratio of related tests requests.

Free PSA/PSA (FPSA/PSA), aspartate aminotransferase/alanine aminotransferase (AST/ALT), direct bilirubin/total bilirubin (BILD/BIL), folate/B12 vitamin (FOL/B12), free thyroxin/thyrotropin (FT4/TSH), urea/creatinine (UREA/CREA)

RESULTS: In spite of the differences observed, CBC, glucose and urate were less requested and TSH more requested in the second period, no significant differences were found in any of the studied tests.

DISCUSSION: Our results suggest that test requesting in Primary Care in Spain have not varied in a two year period. At least achieving targets in related tests requesting ratios, as AST/ALT, is necessary. The showed figures can be used as a pillar foundation to be based in ulterior interventions to achieve appropriate requesting.

CONCLUSION: A more active Clinical Laboratory behavior is necessary to lead to a better laboratory tests appropriate Primary Care requesting.

	2010			2012			p value
	Mean	*Standard deviation*	*CI95%*	*Mean*	*Standard deviation*	*CI95%*	
Tests per 1000 inhabitants							
ALP	139,12	74,28	114,36-163,89	149,97	76,69	132,44-167,49	0,478
ALT	332,05	65,97	310,05-354,04	319,27	66,46	304,09-334,46	0,339
AST	257,58	105,85	222,29-292,87	252,69	111,90	227,12-278,26	0,825
CA	88,22	60,60	68,01-108,42	98,89	71,49	82,55-115,23	0,436
CBC	387,53	93,15	353,95-421,11	359,17	75,45	340,32-378,02	0,112
CRP	60,65	39,38	47,52-73,78	58,10	35,88	49,84-66,35	0,732
CHOL	357,10	57,41	337,96-376,24	339,88	74,74	322,80-356,96	0,220
CREA	351,73	64,38	330,27-373,20	343,05	77,96	325,23-360,86	0,558
ESR	105,53	66,85	81,42-129,63	92,91	55,77	78,86-106,96	0,333
FERR	130,91	33,73	119,33-142,50	127,79	43,68	117,67-137,91	0,710
FOSF	61,00	57,57	41,52-80,48	61,29	53,13	49,15-73,43	0,979
GGT	258,95	79,82	232,34-285,56	251,06	86,66	231,26-270,86	0,642
GLUC	372,61	61,66	352,06-393,17	361,88	72,65	345,28-378,48	0,441
HDLC	274,97	63,21	253,90-296,04	260,71	75,16	243,54-277,89	0,322
HBA1	85,04	22,53	77,53-92,55	89,14	27,59	82,83-95,44	0,435
IRON	131,66	51,13	114,10-149,23	133,53	56,96	120,33-146,72	0,869
LDH	49,69	59,16	29,96-69,41	43,90	49,98	32,48-55,32	0,588
PSA	51,08	14,19	46,35-55,81	52,81	16,52	49,04-56,59	0,586
TSH	174,38	41,71	160,47-188,29	186,58	46,01	176,07-197,10	0,176
TBIL	138,67	67,61	115,79-161,55	151,27	80,08	132,97-169,57	0,416
TG	342,36	53,57	324,49-360,22	325,05	73,80	308,19-341,91	0,206
URAT	298,46	74,06	273,77-323,16	276,77	85,15	257,18-296,36	0,189
UREA	218,25	106,80	182,64-253,86	208,65	116,09	182,12-235,18	0,673
URIA	196,71	75,37	171,59-221,84	210,07	71,50	193,62-226,52	0,363
Related test requested							
FPSA/PSA	0,15	0,10	0,12-0,18	0,22	0,68	0,06-0,38	0,545
AST/ALT	0,78	0,28	0,69-0,87	0,80	0,35	0,72-0,88	0,742
BILD/TBIL	0,11	0,19	0,05-0,18	0,12	0,23	0,07-0,18	0,822
FOL/B12	0,93	0,12	0,89-0,97	0,92	0,14	0,89-0,95	0,710
FT4/TSH	0,37	0,23	0,30-0,45	0,37	0,22	0,32-0,43	0,955
UREA/CREA	0,63	0,31	0,53-0,74	0,62	0,35	0,55-0,70	0,906

B-143

Environmental resource management of an ISO 14000 certified clinical laboratory in Brazil

A. L. T. Araújo, N. B. C. S. Almeida, J. A. R. Vaz, S. S. S. Costa, L. F. A. Nery. *Sabin Laboratory of Clinical Analysis, Brasília, Brazil*

Background: Our clinical laboratory, with headquarters in Brasilia - DF, Brazil, makes 1.7 mi exams per month and it has an environmental resource management system, with ISO 14000 certification, that controls several processes in order to reduce environmental impact in our activities. The purpose of this study is to know the environmental impact that the laboratory's activities had in the ecosystem in 2013 (environmental performance) and to share the monitoring methods, treatments and control used in a health care services institution.

Methods: We measured the consumption of natural resources (water, electric energy and paper), fossil fuel for sample transportation, plastic bags, biomedical and solid waste, paper and electronic waste recycling, printing savings through exam results checked online (lab's website). In short, the biomedical waste is taken to a waste treatment facility and eliminated using pyrolysis, the effluent is treated through an oxidative process using ferric chloride and sodium hypochlorite solution and the environmental monitoring is performed through measurement of chemical oxygen demand (COD) and biochemical oxygen demand (BOD).

Results: The laboratory's environmental performance in 2013 can be seen in the table below:

Environmental performance - 2013	
Water consumption (M^3)	5497
Electric energy consumption (kW)	2.087.898
Paper consumption - white paper (unit)	12.920.000
Paper consumption - recycled paper for reports (unit)	4.191.662
Printed pages (unit)	21.126.179
Waste sorting (recycling) (Kg)	4.038
Fuel consumption (liters/year)	91.744
Plastic - plastic bags for waste (unit)	338.7
Plastic - Oxo biodegradable plastic bags (unit)	956.053
Plastic - plastic cup (unit)	3.001.800
Recycled electronic waste (Kg)	824
Chemical residue treatment (Liters/day)	2000
Biomedical waste treated (Kg)	203.166,25
Exam results checked online	794.614
Printing and paper savings (unit)	6.356.912

Conclusion Identifying the environmental performance of the clinical laboratory allowed us to see the impact of the activities in the ecosystem. This knowledge encourages all workers to engage in different processes, the involvement of service users, and it spreads the environmental resource management practices of the ISO 14000 certification. The environmental performance is assessed every semester and the results are available in the company's website and in the sustainability report published by the United Nation Global Compact every year, in their website.

B-144

Critical laboratory tests values notification according to the Internacional Patient Safety Goals by Joint Comission in a general Private Hospital in São Paulo, Brazil.

L. R. Almeida. *DASA, São Paulo, Brazil*

Background: A laboratory critical value refers to an extremely abnormal laboratory test result which may be life threatening if treatment is not initiated immediately. The number of critical values results will influence laboratory workload, clinical care and patient treatment. International Paciente Safety Goals (IPSG) has been introduced in Brazil recently to reach the Joint Comission acreditation standards. The notification of the critical laboratory tests results, recommended by the goal number 2 (improve effective communication) of IPSG, is one of the most challenging surveys for the clinical laboratories.

Methods: Critical laboratory tests results of a 300 bed general hospital were analyzed from August 2013 to December 2013 using the laboratory information system. The five main tests were selected to understand the Hospital profile according with the tests results and to establish a monthly follow up of the notifications registered on the laboratory system according with the TJC recommendation.

Results: From August to December 2013, 5834 tests from a total of 441.571 (1,3%) fulfilled the definition of a critical value. The five more representative tests that showed critical results were: Prothrombin time with 580 (11%), Partial thromboplastin time with 580 (11%), pH with 285 (5%), Troponin with 216 (4%) and K with 118 (2%). The notification of these values was improved along the months reaching 87% in the last month of this series. The final objective is to reach 100% notification according to the recommendation of TJC.

Conclusion: IPSG is very important to improve the patient safety and can also be used to improve the laboratory procedures and the communication with the hospital staff. The results we have obtained in this study were compared with the ones from another Hospital that has already reached the second period of accreditation by TJC. We noticed that goal number 2 from the IPSG was improved among the months in that hospital, reaching 100% of successful communication of the critical values. The main driver that supported this goal was a very effective continuous education of the laboratory team.

B-146

Quality Assessment and Management in Clinical Diagnostic Laboratory Medicine

J. Kalra[1], R. Sachdeva[2], N. Kalra[2]. *[1]University of Saskatchewan and Royal University Hospital, Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada*

Background: Laboratory results play a major role in guiding decisions in patient management. In laboratory medicine, meaningful, accurate and precise measurements are essential for diagnosis, risk management and treatment. Various strategies have been adopted to reduce laboratory errors including internal quality control (QC) procedures and external quality assessment (QA) programs. Autopsy services also play a major role in contributing to clinical knowledge, medical education and quality assurance programs. The purpose of this study was to assess Proficiency Testing (PT) programs and to determine the rate of concordance and discordance between clinical diagnoses and post-mortem findings.

Methods: Our clinical laboratory participated in two external PT programs: Provincial Health Metrx (mandated by College of Physicians and Surgeons of Saskatchewan) and College of American Pathologists (CAP) survey. The clinical laboratory tests commonly used to manage patients were examined for the period of one year for any discrepancies. We also retrospectively reviewed the records of the medical and autopsy charts for all the deceased adult in-patients admitted during 2002 to 2004 in the hospitals of Saskatoon Health Region (SHR). A total of 3416 in-patient deaths were registered during the study period. In accordance with selection criteria, 158 cases were included in this study. The mean age of subjects was 66.6 ± 15.3 years with a range of 16-94 years. The study group consisted of 92 males (58.2%) and 66 females (41.8%) with an average length of stay at the hospital of 12.9 ± 10.9 days. In addition, we evaluated the impact of diagnostic modalities such as Computerized Tomographic Scanning (CT) and Magnetic Resonance Imaging (MRI) on clinical diagnoses.

Results: In the Health Metrx PT, 5 groups of 43 analytes were analyzed and from 598 tests, 5 discrepancies resulted, yielding a total discrepancy rate of 0.84%. In the CAP survey PT, 12 groups of 58 analytes were analyzed and of 431 tests, 3 discrepancies resulted, yielding a total discrepancy rate of 0.70 %. In both surveys the remaining tests had no discrepancies. The autopsy results showed that the concordance rate between clinical and autopsy diagnosis was 70.9%. The discordance rate was 24% and in 5.1% of the study population a conclusive clinical or autopsy diagnosis was not finalized. CT scans and MRI were found to be confirmatory or diagnostic in 85% and 93% of the autopsy patients in which these modalities were used.

Conclusion: The quality in the clinical laboratory is maintained in a satisfactory manner, meet the performance criteria and requirements set up by provincial regulatory agencies. It is prudent to monitor, promote and enhance quality services for our patients. The study confirmed that the concordance and discordance rates between clinical diagnosis and post-mortem findings in SHR are consistent with those reported in the literature. Also, despite the technical advances in diagnostic modalities, diagnostic discrepancies remain prevalent in the present day health care system. The study also emphasizes the value of PT programs and autopsies as an effective quality improvement and educational tool with a strong impact on quality management.

B-148

Sigma metric's impact on analytical performance in a chemistry area of a reference clinical laboratory A case study at the clinical laboratory of Hospital Pablo Tobon Uribe " the hospital with soul "

A. PORRAS[1], A. M. BEDOYA[2], S. LOPERA[2], S. JARAMILLO[2], D. RIOS[2]. *[1]QUIK, BOGOTA, Colombia, [2]HOSPITAL PABLO TOBON URIBE, MEDELLÍN, Colombia*

Sigma metric's impact on analytical performance in a chemistry area of a reference clinical laboratory A case study at the clinical laboratory of Hospital Pablo Tobon Uribe " the hospital with soul " Key words: analytical performance, sigma metrics. Background: The most frequently method used to validate analytical runs in clinical laboratories is the Levy Jennings (LJ) graph, but this tool can control only the stability of the process, not the size of the analytical errors. Lack of control on the size of the analytical error, could produce misleading or misinterpretation, resulting in wrong laboratory results. Controlling analytical errors contributes to increase the reliability and clinical utility of the results. Analytical error control is time-consuming and demands effort, causing discouragement sometimes. The sigma metrics allow control of analytical errors for a big group of analytes with different total allowable errors, contributing to the improvement of analytical performance in clinical laboratories. Methods: At the Clinical Laboratory of Hospital Pablo Tobon Uribe in Medellín, Colombia on June, 2011, the sigma metric approach was implemented to control the performance of 25 analytesof clinical chemistry by awareness concepts, personnel training, using a specialized software, implementing connectivity for quality control data and showing new graphing tools such as performance per percentiles, sigma metric and total error integrated with Levy Jennings in a specialized software . For the initial diagnosis, the sigma metric was measured using a variety of quality control specifications as biological variability, RilliBAK, CLIA and the state of the art by percentiles. After this, multiple corrective actions were implemented and quality specifications were initially selected, but later on were changed to increase the constraint with the objective to monitor analytic imprecisión. Results: From June 2011 to October 2013,the performance improvement of the analytes, controlled through sigma metricwas considerable. The decrease of the imprecision, bias and analytical total error was observed in the majority of the analytes, the percentage of the analytes with sigma metric >5 increased from 68% to 79.4% and the percentage of the analytes with sigma metric <1.96 decreased from 14% to 2.94%. The trend of performance improvement is clearly observable during these two years as a result of the homogenization of knowledge, process standardization, continuous monitoring, rigorous analysis and corrective actions. Conclusions:The implementation of sigma metricsand its graphs shows the errors as errors per million of opportunities that exceed the total error allowable defined. Additionally the behavior of analytes is displayed, through a "quick glance", contributing to a decrease in analytical errors. The diversity of situations that occur at clinical laboratories, such as differences in workloads, stabilities of reagents, adjustment or calibration times and clinical metrological requirements,influencesthe analytical performance. These situations can be controlled by sigma metric, providing a standard measure that allows for the monitoring of the performance of a large amount of analyte, even despite the diversity.

B-149

Collaboration between ER and Lab to Improve Troponin Turnaround Times

A. M. Boelstler. *St. John Hospital & Medica Center, Detroit, MI*

Purpose: The purpose of this project is to improve the care of Acute Coronary Syndrome (ACS) patient by developing a collaborative relationship with the Emergency Room (ER) and laboratory staff in order to decrease the turnaround time (TAT) of door to troponin result. The goal is door to troponin result in less than 30 minutes. Early diagnosis and medical management of patients with ACS improves the overall outcome in patients presenting to the ER with a complaint of chest pain. Cardiac markers are used in the diagnosis and risk assessment of patients with chest pain.

Design: A multidisciplinary team was formed to create a process and guidelines for the ACS patient that presents to the emergency room. This team worked collaboratively to initiate change within the triage area to reduce draw times, result times, and decrease hemolysis rates.

Participants: A Chest Pain Committee encompassing physicians, registered nurses (RN), emergency room technicians (ERT) , phlebotomists, and lab representatives.

Methods: Multiple meetings were held between the lab and the ER to understand each other's processes in relation to the entire procedure from door to troponin result. Each area worked together, not in silos, to improve the time for each step. Well-defined guidelines were developed for the initial treatment of the ACS patient in triage. Based on these guidelines, the triage RN quickly determines if the patient meets Cardiac Markers guidelines and directs the ERT and phlebotomist accordingly. A phlebotomist is stationed in triage at all times. If cardiac marker guidelines are met, the cardiac markers will be drawn by the phlebotomist while the ERT is performing an EKG. The ERT or RN will be responsible for the lab draw if multiple draws are needed. The introduction of a Mint Green lab tube was added specifically for Troponin levels in the ED. Troponin specimens from this tube can be processed immediately and help us to meet the <30 min Troponin TAT goal. Following the initiation of this process, data was collected and interpreted to determine the success of our process improvement.

Results/Outcomes: Emergency Room and Lab developed a collaborative relationship of professionalism and partnership. Data was received that exemplifies how we have improved the care of the ACS patient.

Order to draw times has decreased from 55 minutes to 8 minutes.

Draw to received specimen has decreased to 3 minutes.

Our door to Troponin result time is averaging 47 minutes with data collection continuing.

Troponin hemolysis rates have decreased from 15% to 1%

Implications: A collaborative Team Approach is being utilized in the care of the ACS patient to improve both patient and associate satisfaction. The care of the ACS patient has improved significantly as evidenced by our data interpretation. Data continues to be collected to evaluate the ongoing effectiveness of our process improvement.

B-150

Designing QC Rules for Multiple Instruments: Should a QC Rule be Centered on Individual Instrument Means or on a Fixed Mean? Should a the limits be based on Individual Instrument SD's or on a Fixed SD?

L. S. Kuchipudi, J. C. Yundt-Pacheco, C. A. Parvin. *Bio-Rad Laboratories, Plano, TX*

Background: Objective - Compare performance of QC strategies centered on the individual instrument mean versus a fixed mean and limits based on individual instrument SD versus a fixed SD when applied to multiple instruments testing the same analyte.

Relevance - Using a fixed mean and SD appears to be a common practice when multiple analytic units evaluate the same analyte. The comparative efficacy of this approach has not been formally evaluated. We compare the expected number of unreliable final results reported due to the occurrence of an out-of-control condition, $E(N_{uf})$, when the QC rule means are centered on the instrument means versus a fixed mean and when the QC rule limits are established based on the instrument SD's versus a fixed SD.

Methods: We consider 4 analytic units in the laboratory evaluating the same analyte. We assume each instrument evaluates 2 QC levels using a $1{:}3_s/2{:}2_s/R{:}4_s$ QC rule every 100 patient examinations. The fixed mean is set to the average of the instrument means. The fixed SD is set so the overall false rejection rate across the 4 instruments is 0.0097. We investigate a range of means and SD's for the 4 instruments. The resulting instrument CV and bias combinations have sigma values $[(TE_a - bias)/CV]$ ranging from 3 to 6. We determined the maximum value for $E(N_{uf})$ and area under the $E(N_{uf})$ curve for the 4 different cases:

1. QC rule means centered on instrument means and QC rule limits based on instrument SDs

2. QC Rule means centered on a fixed mean and QC rule limits based on instrument SDs

3. QC rule means centered on instrument means and QC rule limits based on a fixed SD

4. QC rule means centered on a fixed mean and QC rule limits based on a fixed SD

In each of the above cases we design the QC rules so the overall false rejection rate across the 4 instruments = 0.0097, which is the false rejection rate for the $1{:}3_s/2{:}2_s/R{:}4_s$ QC rule.

Results: Tables of results are computed for the simulations. In general, the maximum $E(N_{uf})$ and the area under the $E(N_{uf})$ curve were lowest for rules using a fixed mean and fixed SD for the instruments.

Conclusion: Using a fixed mean and fixed SD for the QC rule had the best performance. The fixed mean appears to balance the risk of reporting unreliable results across multiple instruments while the fixed SD allocates more false rejection rate to poorer performing instruments resulting in a lower overall risk of reporting unreliable results when individual instruments have moderate to good process capability (3-6 sigma).

B-151

Evaluating the Reproducibility of Analysis in the Clinical Laboratories. Results from a proficiency testing (PT) scheme and comparison with biological variability.

D. Rizos[1], O. Panagiotakis[2], K. Makris[3], A. Haliassos[2]. *[1]Hormone Laboratory, Aretaieion Hospital, Medical School, University of Athens, Athens, Greece, [2]ESEAP Greek Proficiency Testing scheme for Clinical Laboratories, Athens, Greece, [3]Clinical Biochemistry Department, KAT General Hospital, Kifissia, Greece*

The quality criteria for the proficiency testing (PT) schemes in Laboratory Medicine include the evaluation of the accuracy of the participating laboratories, the use of human origin commutable testing materials (sera) that minimize matrix effects, the evaluation of linearity of assays by using at least two samples per distribution and the estimation of the reproducibility of measurements.

In order to fulfill these criteria, ESEAP (the Greek PT scheme in Laboratory Medicine) introduced the measurement of reproducibility using the analysis of the same sample four times during a yearly cycle. The quality goal for the participating laboratories, is the reproducibility to be at least 50% of the biological variability for each analyte. (Ref: Ricos et al. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500, revision 2014).

In this study we used results from the 290 laboratories of ESEAP in Greece and Cyprus and we evaluated them against the proposed limits derived from the biological variability for each of the analytes included in the "clinical chemistry" scheme.

We excluded the results from laboratories that haven't reported all the four samples, from the laboratories that where excluded from the normal bias analysis (elimination in two-passes of all results > or <2.5SD of the consensus mean value) and the laboratories that reported a method change during this cycle. We finally processed 4 results from 209 laboratories and for 21 different parameters and we calculated the mean value of reproducibility for each parameter.

These results are presented at the following table:

Analyte	Biological Variability (%)	Proposed limits (%)	Measured Reproducibility (%)
Glucose	5.60	2.80	2.31
Urea	12.10	6.05	3.36
Creatinine	5.95	2.98	***4.16***
Sodium	0.60	0.30	**1.42**
Potassium	4.60	2.30	1.78
Total Protein	2.75	1.38	***2.55***
Albumin	3.20	1.60	***2.85***
Cholesterol	5.95	2.98	2.68
HDL- Cholesterol	7.30	3.65	3.57
Triglycerides	19.90	9.95	2.89
Uric Acid	8.60	4.30	2.82
Total Bilirubin	21.80	10.90	4.14
Calcium	2.10	1.05	**2.55**
Phosphate	8.15	4.08	2.63
Magnesium	3.60	1.80	***3.60***
Iron	26.50	13.30	4.49
SGOT (AST)	12.30	6.15	3.13
SGPT (ALT)	19.40	9.70	5.30
γ-Glutamyl Transferase	13.40	6.70	4.59
Creatinine Kinase	22.80	11.40	8.90
Amylase	8.70	4.40	3.08

Our results show that all the currently used methods outperform the proposed quality goals, except for the cases of **Sodium** and **Calcium** as also as of ***Creatinine, Total Protein, Albumin*** and ***Magnesium*** where the results are below the biological variability but not below the quality goal of 50% of the biological variability.

B-152

Relational Data Modeling Approach to Demonstrate Value of the Clinical Laboratory in Healthcare Systems

S. Chekuri, B. Clements, B. Brimhall. *University of Mississippi, Jackson, MS*

Background: Adoption of electronic health records has will likely expand over the next several years due to incentives and penalties in the American Recovery and Reinvestment Act. Furthermore, there is growing interest in mining and analyzing this data to evaluate costs, practice patterns, and clinical effectiveness. We evaluated the impact of the ability to efficiently link laboratory data to external sources for large hospital projects. We also developed an appropriate data model to facilitate such data integration.

Methods: We extracted and linked clinical and operational data from existing sources (EPIC Systems®, Cerner CoPath®) which were also joined to detailed financial data (Allscripts®) for each department as well as the hospital system as a whole as well as external data documents (e.g., external price proposals). Linked data were used prospectively to evaluate the pro forma return on investment (ROI) for hospital projects involving the laboratory as well as the impact of such projects on workflow and quality measures when applicable. Linked data were employed retrospectively to evaluate the actual ROI and impact on clinical operations. We specifically looked at two large recent projects (2013-2014): a proposal to purchase a new microbiology system (prospective) and the results of a project to streamline radiology patient flow using POCT (retrospective). In each case, we examined the impact of absent, or poor, linking of laboratory to external data sources on each project. Based on our work we constructed a relational data schema with Navicat® software to connect disparate clinical, operational, and financial data sources.

Findings: The first project was an evaluation to purchase a MALDI-TOF instrument for bacterial identification. Without efficient links to external data sources we had to rely on cost savings resulting from lower reagent costs. With only this information projected measures of ROI were: payback period = 20.6 years, net present value (NPV) = ($228,519), and modified internal rate of return (MIRR) = -22.34%. None of these measures argues financially to pursue the project and one would have to rely on non-financial factors to make the case for purchase. We then re-evaluated the project including of cost savings resulting from earlier adoption of appropriate treatment of patients with sepsis (MSDRGs 371-373) and earlier discharge (based on actual costs and volumes at our hospital system). With this linked information, measures of ROI were: payback = 12.62 weeks, NPV = $5,471,954, and MIRR = 80.79%. All of these measures are highly favorable for the project. For the second project we evaluated the impact of a joint laboratory/radiology project on net revenue for radiology contrast studies. After implementation of the POCT intervention, the annual patient volume for these studies increased by 906 resulting in a net contribution of $410,776. As a project the actual payback period was 22.1 days. Patient satisfaction measures were also demonstrably better.

Conclusion: Linking laboratory data and data from external sources allows laboratory professionals to 1) provide optimal data for making clinical and operational changes, 2) credibly demonstrate value to hospital administrators, and 3) foster collaborative projects throughout the hospital system.

B-153

Comparison of critical results frequency for point-of-care testing (POCT) vs. STAT core laboratory testing among critical care unit patients: a management review regarding POCT utilization

S. K. Mardekian, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: A point-of-care analyzer (EPOC, Epocal, Ottawa) was recently introduced into a critical care unit (CCU) at our institution to measure blood gases (pH, pO2, pCO2), electrolytes (Na, K, ionized Ca), metabolites (glucose, lactate), and hematocrit (HCT) in select patients. Because of cost considerations, POCT was originally restricted for use in patients meeting conditions either of use of extracorporeal oxygenation (ECMO), use of a ventricular assist device (VAD), need for resuscitation ("code"), or known hemodynamic instability. In a setting where STAT testing is frequently warranted, selective use of POCT raised the issue of whether uniformity of standard of care was compromised when POCT was not universally deployed. We performed a six-month review of data to compare frequency of critical values between CCU samples for which POCT was utilized (POCT) and those CCU samples for which STAT core laboratory testing (CORE) was performed instead, to assist in management evaluation of whether lesser restriction on use of POCT should be advocated in this setting.

Methods: POCT and CORE laboratory test results for CCU patients for a six-month interval were obtained from electronic records. Glucose was excluded from the analysis because of overlap with extensive use of separate POCT glucose analyzers. The number and percentage of critical results among POCT and CORE samples were determined for 8 analytes as follows (analyte (critical ranges)): pH (<7.2 or >7.6); pO2 (<40 mm Hg); pCO2 (<20 mm Hg or >70 mm Hg); Na (<120 mmol/L or >160 mmol/L); K (<2.5 mmol/L or >6.5 mmol/L); iCa (<3.3 mg/dL or >6.5 mg/dL); lactate (>3.3 mmol/L); HCT (<20).

Results: POCT data comprised 261 panels of 8 analytes (2088 measurements) from 64 patients. CORE data comprised 11711 measurements from 206 patients. 58.6% of all 261 POCT panels had one or more critical results, with an overall critical result percentage of 11.9% among the 8 analytes. CORE test results had an overall critical result percentage of 3.1%. However, the ratio (R) of absolute number of critical results for POCT/CORE was 249/367 (R=0.67). This low value for R is likely to be an upper limit (that is, R is at most 0.67), in consideration of the fact that POCT results are bundled within a panel rather than ordered independently (i.e., some number of POCT critical results are likely to be an overcount due to untargeted repeat testing).

Conclusions: CCU use of POCT demonstrated high preselection for critical results. However, absolute numbers of critical results in the CCU were substantially greater from among CORE (non-POCT) results (R<1). Given R<1, and in consideration of the difference in expected turn-around-times between POCT and CORE testing (nominally, <10 min for POCT vs. up to 60 min for certain CORE analytes), use of CORE testing in the CCU might be regarded as a lesser service to patients relative to use of POCT. These findings supported a management recommendation that use of EPOC POCT in the CCU should be less restricted in order to maintain a more uniform standard of care.

B-154

Monitoring the Quality of Results from the ARCHITECT Analyzer Using Six Sigma Metrics Generated by EP Evaluator Software

S. Cox[1], S. Westgard[2], A. Carrillo[3], R. Wonderling[3]. *[1]Saint Francis Health System, Tulsa, OK, [2]Westgard QC, Inc., Madison, WI, [3]Abbott Diagnostics, Abbott Park, IL*

Quality of lab results has a direct impact on patient care. Six Sigma is a universal benchmark to measure quality. We used Sigma metrics as a quality indicator to objectively quantitate the performance of 26 chemistry assays on the Abbott ARCHITECT c8000 and c16000 instruments. We calculated Sigma metrics using the equation: Sigma Metric = (TEa - Bias ^{observed}) / CV ^{observed} Total allowable error (TEa) was obtained from either CLIA or the RICOS database, our bias compared to our peers was derived from the BioRad Unity database and CV's were calculated periodically from our lab results. We have been monitoring Sigma metrics quarterly for over a year using the EP Evaluator software. This software is user-friendly and allows the user to input specific TEa goals. We currently compare sigma metrics between 3 analyzers to monitor performance across the instruments. Using laboratory Quality

Control (QC) data generated for the chemistry assays on the ARCHITECT c8000 and c16000 instruments, we observed on average, 97% of the assays were greater than 4 Sigma and 71% were greater than 6 Sigma. None of the assays were less than 3 Sigma and 3% of the assays were between 3 to 4 Sigma. As an example, Sigma metrics for 26 chemistry assays from a quarterly monitoring check on one of the ARCHITECT c16000 instrument is shown in Table 1. In conclusion, the Abbott ARCHITECT instruments provide high quality results for 97% of the chemistry assays. Our next step is to implement streamlined Westgard rules to assist us in reducing the amount of QC checks currently performed in our lab. We plan to further automate the monitoring of Sigma metrics when EP Evaluator would have the capability to automatically download the bias values from the BioRad Unity database.

Table 1. Six Sigma Metrics assays on one of the ARCHITECT c16000 analyzer (April-June 2013)

Test	TEa%	Bias%	CV%	Sigma	Test	TEa%	Bias%	CV5	Sigma Metric
Albumin	10	0.4	2.2	4.5	Creatinine	37.28	-0.3	1.8	20.4
Alk.Pkos.	30	2.9	2.0	13.7	GGT	22.7	-7.9	1.9	8.0
ALT	20	5.3	1.7	8.6	Glucose	10	-0.2	1.4	7.1
Amylase	30	-1.3	1.1	25.4	HDL	30	0.7	2.2	13.4
AST	20	2.4	2.4	7.3	Lipase	37.88	-1.4	2.2	16.6
Bili D	45	4.1	5.0	8.1	Magnesium	25	-2.1	3.5	6.6
Bili T	30.69	1.0	4.1	7.2	Phosphorus	10.11	-0.2	2.1	4.7
Calcium	8.29	-2.2	1.4	4.4	Potassium	12.71	0.7	1.4	8.8
Chloride	5	0.4	0.9	5.3	Total Protein	10	0.0	1.3	7.9
Cholesterol	10	-0.1	0.7	13.5	Sodium	3.25	0.7	0.7	4.4
D-LDL	12	-1.4	2.5	4.3	Triglycerides	25	-1.4	1.4	17.4
CK	30	2.2	1.0	27.2	Urea (BUN)	9	-1.7	2.0	3.7
CO2	25	0.4	4.3	5.7	Uric Acid	17	-1.9	1.3	11.4

B-156

Managing Emergency Department Add-on Laboratory Orders

E. L. Ryan, R. J. Molinaro. *Emory Univerity School of Medicine, Atlanta, GA*

Background: Laboratory test orders placed on specimens after the initial orders have been placed (addedon orders) can tax resources and are impacted with reporting delays which may impact patient care. These added-on orders disrupt normal workflow and can also impact staffing needs.

Methods: We examined 31 days of Emergency Department (ED) orders at two institutions to explore differences between initial ordering patterns and the add-on orders to determine if automation storage and laboratory processes could decrease these delays. We assessed time to results for both initial orders and add-on orders on Troponin, Magnesium, and Lipase orders. “Time to result” for add-on orders was defined as the time from order electronically placed to results sent electronically, since the specimen had already been received. “Time to result” for initial orders was defined as the time from specimen receipt to results sent electronically. We compared “time to result” for add-on orders at the two institutions to determine if automated storage decreased reporting delays.

Results: Over the 31 days, there were 35,840 ED orders at the two institutions and 627 (1.7%) of these were addon orders. Most of the laboratory add-on orders were chemistry tests (62%). The following three tests totaled 45% of the addon orders: Troponin, Magnesium, and Lipase. For each of these three tests, add-on orders were significantly delayed. One institution had refrigerated storage on the automation line (Hospital #1) while at the second institution sample storage was off-line (Hospital #2). For Troponin, an off-line test at both institutions, no difference was observed. For Magnesium and Lipase, both on-line tests, there was an additional 0.36 to 2.21 hour delay in result reporting for add-on when the sample storage was off-line. For example, Magnesium addon median time to result at Hospital #1 was 0.57hr (IQR 0.38, 1.07) while at Hospital #2 it was 2.37hr (IQR 1.00, 3.38) $p<0.0001$. The second approach to improving addon time to results was a cost-benefit analysis of adding Magnesium to all comprehensive metabolic panels and only reporting the result in instances where it was ordered. Depending on the institution, it was estimated $100 - $200 a month in unbillable results would be incurred in order to eliminate the approximately 1-2 hour delay in result reporting..

Conclusion: In our assessment, the addition of automated storage is the optimal approach to decrease reporting delays of addon test orders.

B-157

Strategies to Improve Staff Competency and Turnaround Time in Haematology Slide Review

L. Lam, J. Lee, L. Tan, C. Soon. *Alexandra Hospital, Singapore, Singapore*

Background: Turnaround time (TAT) is one of our laboratory’s key performance indicators. We noticed that majority of our Full Blood Count (FBC) tests that exceeded our 1 hour TAT target were related to manual slide reviews. There seemed to be little correlation among the complexity of cases or seniority of staff.

Method: We reviewed all manual slide reviews for April 2012 (n=651) and grouped the degree of difficulty into easy, moderate and difficult categories. In each of the categories, we further analyzed the time taken by our staff based on their job grades: New hire, Junior Medical Technologist, Medical Technologist and Senior Medical Technologist. In July 2012, we proposed a “recommended” slide reading time based on the complexity of cases and the seniority of staff. All cases exceeding the new slide review time target were reviewed and retraining given to concerned staff.

Results: In August to October 2012, the TAT for FBC is 96.4% (78 cases out of 2175 exceeded TAT target). In November 2013 to January 2014, the TAT for FBC is 99.2% (22 cases out of 2846 exceeded TAT target). We applied chi square test and demonstrated a p-value (2-tail) of <0.0000001.

Conclusion: We were able to objectively guide staff competency and set slide reading time according to case complexity and seniority of staff, and propose specific and targeted retraining. More importantly, we realized a definite and sustained improvement in our FBC TAT, ultimately providing better patient care.

B-158

Evaluation of Quality OptimiZer quality management software (Awesome Numbers Inc.) to minimize patient risk, reduce clinical cost and implement EP 23 recommendations.

Z. C. Brooks[1], R. Gerz[1], N. Lepage[2], D. Plaut[3]. *[1]AWEsome Numbers Inc., Worthington, ON, Canada, [2]Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, ON, Canada, [3]David’s Consulting, Plano, TX*

Objectives to evaluate the efficacy of Quality OptimiZer™ software to assess analytical process quality, verify clinical effectiveness of Q.C. processes and recommend a comprehensive Q.C. strategy, to quantify the impact of OptimiZer Q.C. processes on patient risk, clinical and laboratory costs, and to compare Quality OptimiZer reports to EP 23 recommendations.

Relevance: Ineffective quality control practices expose patients to the risk of incorrect or delayed diagnosis and/or treatment. CLSI EP23 requires labs to “ensure test result quality is appropriate for clinical use;” validate “the ability of the QC procedures to detect medically allowable error;” and assess “potential costs both in terms of the patient’s well-being and financial liability.”

Methodology: We examined analytical processes, Q.C. processes, patient volumes and costs from two laboratories x two instruments x five analytes. For each Q.C. sample, we gathered : A. The four numbers required to evaluate analytical process quality: **1.** Measured mean; **2**. Measured SD; **3**. Peer mean; **4**. TEa limit, and B. The three numbers that determine Q.C. process effectiveness: **5.** Q.C. Chart assigned mean; **6.** Assigned SD; and **7.** Q.C. rule(s) Quality OptimiZer: - rated analytical process quality based on Total Error and Margin for Error recommended a 5-part Q.C. strategy - simulated a shift that would cause 5% of results to fail TEa limits. - compared the effectiveness of current and recommended QC processes to detect this significant shift - quantified patient risk, clinical and laboratory costs of each QC process. We selected one sodium control to illustrate the importance and interaction of the seven numbers required to manage quality.

Results For the selected sodium example: - Laboratory practice for all controls on all tests was to use a 1-2 and 9x rule as warnings, and 1-3, 2-2, 2/3-2, R4 rules as rejects. - The assigned mean was 0.7 SD below the measured mean; the SD was assigned at 2.1 x the measured SD. - The OptimiZer Q.C. strategy would detect a clinically-significant change sooner, prevent risk to 1330 patients, save 66 patients from clinically-misleading results and result in a net saving of $1,062.00. Quality OptimiZer reports satisfied EP 23 recommendations to: - ensure test result quality is appropriate for clinical use, - determine statistical limits that will identify unacceptable changes in performance of the measuring system, - prove effectiveness of quality control - quantify patient risks and costs of control quality, - implement and modify a 5-part Q.C. strategy.

Conclusions OptimiZer Q.C. processes met EP23 requirements, decreased patient risk, and reduced clinical costs. Error detection is impeded by the common practice of assigning mean and SD values from inappropriate sources and using outdated Q.C. rules. Laboratory quality would benefit from increased staff focus on clinical quality and the interaction of the seven numbers that drive and assess that quality.

B-159

Generation of statistical protocols derivated from sigma metric in a clinical laboratory

A. PORRAS[1], O. MARTINEZ[2], M. F. LEON[2], M. ISAZA[2], M. ROMERO[1], J. FANDIÑO[1]. *[1]QUIK, BOGOTA, Colombia, [2]COLSANITAS, BOGOTA, Colombia*

OBJECTIVE: To demonstrate the usefulness of the sigma metric to generate statistical protocols to control the analytical performance of measurement systems in clinical laboratories.

KEY WORDS: statistical protocols, sigma metric, clinically useful results, analytical performance.

BACKGROUND: Historically, the clinical laboratory has used different strategies for evaluating the performance of analytical runs since Levy Jennings graphs, statistics rules on the same graphs and analytical performance limits (CLIA, RilliBAK, Biological variation, etc.). However, standardizing the selected strategy into a daily routine becomes a challenge for the clinical laboratory.Recently, some laboratories in Colombia have invested resources and efforts for the implementation of the sigma metrics as an indicator of measurement systems efficiency, with the benefit of including the automatic generation of statistics from the sigma metric protocols, which has helped to increase the safety of delivering clinically useful results.

METHODS: 1,192 statistical protocols, corresponding to 437 analytes from chemistry, hormone and coagulation areas from a network of 12 clinical laboratories, were designed over a period of 12 months with specialized software. At the end of this period the protocols were ordered and grouped in ranges according to their sigma metric, defining the protocols most commonly used in each range and finally generating algorithms to select the protocol to implement.

RESULTS: 6 sigma metric ranges were obtained. The most commonly protocols obtained for each range of sigma metric were ≤3σ sigma: $1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_X$ (55%) and $1_{3s}/2_{2s}(2_{of32s})/R_{4s}/3_{1s}/6_x$ (43%) of cases, for the range 3σ to 3.9σ :$1_{3s}/2_{of32s}/R_{4s}/3_{1s}/6_x$ (38%) and $1_{3S}/2_{2S}/R_{4S}/4_{1S}/8_X$ (25%), for the range 4.0 σ-4.6σ: $1_{3s}/2_{2s}/R_{4s}/4_{1s}$ (34%), $1_{3s}/2_{2s}(2_{of32s})/R_{4s}/3_{1s}/6_x$ (38%), $1_{3s}/2_{2s}(2_{of32s})/R_{4s}/3_{1s}/8_x$ (25%), for the range 4.6σ-4.9σ: $1_{2.5s}$ (72%), 1_{3s} (23 %) y $1_{3.5s}$ (5%), for the range 5.0σ -5.9σ: $1_{2.5s}$ (39%), 1_{3S} (51%) and $1_{3.5S}$ (10%) and finally for ≥6σ: $1_{3.5s}$ (94%).

CONCLUSIONS The use of statistical protocols contributed to the improvement of performance in laboratory tests achieving an increase of 93% to 96% in the number of measurements with sigma metrics > 1.96 over a 12 month period. These protocols were automated in correlation with the sigma metric, thereby decreasing the investment of time, false rejections and false acceptations produced by inadequate protocols and poor timing. Additionally, the retrospective analysis led to the conclusion that for analytes with < 4.7σ multi-rules protocols are required and for > 4.7σ a single rule is applicable.

B-160

Sigma metrics to assess analytical quality: Importance of allowable total error (TEa) target.

D. Armbruster[1], S. Westgard[2]. *[1]Abbott Laboratories, Lake Villa, IL, [2]Westgard QC, Madison, WI*

Background. Six Sigma metrics were used to assess the analytical quality of automated clinical chemistry tests in a large clinical laboratory and examine the impact of different allowable total error (TEa) goals on the metric. Clinical laboratories are challenged to maintain the highest analytical quality but it is difficult to measure it objectively and quantitatively. Methods. The Sigma metric is estimates quality based on the traditional parameters used in the clinical laboratory: allowable total error (TEa), bias, and precision. Sigma metrics were calculated for 41 clinical chemistry and immunoassay tests, including serum and urine matrices, on five ARCHITECT c16000 chemistry analyzers. Controls at two analyte concentrations were tested to calculate precision, bias was estimated as the difference between observed results and the control target values, and Sigma metrics were calculated using three different TEa targets (Ricos biological variability, CLIA, and RiliBÄK) using the following equation: Sigma = (TEa – bias)/CV (all values expressed as %) Results. Sigma metrics varied with the analyte concentration, TEa target, and between/among analyzers. Sigma values identified assays that are analytically robust and require minimal QC rules and those that exhibit more variability, requiring more complex QC rules. Analyzer-to- analyzer variability was also assessed using Sigma metrics. As an example, Fig. 1 demonstrates the effect of the TEa target on the Sigma metrics for albumin.Conclusions. The Sigma metric is an efficient means to measure quality and optimize QC rules based on observed quality. Lack of TEa targets for some analytes and the variability of TEa from different sources can cause inconsistent estimates of Sigma for the same anlayte. Architect analyzers demonstrated generally high Sigma values and comparable analyzer-to-analyzer performance. Sigma metrics are a valuable means for comparing the analytical quality of two or more analyzers to ensure the comparability of patient test results.

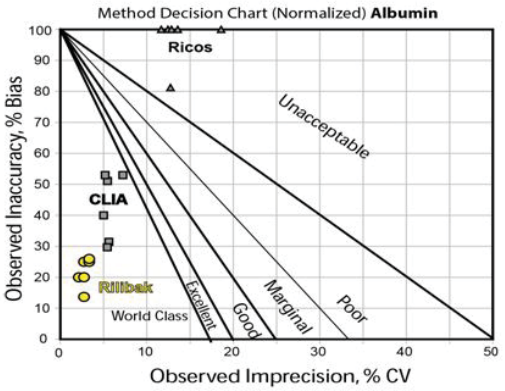


Figure 1. Normalized method decision chart for albumin comparing Sigma metrics calculated using biological variability (▲), CLIA (■) and RiliBÄK (●) TEa targets. Data points represent the Sigma value for controls at two concentrations and from three different analysers.

B-161

Performance of Analysis Teams of Blood Collection after Training in Pre-Analytical phase and Phlebotomy

A. G. L. Guimarães, J. P. D. Padilha, S. V. Paulino, J. Z. Costa, J. B. L. Filho, A. C. S. Ferreira. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Pre-analytical conditions are the key factors in maintaining the high quality of tests results. They are necessary to accurate reproducibility of laboratory tests for clinical diagnosis. In research at private's clinical laboratories, we evaluated the impact of some pre-analytical drivers before and after training, in the team of blood collection.

Objective: Analyze the points of improvement during the process of phlebotomy, and proposing a tool for continuous improvement that can aggregate in the preparation of this professionals working in this area. Promote the training of these phlebotomists and assess how this continuing education can impact not only on the quality of the tests report, but also the performance of these professionals to the client.

Methods: Pré-Analytical errors are attributed to a lack of patient preparation, care, collection and identification. Their becoming frequent because, of the low training and the existence of different degrees of involvement of several people in the process. The study conducted quantification in percentage of errors (points of improvement) committed in the collection procedure in three clinical pathology laboratories, in the South, Midwest and Northeast regions of Brazil, before and after the theoretical and practical training in venipuncture, following the most current practices described in the literature. The data were divided into three phases: pre-collecting, collection and pos-collection.

Results: The request of the patient's identity, verification of the test request form, preparation of client and antisepsis had opportunities of improvement raging from 6-31% before training and 1-7% after the training. The blood collection in a closed system totaled 75% of the punctures before the training and 86% in the post training, had 13% and 44% respectively of opportunity for improvement. The time using tourniquet and homeostasis showed 17% and 60% of errors before training, versus 1% to 4% after. The overall mean compliance before training was 75% and after the educational activity was 98%.

Conclusion: We conclude that the improvement of the team after the training was considerable and has an evident gain in the pre-analytical process and the quality of the request report result.

B-162

Disclosure of Medical Errors: An Approach towards Improving Quality in Laboratory Medicine

J. Kalra[1], J. Steeg[2], N. Kalra[2], R. Sachdeva[2]. *[1]University of Saskatchewan and Royal University Hospital, Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada*

Background: The quality of healthcare is an emerging concern worldwide. Despite the advancement in medical field, adverse events resulting from medical errors are relatively common in healthcare system. We have previously reported a non-punitive,"no-fault" model for reporting medical errors in clinical laboratory medicine. There are several barriers to disclosure including the risk of legal action faced by the physicians and often strained physician-patient relationship. It also jeopardizes the opportunity to enhance quality improvement in health care, as many medical errors are the result of systemic problems that are difficult to detect unless the errors are reported. However, an appropriate disclosure is vital to overcome these barriers and manage the consequences of an adverse event**.**

Methods: In order to analyze the progress made in the area of medical error disclosure and to understand the rationale for effective error disclosure policies, we reviewed and evaluated various error disclosure initiatives across Canada and other parts of the world (Australia, New Zealand and United States of America).

Results: The majority of provincial regulatory bodies in Canada have adopted some form of disclosure policy. However, these Canadian provincial initiatives remain isolated because of their non-obligatory nature and absence of federal or provincial laws on disclosure. In Australia, disclosure policy integrates the disclosure process with risk management analysis towards investigating the critical events. In New Zealand, in any adverse event, patients are rehabilitated and compensated through a no-fault state funded compensation scheme. This disclosure model supports the health care providers and strengthens the policy of honest disclosure. The United States Joint Commission on Accreditation of Healthcare Organizations mandated an open disclosure of any critical event during care to the patient or their families. By following an open disclosure policy, patient's autonomy can be preserved and malpractice claims can be reduced effectively. The complexities of medical error disclosure to patients present ideal opportunities for medical educators to probe how learners are balancing the ethical complexities involved in error disclosure with other related fields.

Conclusion: The correction of flaws in the healthcare system and the subsequent protection of patients' health should be the industry's top priority, rather than applying punitive measures to physicians and health care providers who make inevitable errors. We believe that the disclosure policies can provide framework and guidelines for appropriate disclosure which can lead to improved quality care and more transparent practices in clinical laboratory medicine and overall healthcare system. We suggest that disclosure practice can be improved by creating a uniform policy, centered on honest disclosure and addressing errors in a non-punitive manner.

B-163

Improvement of pneumatic tube system specimen submission from the Emergency Department to the Core Laboratory using Lean Six Sigma tools

C. Henemyre-Harris, L. Fujimoto, R. Dotson, J. Waynick, M. Angeles, A. Brinson. *Tripler Army Medical Center, Tripler AMC, HI*

Background: Pneumatic tube systems (PTS) can eliminate the need for clinical staff to leave the ward to deliver specimens and can save time in transporting specimens to the laboratory. However, once the PTS carrier arrives in the lab, the onerous for submission compliance rests heavily on lab staff. Implementation of a hospital-wide PTS resulted in many specimens arriving in the lab without proper order entry. Review of the PTS usage records indicated that the Emergency Department (ED) submitted the greatest number of specimens overall and the greatest number of specimens missing test orders. In addition, ED staff felt that delays in specimen processing were increasing the overall wait time for ED patients. This management project focused on strengthening the relationship between the ED and Core Laboratory to both meet the College of American Pathologist's requirement for orders for all lab tests and to decrease the specimen processing time for ED samples.

Objective: To assess and improve the ED's process for submission of specimens to the Core Lab via PTS.

Methods: The define-measure-analyze-improve-control (DMAIC) tollgates of a Lean Six Sigma (LSS) project were used to identify and implement solutions for the ED to submit specimens with electronic orders. Data captured by lab staff estimated the error defect rate for ED specimens missing test orders. Process and value-stream mapping identified the extra steps both lab and ED staff took when samples were missing orders. A root cause analysis was used to identify critical factors relating to the delay in processing ED samples. Solutions were selected and a new process was defined. Pilot studies tested the proposed solutions to ascertain improvement significance and full-implementation potential. Finally, a control/response plan was initiated to sustain the gains obtained in this management project.

Results: The test orders defect (missing + partial orders) rate of ED specimens submitted by PTS to the Core Lab was 16.8%. Reworking of ED specimens missing orders between the ED and Core Lab staff accumulated to one full-time equivalent (FTE) ED clerk/medical laboratory technician. Value-stream mapping identified some quick wins in the submission process such as a change in the default setting for label printers in the ED. The root cause analysis generated three solutions: 1) lab order entry granted to ED medical support assistants, 2) new employee orientation revised to ensure new ED staff received the proper laboratory information system (LIS) signature class, 3) "rainbow" specimen draw SOP initiated for the ED. These solutions reduced the specimens submitted by PTS without orders defect rate down to 3.5%.

Conclusion: The LSS structure to this management project provided a forum for open communication and buy-in from end-users in both the ED and Core Lab. Leadership support from both departments fostered a culture of change which resulted in a cost avoidance of one FTE, an enhanced process for specimen submission, and improved CAP compliance for lab test orders. Lessons learned from this management project can be applied to other clinics and wards in the medical center.

B-164

Inventory Automation for the Lab and Cost Savings Using an Inventory Management System

S. Cox[1], A. Carrillo[2], R. Wonderling[2]. *[1]Saint Francis Heath Sysem, Tulsa, OK, [2]Abbott Diagnostics, Abbott Park, IL*

Today's laboratories share similar inventory management problems spending too much time ordering and managing inventory. Low volume usage products, like calibrators, expire unnoticed requiring urgent orders with overnight shipping expense. Manual check-in and check-out processes lead to errors. Physical inventories are required to reconcile discrepant levels. Managing expiration dates and sequestering new reagent lots requires constant vigilance. Most laboratories are also resource-limited and technical staff is needed for complex testing issues.

We introduced the Abbott Inventory Manager at Saint Francis Health System to automate our inventory management. Each item is assigned a Serialized Global Trade Item Number (SGTIN) tag that is tracked using Radio Frequency Identification (RFID) technology. This system combines the use of RFID, electronic connectivity with Abbott and software management to track inventory starting from Abbott's warehouse to the hospital. Electronic Data Interchange (EDI) connectivity with Abbott enables our lab to access product catalog, create purchase orders, receive order acknowledgement and advance shipping notices. The software monitors on-hand stock levels, lot numbers, expiration dates with critical alerts and triggers auto-replenishment suggestions. It configures user roles and approval levels and also maintains an audit trail. Since Inventory manager is an open system, we are able to include non-Abbott items as part of the inventory.

Post implementation, our hands-on time of unpacking, labeling and logging-in our inventory has been reduced by 60% and our error rate has declined from 27% to 1.1%. Table 1 reflects the annual lab staff labor savings of $21,606.47 as documented during pre and post implementation. We anticipate, after one year, to see additional savings due to reduction of expiring reagents and emergency overnight orders.

In conclusion, automation of inventory management in our lab has minimized manual intervention of labeling, counting, tracking and ordering inventory. We reduced our error rate and realized both time and cost savings.

Table 1. Lab Staff Labor Savings Pre and Post Implementation of the Inventory Management System

Dept.	Activity	Pre IMS		Post IMS		Savings	
		Annual Labor Hours	Annual Labor Dollars	Annual Labor Hours	Annual Labor Dollars	Annual Dollars	%
Receiving Dock	Physical Count	57.4	$969	8.2	$138	$831	86%
Receiving Dock	SAP Goods Receipt	12.2	$206	18.3	$309	($103)	-50%
Lab	Receive Product into Lab	306.5	$9,195	121.4	$3,642	$5,553	60%
Lab	Consume Products	27.0	$809	22.5	$675	$135	17%
Lab	SAP Decrement	44.9	$1,347	12.0	$359	$988	73%
Lab	Physical Inventory	261.7	$7,850	24.9	$746	$7,104	91%
Lab	Check/Order Products	65.5	$1,965	33.2	$996	$969	49%
Lab	Yellow Sticker Generation	204.3	$6,130	0.0	$0	$6,130	100%
Receiving Dock	Transport to Main Lab (CC-IA)	19.4	$328	19.4	$328	$0	0%
Receiving Dock	Transport to Lab Storage (Heme)	6.9	$116	6.9	$116	$0	0%
Total Annual Hours and Dollars		1,218.5	$28,915	254.76	$7,308.53	$21,606.47	75%

B-165

Re-engineering Critical Laboratory Testing for Timely Chemotherapeutic Management.

X. Yi, R. Wolsky, C. VanSlambrouck, D. Mika, J. Leanse, E. K. Y. Leung, C. Nabhan, K. T. J. Yeo. *The University of Chicago, Chicago, IL*

Background: Delivery of cytotoxic therapy is a complex multifaceted process that involves harmonized collaboration between all systems involved. Laboratory assessment is an essential component of assuring that patient care is efficient, timely, and accurate. As the volume of oncology patients is increasing, providers and patients are faced with long wait times until laboratory results are available before chemotherapy can be safely administered. Optimizing laboratory turn-around time (TAT) assures timely delivery of chemotherapy, which would subsequently translate into improved outcomes and satisfaction. In this study, we aimed to investigate how to reduce the laboratory TAT for key laboratory tests so as to optimize the timely administration of chemotherapy. **Method**: The TAT can be affected by several factors including phlebotomy, clinical laboratory distribution, and test operation. We collected time data in each step in this process, including the time from specimen collection to specimen receiving in the central laboratory (Col-Rcv), specimen receiving to result release (Rcv-Res), and the overall TAT from specimen collection to result release (Col-Res). **Results**: The median TATs before our process re-engineering were: 31 min (Col-Rcv), 40 min (Rcv-Res) and 74 min (Col-Res) for a comprehensive metabolic panel (CPNL), and 34 min (Col-Rcv), 9 min (Rcv-Res) and 50 min (Col-Res) for a complete blood count (CBC). After reconfiguring the specimen transfer and analytical workflows to have specimens tubed directly to the central laboratories and assayed immediately, the CBC showed a significant reduction in the median (Col-Res) TAT of 20 min ($p<0.0001$). For CPNL, by processing all specimens as STAT samples, the median (Col-Res) TAT was significantly reduced from 74 min to 54 min ($p< 0.0001$). To investigate if we can further improve on the (Col-Res) TAT for chemistry tests, we engaged our clinical colleagues who identified 2 key CPNL analytes, total bilirubin (TBil) (to monitor liver toxicity) and creatinine (Creat) (to monitor renal toxicity), that, if we are able to deliver the results within the same timeframe of the CBC, would allow for faster decision-making for drug infusions. We evaluated the suitability of the whole blood ABL 8000 analyzer (Radiometer, OH) for this purpose and found that both whole blood TBil (WB-TBil) and Creat (WB-Creat) can be routinely resulted within 2 min of sample introduction. Correlation studies (Passing-Bablok and Bland-Altman plots) between whole blood and plasma samples from this cancer population showed: [ABL WB-TBil] = 2.00 [Roche plasma TBil] – 0.60, (range 0.1-2.1 mg/dl, n=164) and [ABL WB-Creat] = 1.08 [Roche plasma Creat] – 0.04 (range 0.4-3.1 mg/dl, n=166). There was 90% concordance of WB-TBil results when compared to plasma TBil results <1.1 mg/dL. The CV for WB-Creat was <10 % (0.43 mg/dL), and WB-TBil was 20% (0.5 mg/dL). **Conclusion**: Careful workflow analysis and re-engineering of transport and analytical process for key laboratory tests significantly reduce median overall TAT to a ~20 min that will facilitate timely delivery of chemotherapy.

B-166

The Effect of Patient Immune Status on QuantiFeron Test Results: An Investigation

T. K. Jackson[1], H. K. Lee[2]. *[1]Geisel School of Medicine at Dartmouth, Lebanon, NH, [2]Dartmouth-Hitchcock Medical Center, Lebanon, NH*

Background: Our laboratory tests patient specimens for latent tuberculosis using a test kit to measure cell mediated immune response. Latent tuberculosis is non-communicable and asymptomatic, but can develop into active tuberculosis at a later time and therefore is important to diagnose to prevent disease development.

The test is a two-part process. The patient's blood is drawn into a series of three tubes: a tube containing tuberculosis antigen, a tube containing nothing, which serves as a negative control, and a tube containing mitogen, which is a non-specific stimulator of T-cells to trigger a gamma interferon response and serves as a positive control. The tubes are incubated at 37°C for 16-24 hours and then the plasma is analyzed using an ELISA test. There is a software calculation against a standard curve that determines IU/mL of the nil, TB antigen and mitogen tubes. The results are then analyzed compared with a variety of permutations to determine if the results are positive, negative, or indeterminate. Infectious disease providers are interested in tracking the individual IU/mL results that the software uses in the interpretation and therefore the nil, TB antigen minus nil, and mitogen minus nil results are reported in addition to the qualitative interpretation.

New lots of kits are checked for similar reactivity with specimens that have already been tested with the kit currently being used prior to patient testing.

It was noticed in June of 2013 that, although the lot-to-lot check had been acceptable, the newer lot of kit being used was yielding more indeterminate and low-reactive results. It was important to determine if this was due to a problem with the testing kit or the immune status of the patients.

Methods: All results over a nine month period were tracked. Any patient with an indeterminate or low-reactive result (mitogen minus nil of less than 1.0) was checked for a diagnosis suggesting immunocompromised status.

Results: From May 2013 until January 2014, a total of 1648 samples were tested. Of those, there were 44 (2.67%) indeterminate results and 14 (0.85%) negative results due to low mitogen reactions. Case histories of these patients revealed that in fact, they were immunocompromised and the test results were concordant with clinical findings. All but one result were explained by the patients being immunocompromised due to various disease states. The one that could not be explained was a pre-employment sample with no charted information.

Conclusion: In conclusion, it has been determined that the increase in indeterminate results that was observed was due to immunocompromised patients and not sub-optimal test kits. For patient safety and quality of results, it has been decided that we will continue our practice of tracking the indeterminate and low-reactive results to ensure that the immune status of the patient is the cause and not a problem with kit or sample tube integrity, particularly because infection disease providers are using the calculated values in their clinical decision-making.

B-167

Use of a Decision Matrix and Positivity Rates to Substantiate Test Scope: Propoxyphene - A Case in Point

L. Labay, S. Noel, M. Welsh. *NMS Labs, Willow Grove, PA*

Background: Any toxicology test ordered should have value to the individual requesting the test and to the patient. It is, therefore, critical to optimize analytical scopes so that relevant testing is performed. Otherwise, there is consumption of resources with little value added. Since reference laboratories often serve wide client bases with competing needs, it is advantageous to have an objective means of determining if an analyte should be maintained in a particular test. A decision matrix (DM) was created to quantify the considerations involved, and used to evaluate the necessity of maintaining propoxyphene in limited-scope immunoassay panels.

Propoxyphene was approved by the US FDA in 1957 for use as an opioid analgesic, but was withdrawn in Nov 2010 due to risk of serious or fatal heart rhythm abnormalities.

The drug was frequently prescribed, and associated with abuse, overdoses and lethal outcomes. While past toxicological relevance cannot be argued, is it appropriate to now remove propoxyphene from immunoassay tests or should the practice continue? To address this question, the DM was used in conjunction with a multi-year positivity rate evaluation.

Methods: The toxicology panels evaluated were immunoassay based (ELISA or EMIT) and used for several matrices. DM assessment criteria with the associated factors were: a) legal availability in the US [yes:5; no:1], b) legal world-wide availability [yes:5; no:1], c) length of time unavailable [>10 yrs:1; 5-10 yrs:2; 2-4 yrs:3; 1-2 yrs:4; <1 yrs:5], d) illicit drug sources [yes:5, no;1], e) current positivity rate [75-100%:5; 50-74%:4; 25-49%:3; 1-24%:2; <1%:1] and f) other testing means within the laboratory [yes:1; no:5]. The factors for propoxyphene were entered for each matrix type to obtain the Total Score (TS). Using the DM, the minimum and maximum possible TS are 6 and 30, respectively. The recommendations based upon the TS are: 6-9 (remove from scope), 10-19 (monitor) and 20-30 (maintain in scope). An evaluation of positivity rates over a 3.5 year period was also performed.

Results: The TS for propoxyphene was 8 out of 30 in blood, serum/plasma, urine, tissue, hair, stool and meconium, and 9 out of 30 for fluid. Positivity rates by matrix type by year, from 2010 to mid-2013, were: blood (2.03%, 0.32%, 0.18%, 0.10%); serum/plasma (1.79%, 0.21%, 0.13%, 0.0%); urine (2.65%, 0.56%, 0.28%, 0.22%); tissue (1.48%, 0.86%, 0.0%, 0.0%); fluid (3.86%, 1.62%, 1.56%, 2.32%); hair (1.19%, 0.81%, 0.0%, 0.0%); stool (7.5%, 0.0%, 0.0%, 0.0%); meconium (19.62%, 22.45%, 9.53%, 0.0%).

Conclusion: Using the above determinations, our working group made two recommendations: 1) remove propoxyphene from the immunoassay panels and 2) treat propoxyphene in a similar fashion to most other drugs by using a broad-spectrum screening approach (TOF or GC/MS) or a directed analysis when warranted by case history. The severe decline in positivity rates suggests that the continual need to monitor the drug is no longer required. When the challenge of assessing scope relevancy arises, it is prudent to consider all available data including positivity rates and to employ a DM to substantiate any operational decisions.

B-168

Comparison of Inpatient and Outpatient Genetic Test Utilization in a Pediatric Tertiary Care Center

P. C. Mathias[1], J. H. Conta[2], D. L. Sternen[2], M. L. Astion[2], J. A. Dickerson[2]. *[1]University of Washington, Seattle, WA, [2]Seattle Children's Hospital, Seattle, WA*

Background: Increasing rates of send out testing represent a growing financial burden for hospital laboratories. In the pediatric tertiary care setting, genetic testing for rare inherited diseases is costly, and many such tests are infrequently ordered, which increases the probability of an order error. In addition, these tests are occasionally ordered on inpatients, so their cost is not reimbursed. In an effort to better utilize resources, some medical centers are actively monitoring test utilization for tests fulfilling certain criteria such as cost. The goal of this work was to analyze the data in a laboratory utilization database and compare genetic tests ordered in both inpatient and outpatient settings to determine if inpatient genetic test orders should be treated differently than outpatient orders.

Methods: A laboratory-generated test utilization database maintained by faculty and genetic counselors containing over 750 orders for genetic tests over a period of more than 2 years was analyzed. Send out tests were recorded in the database if they met the following criteria: 1) cost > $1000, 2) multiple genetic tests on one requisition, 3) request to send to nonpreferred laboratory, or 4) request to send out a test performed in-house. At the time of order, patient demographics (including inpatient vs. outpatient), test information, ordering provider specialty, and test cost were recorded. After review by the appropriate staff, the approval or modification of a test (including indications for doing so), cost savings, and the results of the tests were also entered into the database. Based on the indication for ordering the test, results were classified as positive, negative, uncertain significance, or pending. For tests cancelled or modified after review, orders determined to be non-indicated or ordered incorrectly were recorded. Data on inpatients and outpatients was compared and the proportions of test approvals, positive results, cost, cost savings, and error rates were compared, with tests for equality of proportions applied when relevant.

Results: Data from 147 inpatient and 632 outpatient genetic test orders was analyzed. The rate of test approval without modification was similar for both groups, at 66% for inpatient orders and 71% for outpatient orders (p=0.27). The proportion of positive results was also similar between the two groups, at 29% for inpatients and 27% for outpatients (p=0.78). The mean cost for inpatient genetic tests reviewed was $660 higher and the mean cost savings per order reviewed was $120 higher in inpatients (cost savings of $550 vs. $430 per order reviewed). The error rate, which was calculated as a percentage of tests that were either cancelled or modified because an incorrect test was ordered, was not significantly different, at 6.8% for inpatient and 5.1% for outpatient genetic test orders (p=0.52).

Conclusion: The rate of test approval and proportion of positive results was similar between inpatient and outpatient genetic tests orders, although cost savings per inpatient order was higher. The order error rate of greater than 5% for both patient groups, however, suggests that maximizing the proportion of genetic test orders under review would prevent errors in diagnosis and patient management.

B-169

QC Rules for High Sigma-Metric Processes

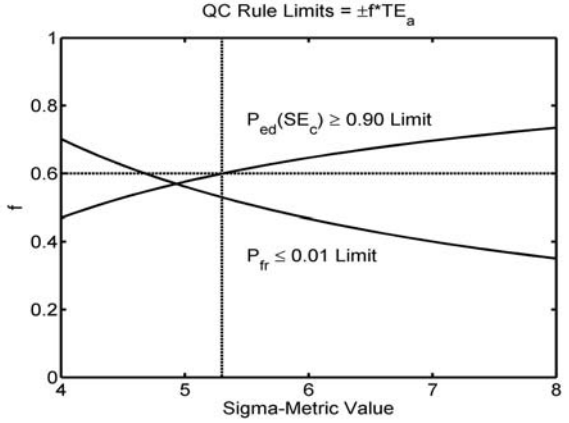
C. A. Parvin. *Bio-Rad Laboratories, Plano, TX*

Background: Objective: Define a simple, yet effective QC rule for high sigma-metric processes and determine the minimum sigma-metric threshold for the rule.

Relevance: High sigma-metric processes are generally easy to quality control. A simple effective QC rule that can be confidently applied in all high sigma-metric situations would be valuable.

Methods: QC rejection limits are defined as $\pm f*TE_a$ (a fraction of the allowable total error specification for the analyte). Probabilities of false rejection, P_{fr}, and critical systematic error detection, $P_{ed}(SE_c)$, were derived assuming either 2 or 3 QC concentration levels are evaluated. SE_c is defined as an out-of-control condition that would produce 5% patient results containing measurement error exceeding TE_a. QC rule rejection limits were required to provide $P_{fr} < 0.01$ and $P_{ed}(SE_c) > 0.90$.

Results: Validation: Mathematically derived probabilities were validated by computer simulation. Conclusion: The range of values for f that meet the false rejection and error detection constraints when 2 QC concentration levels are evaluated as a function of sigma-metric value is shown in the figure. A QC rule with rejection limits given as $\pm 0.6*TE_a$ provides as least 90% critical systematic error detection with a false rejection rate less than 1% for any process with sigma-metric value greater than 5.3. The false rejection rate of the QC rule will decrease and the error detection probability of the rule will increase for sigma-metric values greater than 5.3.



B-170

Total Laboratory Automation (TLA) to Improve Efficiencies: Before and After study at a 2,941 Bed Medical Center in Taiwan.

P. Liu, M. Shieh, W. Liu, C. Lin, M. Lee. *Department of Pathology and Laboratory Medicine Central Lab, Taipei Veterans General Hospital, Taipei, Taiwan*

Objective: To assess the increase in efficiency of TLA by implementing the ACCELERATOR APS to replace multiple separate work processes including better Turn Around Time goal achievement, optimized workflow, reduction in FTE, reduced consumables and increased space utilization.

Relevance: ACCELERATOR APS is an automated track system that manages pre-analysis, analysis, as well as post-analysis operations. It centralizes all these operations.

Methodology: This study analyzed one month's data of % that achieved the TAT goal and consumables usage of 29 clinical chemistry assays for STAT outpatient

sample from laboratory information system. The number of working steps and staff requirements were obtained by workflow observation and space require, all metrics performed before and after implementation.

Validation: The facility has a TAT goal of 60 minutes for 29 STAT Chemistry assays for outpatient sample. The percentage of TAT achievement and consumables usage were analyzed using LIS data. Working steps and personnel required were collected via workflow observations. Space utilization is from the calculations done by engineers.

Results:

Metrics	Before Implementation	After Implementation	% of increase and decrease
No. of tests	27,866	32,286	15.8%
% Achieved TAT goal	96	99.3	3.3%
Working steps	21	5	-76.0%
Personnel	10	4	-60.0%
Sample tube usage	26.058	25,608	-1.7%
Aliquot tip and cup usage	5,211	0	-100%
Space required (sq. meter)	1,397	597	-57.2%

Conclusion: The implementation of the ACCELERATOR APS to combine pre-analysis, analysis and post-analysis in one platform, resulted in an improvement of laboratory efficiencies in all key metrics. This was achieved despite the 15.8% increase in testing volume observed after implement of the ACCELERATOR APS.

• 3.3% increase in tests achieving their TAT goal

• 76%, 60%,1.7% and 52.7% are reduction in working steps, personnel, sample tubes and space required respectively

• 100% elimination of aliquot tips and cups due to consolidate testing.

B-171

Use of Technological Advances to Improve Laboratory Turn Around Times

P. B. Kyle, K. B. Brown. *University of Mississippi Medical Center, Jackson, MS*

Background: Timely laboratory results can be essential for quality patient care. As such, laboratory turnaround times (TAT) are often cited as performance measures and quality indicators. We sought to tabulate changes in TAT observed after the implementation of new processes and technologies into our laboratory. These included personalized productivity reports, status display monitors, result autoverification, preanalytical automation, and the use of plasma instead of serum.

Methods: The TAT of basic chemistry profiles were determined before and after each change was implemented. The TATs of samples analyzed from 0700-1530 weekdays only were tabulated as per institutional review board approval at the University of Mississippi Medical Center, Jackson, MS. Each experimental group included over 4000 samples collected during a given eight week period. A minimum of two weeks were allowed to pass in order for technologists to adapt to each change, whereas three months were allowed for the acclimation to new instrumentation. After baseline measures were collected, the first change involved daily dissemination of individualized performance reports. Each report included the number of samples analyzed and average TAT of STAT and routine results of each technologist. Secondly, an LCD monitor was installed within view of the technologists. The LCD monitor displayed the status of all pending samples. Next, the results of autoverification was recorded where only normal patient results were autoverified. The TAT changes associated with preanalytical automation and use of plasma were also tabulated.

Results: The greatest reduction in TAT, 13.6 minutes, was obtained after the implementation of preanalytical automation. Reductions of 6.81, 6.65, and 5.99 minutes, respectively, were noted with implementation of autoverification, the status display monitor, and productivity reports. Autoverification resulted in the best cost:benefit ratio at no cost, whereas autoverification was the most costly at hundreds of thousands of dollars.

Conclusion: Preanalytical automation afforded the greatest reduction in TAT, but also came with significant monetary and space requirements. Laboratories can significantly reduce their TAT via several methods inexpensive methods. Solutions include autoverification, personnel productivity reports, LCD displays, and alternate sample collection tubes.

B-172

Eliminating non-value added pre-analytic processes to improve laboratory turnaround time and patient safety for emergency room patients

N. I. Rush[1], A. Planker[2], M. K. Zimmerman[1]. *[1]Indiana University School of Medicine, Indianapolis, IN, [2]Indiana University Health, Indianapolis, IN*

Background: Our hospital system has been looking at various ways to eliminate non-value added processes to improve patient care and decrease costs. One cause of emergency department (ED) patient dissatisfaction is extended length of stay (LOS) from delays in test ordering to test result availability time. A joint ED and laboratory team sought to decrease LOS by utilizing value stream mapping (VSM). VSM is a lean manufacturing method to analyze and improve the flow of information and materials across a process. We focused on an ED with no stat lab: all specimen testing outside of a very limited ED point-of-care menu was sent via pneumatic tube to the main lab. In the main lab, we utilized VSM to optimize the flow of ED specimens from pneumatic tube receipt to automated chemistry or hematology line log-in time.

Methods: The measuring timeframe criteria for the laboratory portion of the project were established. The tests tracked were the basic metabolic panel, complete metabolic panel, hepatic panel, lipase, and complete blood count. Measurements were (1) laboratory pneumatic tube station receipt to stat specimen log-in (SL) time, (2) SL to automated chemistry line log-in (CL) or hematology line log-in (HL) time. Percent of ED relabeled (%R) tubes was determined by manually tracking relabeled specimens over 24 hour periods. Specimen processing personnel motion was observed and spaghetti charts were made. After corrective interventions, times for (1) and (2), %R, and spaghetti chart walk paths were reassessed.

Results: The initial state showed that SL was 2:00 minutes, CL was 13:20 minutes, HL was 05:20 minutes, and %R was 46%. Major causes of pre-analytical phase delay were lack of prioritization for stat specimens, issues with label placement on specimen tubes, and work interruptions and non-value added motion by specimen processing personnel. The intervention for ED personnel was training on correct label positioning techniques using visual aids to demonstrate the spatial relationship between specimen label, automated line bar code reader, and specimen puck. The specimen processing area interventions included establishment of a STAT bench, establishment of a separate Problem bench to eliminate unnecessary interruptions, and designated specimen tube runners. After intervention, SL was 00:48 minutes (60% decrease); CL was 09:20 (30% decrease); HL was 03:44 (30% decrease); and %R was 0 (100% decrease). A comparison of the initial state spaghetti chart to that of the confirmed state showed an 87% decrease in walk paths. The projected annual monetary savings for the laboratory portion was $33,396.

Conclusions: Small yet significant interventions implemented via this VSM project by ED and laboratory personnel improved the specimen flow from the ED to the laboratory automated lines. The interventions in the current study decreased pre-analytical phase delays, improved patient safety and satisfaction, and resulted in potential monetary savings.

B-173

Quantifying of the Cost of Unnecessary Clinical Laboratory Testing for Hospital Systems and Healthcare Payers

S. Chekuri[1], A. Mandel[2], C. Hightower[2], B. Brimhall[1]. *[1]University of Mississippi, Jackson, MS, [2]CareCore National, LLC, Bluffton, SC*

Background: Diagnostic laboratory testing represents the largest source of structured medical information in most healthcare systems. Testing with little or no diagnostic value is a significant source of unnecessary cost to the health care system. Using data available from two hospital systems (>15 hospitals) and four large regional payers (>6 million covered lives) we evaluated testing patterns for several diagnostic test scenarios to quantify the cost of unnecessary testing.

Methods: Using available evidence-based medical knowledge we determined test scenarios for which at least one test was largely unnecessary. We aggregated 14 test scenarios into 7 diagnostic categories: thyroid (e.g., concurrent free T3 and/or free T4 given TSH within the reference range), liver function (e.g., concurrent GGT and alkaline phosphatase given ALKP within the reference range), suspected pancreatitis (e.g., concurrent serum amylase and lipase), vitamin D status, iron status, general inflammation (e.g., concurrent CRP and erythrocyte sedimentation rate), and myocardial injury (e.g., concurrent troponin and CKMB).

Combining laboratory, operational, and detailed financial data we calculated costs based on available cost accounting categories for each hospital system. We also queried claims databases for four US regional payers and extrapolated ordering

patterns from hospital data. Hospital data and payer data covered at least 12 months (24-48 months in most cases) during the period 2008-2013 for hospitals and 2009-2012 for payers.

Findings: The mean annual volume of unnecessary testing in the hospital systems was 270,517 tests representing variable plus labor costs of $1,824,805 and total costs of $2,514,822. For payers, the mean annual volume of the same unnecessary testing was 1,261,817 tests worth $30,497,028 in net paid out claims (see table).

Conclusion: The cost of unnecessary testing is substantial to both hospitals and payers. There is great opportunity for laboratory professionals to quantitatively demonstrate value by improving test utilization patterns.

Annual volume and cost of unnecessary testing for hospitals (a) and payers (b)

Diagnostic Category	Volume(a)	Variable Cost(a)	Total Cost(a)	Volume(b)	Paid Claims(b)
Thyroid	109,209	$645,389	$913,858	536,097	$12,252,830
Liver Function	17,542	$80,989	$109,361	30,963	$458,420
Suspected Pancreatitis	23,179	$118,793	$164,338	172,798	$3,606,089
Vitamin D Status	4,610	$175,018	$229,000	33,864	$2,166,743
Iron Status	27,292	$145,967	$202,811	145,186	$4,503,433
General Inflammation	31,108	$194,863	$298,743	196,372	$1,868,772
Myocardial Injury	63,077	$463,786	$596,711	146,537	$5,640,741
TOTAL	270,517	$1,824,805	$2,514,822	1,261,817	$30,497,028

B-174

Sample Size Requirements for QC Lot Cross-Over Studies

C. A. Parvin. *Bio-Rad Laboratories, Plano, TX*

Background: Objective: Determine the number of QC results required in a cross-over study to provide a good estimate for the mean (and possibly SD) for a new lot of QC material.

Relevance: Establishing the mean concentration for a new lot of QC material is an important laboratory activity. A major concern is that a poorly estimated QC mean will lead to a QC rule that gives too many false rejections. Designing cross-over studies that provide good estimates with a minimum number of replicates is important.

Methods: Simulations were employed to estimate the influence of cross-over study sample size on a QC rule's false rejection rate when the QC rule is based on estimates obtained from the cross-over study. Three cases are considered; 1) the QC mean is estimated, but the SD is known, 2) the QC mean is estimated and SD is computed as estimated mean * known CV, and 3) both QC mean and SD are estimated. The outcome metrics evaluated are the expected probability of false rejection, $E(P_{fr})$, when cross-over study estimates are used in the QC rule, and the probability the false rejection rate is not greater than twice the false rejection rate of the QC rule with known QC mean and SD. One million simulations were employed to obtain estimates.

Results: The table shows some of the results.

Conclusion: If the SD or CV are known and only the QC mean is estimated, then a cross-over study based on N=10 results can provide a QC rule that has at least an 80% chance that the false rejection rate of the rule will be no greater than twice the false rejection rate using the true QC mean and SD. If SD must be estimated from the cross-over study, then at least 60 QC results are required.

1-3s/2-2s/R-4s QC Rule (Using known mean and SD, $P_{fr} = 0.01$)

Case	N	$E(P_{fr})$	$P(P_{fr} < 2 * 0.01)$
SD known	7	0.017	0.75
SD known	**10**	**0.015**	**0.86**
SD known	14	0.013	0.93
SD known	21	0.012	0.98
CV known (10%)	7	0.018	0.73
CV known (10%)	**10**	**0.015**	**0.83**
CV known (10%)	14	0.013	0.90
CV known (10%)	21	0.012	0.96
SD estimated	20	0.025	0.55
SD estimated	40	0.016	0.71
SD estimated	**60**	**0.014**	**0.81**
SD estimated	80	0.013	0.86

B-175

Investigation on Causes and Impact of Specimen Rejection in Clinical Chemistry Laboratory

M. Chen, R. Phipps, R. Del Guidice, E. Wagar, Q. H. Meng. *University of Texas MD Anderson Cancer Center, Houston, TX*

Background: Accurate results are critical to patient management and quality care. Errors occurring in the pre-analytical phase account for up to 90% of total laboratory errors. Of those, inappropriate specimen due to quality and quantity account for 70%. Pre-analytical specimen integrity and quality are extremely important to the final result reported by the laboratory. Specimen rejection is conducted to ensure accurate identification and quality of samples as well as the accurate results. Unlike quality control systems widely applied to ensure the quality of the analytical phase, quality improvement intending to reduce pre-analytical errors as part of total quality management has not been fully implemented and achieved. This study was conducted to investigate the frequency, causes, and impact of specimen rejection in clinical chemistry laboratory.

Methods: Rejected chemistry specimens in our laboratories recorded in LIS during a 4-month period were collected. The number of rejected specimens, reason, and location for rejection were analyzed.

Results: Of the 245,058 chemistry specimens received to the laboratories during the data collection period, 647 (0.26%) were rejected. The most common reasons for specimen rejection were contamination (IV fluid or TPN) (227, 35.1%), unacceptable specimen (wrong collection tube, unlabeled, mislabeled, or inappropriately labeled specimen) (n=179, 27.7%), quantity not sufficient (QNS) (n=98, 15.1%), hemolysis (n=61, 9.4%), clot (n=60, 9.3%), and patient ID error (n=14, 2.2%). The most commonly affected analytes due to specimen rejection occurring after collection were glucose (n=192, 8.82%); calcium (n=153, 7.0%), magnesium (n=148, 6.8%), potassium (n=138, 6.3%), creatinine (n=101, 4.6%), and BUN (n=96, 4.44%). Inpatient areas had the most rejections occurring after collection (45.3%), followed by outpatient areas (24.6%), adult Intensive Care Units (ICUs) (17.2%), and ER (11.0%). When compared with the respective frequency with which they collect specimens, laboratory personnel (phlebotomists) submitted significantly fewer rejected specimens than other in-hospital personnel groups (at a rate of 0.22% vs. 1.34% for floors often collected by other in-hospital personnel groups. Total recollected specimens (n=230, 0.09%) during a 4-month period added an average of 108 minutes delay (from recollect order placed to results completed) to the turnaround time per test. The cost for total of 230 specimen recollection was $5510.80 USD.

Conclusions: Specimen rejection criteria should be followed and specimen rejection should be monitored on a regular basis. Those frequent factors that are associated with rejection and have a great impact on patient results and patient care should be identified. Actions and education should be taken for quality improvement. Efforts should be made to standardize laboratory manuals and procedures as part of continuous quality improvement program. Policy and procedures specifically to specimen requirement, collection, transportation, and preparation should be strictly followed.

Wednesday, July 30, 2014

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

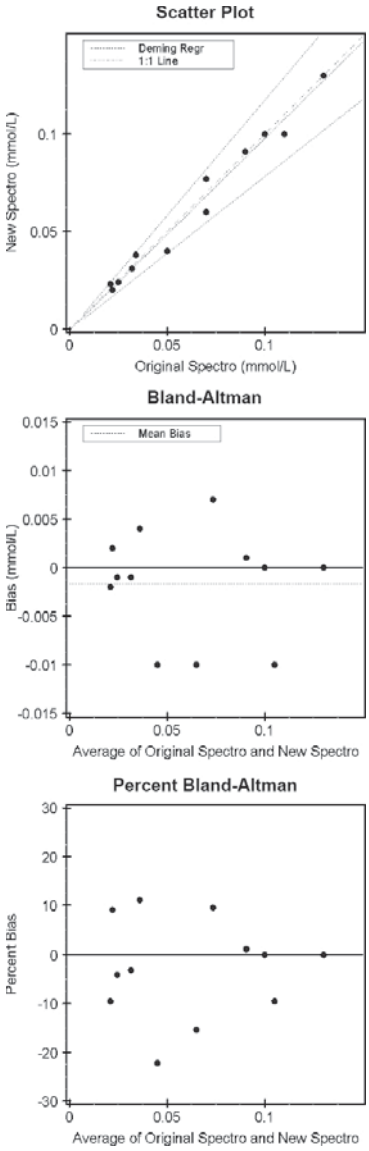
Electrolytes/Blood Gas/Metabolites

B-176

Validation of a spectrophotometric assay for the measurement of pyruvate in whole blood.

S. Albeiroti, J. M. El-Khoury, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Pyruvate plays a major role as an intermediate in carbohydrate and amino acid metabolism. Clinically, an elevated Lactate-to-Pyruvate ratio can be indicative of several diseases including disorders of mitochondrial metabolism and inborn errors of metabolism. In our laboratory, pyruvate is measured by a spectrophotometric assay. NADH used in the assay was prepared freshly before each test by adding Trizma® buffer to a 2 mg NADH bottle from Sigma Aldrich®. Inconsistency in absorbance values among different NADH bottles provided by the manufacturer has been a major challenge in our lab. **Objective:** To develop a pyruvate assay that requires shorter processing time and results in more consistent absorbance values. **Methods**: NADH stock (0.225 mg/ml) was prepared in 1.5 mmol/L Trizma® and stored at 4°C. Deproteinized blood sample was prepared by adding 2 mL whole blood to 4 mL cold 12% trichloroacetic acid. The mixture was mixed, incubated for 10 minutes at 4°C and centrifuged for 7 minutes at 2000 g. 2 mL of the supernatant was mixed with 1 mL 0.225 mg/ml NADH in a 1-cm cuvette. Using Beckman DU 800 spectrophotometer, absorbance was measured at 340nm. 50 µL of 1000 units/mL LDH was then added and absorbance was measured after 10 minutes of incubation. **Results:** NADH stock solution was stable at 4°C over a period of 60 days with an inter-assay precision of 1.8% for the pyruvate measurements in prepared QCs. QCs used were 0.07 mmol/L pyruvate in 5% BSA in saline. The assay had an analytical measurement range of 0.04-0.35 mmol/L with recoveries ranging from 89% to 105%. Average within-run precision was <1%. Pyruvate results of patient samples obtained by this assay (n=12) were comparable with results obtained by the original method (r=0.9891, slope=0.986, intercept=-0.0008, SEE=0.0058). **Conclusion:** The modified spectrophotometric assay for the measurement of pyruvic acid in whole blood is accurate and robust.



B-177

Validation of creatinine test using the standard (SRM967) as reference for two methodologies

A. L. Camilo, F. S. Fukuoka, C. F. A. Pereira. *DASA, São Paulo, Brazil*

Background: The ADVIA Creatinine_2 is used for *in vitro* diagnostic determination of human serum, plasma (lithium, heparin) and urine creatinine activity on the ADVIA Biochemical Systems (Siemens Healthcare Diagnostics). These measurements are used in renal diseases diagnosis and treatment, and also in renal dialysis monitoring. **Objective:** This study aims to perform the validation of Creatinine_2 (CREA_2) ADVIA assay on ADVIA 2400 equipment using as reference the standard (SRM967) of the National Institute of Standards and Technology (NIST). Moreover, the study verifies if results obtained by the method had deviation no greater than the analytical specifications.

Methods: The Creatinine_2 (CREA_2) method is based on the reaction of picric acid with creatinine in an alkaline medium, as described in the original Jaffe´s procedure. The assay range is 0.1 - 25.0 mg/dL (8.84 - 2,210 µmol/L) to serum samples and 1.5 - 300 mg/dL (133 - 26,250 µmol/L) to urine samples.

Results: Data show the total imprecision of measurements using control material and prepared pools. The within-run assay obtained CV=0.74% to 2.6%, and the total assay obtained CV=2.55% to 5.01% on samples serum with creatinine concentrations of

0.48 to 5.81 mg/dL. The within-run assay obtained CV=1.38% to 3.05%, and total assay obtained CV=2.95% to 6.84% on samples urine with creatinine concentrations of 0.73 to 235.12 mg/dL. Dilution linearity of multiple serum samples demonstrated means recoveries of 95.46% - 97.41%. Linearity test by applying eleven (11) points of dilution factors using the Standard Reference aqueous solution (SRM967) of the National Institute of Standards and Technology (NIST) at the following concentrations: 1.0 mg/dL and 30.0 mg/dL and the regression linear equation to this study was y = 0.956x + 0.057 (R^2=1). The comparative results between ADVIA Creatinine_2 and LABTEST Creatinine (Labtest Diagnóstica S.A) were performed in 53 serum samples and 40 urine samples; all known results were within the creatinine analytical measurement range. The obtained correlation coefficient to serum and urine samples was 0.999. The obtained linear regression to serum samples was 1.01384 to the ADVIA Creatinine_2 and -0.005066 to the LABTEST Creatinine. The obtained correlation coefficient to urine samples was 0.999. The linear regression was 0.987 to ADVIA Creatinine_2 and -0.52 to LABTEST Creatinine.

Conclusion: The results show that ADVIA Creatinine_2 test presents the total error estimate less than the allowable total error in all levels of clinical decision. The estimating bias are less than or statistically equal to the defined EQA Bias in a significance level of 2.5%. The evaluated results show that ADVIA Creatinine_2 test is an accurate method to measure creatinine serum and urine samples through a wide range of clinically relevant concentrations and it also shows equivalent performance to LABTEST Creatinine assay.

B-178

Serum creatinine determined by Jaffe and enzymatic method, in regular, icteric and hemolyzed samples

M. Friedrich. *Hospital Universitário, Universidade de São Paulo, São Paulo, Brazil*

Background: Serum creatinine is an important clinical marker for renal clearance. The Jaffe reaction remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations. With the recent introduction of the reporting of estimated glomerular filtration rate (eGFR), inter-laboratory agreement of serum creatinine results has become an important international priority. The aim of this study was to compare analytical performance and practicability of the enzymatic method and Jaffe method for serum creatinine for routine use and to compare the effects of some common interfering substances on both methods.

Materials and Methods: We assessed 221 serum samples obtained for routine clinical care: 106 regular samples (without interfering substances), 69 icteric and 57 hemolyzed samples. 11 samples were both icteric and hemolyzed. Serum creatinine was determined both by kinetic Jaffe's and enzymatic method on Siemens ADVIA Chemistry 1800. The ADVIA Chemistry Creatinine_2 (CREA_2) assay is a Jaffe, alkaline picrate, kinetic method and the ADVIA Chemistry Enzymatic Creatinine_2 (ECRE_2) assay is a Creatininase method. We analyzed the agreement between the two methods and determined the mean difference between them.

Results: Comparison of Jaffe (X) and enzymatic (Y) measurements of serum creatinine levels reveals significant correlation with or without the presence of interfering substances. However, mean differences between enzymatic to kinetic Jaffe's methods were higher for icteric and hemolyzed samples, as shown in the table below.

Conclusions: Serum creatinine can be overestimated by Jaffe's method in the presence of interfering substances, such as hemoglobin and bilirrubin. Enzymatic method is less affected by interferences so it is a better method to measure creatinine.

Table 1. Comparison between serum creatinine measured by Jaffe and enzymatic methods

	Mean (mg/dL)	Median (mg/dL)	SD	Lowest value (mg/dL)	Highest value (mg/dL)	R^2	Linear Regression	Bias
All samples (n=221)								
CREA_2 Jaffé	3.66	1.95	4.65	0.21	30.25	0.997	y = 1.023x - 0.21	-3.82
ECRE_2 Enzymatic	3.52	1.77	4.77	0.25	31.66			
Regular samples (n=106)								
CREA_2 Jaffé	4.85	2.46	5.90	0.21	30.25	0.998	y = 1.025x - 0.18	-1.23
ECRE_2 Enzymatic	4.79	2.27	6.10	0.25	31.66			
Icteric samples (n=69)								
CREA_2 Jaffé	2.52	1.8	2.62	0.46	15.17	0.992	y = 0.991x - 0.18	-8.33
ECRE_2 Enzymatic	2.31	1.58	2.60	0.51	15.05			
Hemolyzed samples (n=57)								
CREA_2 Jaffé	2.62	1.69	2.66	0.37	14.20	0.993	y = 0.997x - 0.20	-8.01
ECRE_2 Enzymatic	2.41	1.46	2.66	0.45	14.34			

B-179

The reference value of non esterified fatty acids determined by enzymatic method in healthy population

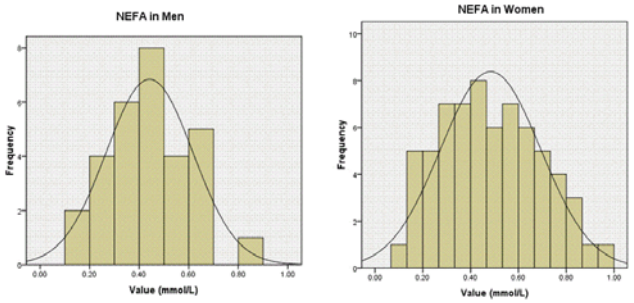
Y. Huang, T. Wang, X. Du, J. Jian, J. Kang, F. Chen, A. Zhu. *Beijing Strong Biotechnologies, Inc., Beijing, China*

Background: Increased circulating levels of nonesterified free fatty acids (NEFA) have been observed in such hyperinsulinemic states as obesity, impaired glucose tolerance, diabetes, and dyslipidemia where they have been causally linked to the development of insulin resistance and hyperinsulinemia.We created liquid enzymatic method for measuring NEFA. Appropriate reference ranges allow for effctive utilization of an assay. The aim of the current work was to establish NEFA reference ranges in healthy population.

Methods: Colleted normal serum specimens for referenc range determinations from 101 healthy individuals, age from 20 to 62 years, who attended Beijing Strong Biotechnologies for annual health check-up. None of these specimans contained Diabetes and hypertersion. Specimens containing high TG and high GLU were expected. The ration of female/male was 70/31, Blood was collected in the morning after fasting for 12 hours. Blood sample were stored on ice. Centrifugation was carried out within one hour. The serum samples were assayed on Hitachi 7180 within 2 hours of cellection. The outliers was excluded using Grubbs statistics mehtod.

Results: NEFA displayed a normal distribution for this reference population both male and female (Figure 1). The reference interval for NEFA using liquid enzymatic method provided different range between males and females, the range for females (n=70) was 0.02 to 1.02µmol/L and for males (n=31) was 0.10 to 0.78µmol/L

Conclusions: The reference range determined in this healthy population was gender dependent with higher levels for females.



B-180

Hydroxocobalamin Interference with Carboxyhemoglobin (COHb) Measurements

P. V. A. Pamidi, H. Yim. *Instrumentation Laboratory, Bedford, MA*

Background: *H*ydroxocobalamin (OHCbl), a vitamin B12 analog, is known to interfere with CO-Oximetry measurements (1). Recent study by Livshits et al (2) refers to two carbon monoxide poisoning patients and claimed that the CO-Oximeter reported falsely low carboxyhemoglobin after hydroxocobalamin therapy. The paper by Livshits et al went on to suggest that the false reading by the CO-Oximeter may lead to incorrect diagnosis and delay of appropriate treatment. However, assessment in out lab prior to this publication (1) showed a smaller interference at normal COHb levels. In this study, impact of hydroxocobalamin at high COHb levels is evaluated.

Methods: Blood samples collected from healthy donors were used to prepare COHb levels (25 -50 %). COHb samples spiked with hydroxocobalamin (1 g/L) were measured on 3 GEM Premier 4000 analyzers. The effect of OHCbl interference on carboxyhemoglobin is evaluated using the measured difference between the unspiked and the spiked samples. To emulate the treatment conditions reported in Livshits reference, blood samples were tonometered with 100% oxygen.

Results: Carboxyhemoglobin data shown in table below confirmed that accuracy is slightly affected by the presence of OHCbl (1-2 units). However, with or without OHCbl, the reduction in COHb is mainly triggered by the oxygen treatment. In addition, spiked samples were appropriately flagged by the iQM software.

Conclusions: Blood samples spiked with 1 g/L OHCbl showed a small interference (1-2%) on carboxyhemoglobin consistent with our prior data in reference 1. Based on these results, the dramatic reduction in the COHb level as reported by Livshits is not due to interference from hydroxocobalamin and oxygen treatment would likely have caused reduction in COHb levels.

References: 1) P.V.A. Pamidi, et al , Clin. Chim. Acta, 401, 2009, 63-67.

2) Z. Livshits, et al, New ENGLAND J. MED 367: 1270 - 1271, SEPTEMBER 27, 2O12

COHb Sample	COHb	COHb + OHCbl 1 g/L	Delta
25% COHb	26.7	25.8	0.9
25% COHb (O_2 Tonometry 0.5 hr)	19.9	20.2	-0.3
25% COHb (O_2 Tonometry 1 hr)	14.3	15.0	-0.7
25% COHb (O_2 Tonometry 2 hrs)	7.3	7.7	-0.4
25% COHb (O_2 Tonometry 3 hrs)		3.7	
50%COHb	52.3	50.7	1.7
50%COHb (O_2 Tonometry 0.5 hr)	41.1	38.1	3.0
50%COHb (O_2 Tonometry 1 hr)	28.9	27.5	1.3
50%COHb (O_2 Tonometry 2 hrs)	15.3	12.3	2.9
50%COHb (O_2 Tonometry 3 hrs)		5.7	

B-181

Demonstration of In-Vitro Synthesized Calcium Oxalate Dihydrate Crystals with Native Octahedral Morphology for Use in Urine Sediment Controls

B. Fernández, C. Grandjean, M. Ghadessi, M. Ban. *Quantimetrix, Redondo Beach, CA*

Background: The microscopic analysis of urine sediment is routinely performed to screen for the presence of red and white blood cells, epithelial cells, microorganisms, casts, and a wide range of crystals. Crystals, regardless of type, result from the precipitation of urine solutes out of solution. When crystals are found in freshly voided urine, they indicate formation in-vivo and are usually clinically significant. Factors contributing to crystal formation include the concentration of the solute in the urine, pH, and flow rate of urine through the renal tubules. Super-saturation can arise from dietary excess, dehydration, or from certain medications. Oversaturation of urine with crystals can lead to stone formation causing pain and an increased incidence of urinary tract infections. Nearly 80% of all urinary stones are calcium compounds, especially calcium oxalate. Calcium oxalate dihydrate (COD) crystals in an octahedral morphology are the most commonly observed crystals in human urine. Urine sediment control materials, used by clinical labs to validate the processing, centrifugation, and microscopy of urine samples, would therefore ideally contain the native octahedral form of COD crystals.

Objective: To synthesize calcium oxalate dihydrate (COD) crystals with the native octahedral morphology for use in urine sediment control formulations.

Methods: Two reagents were prepared: Reagent A was an aqueous solution of sodium oxalate at 1mg/mL and Reagent B was an aqueous solution calcium chloride at 4 mg/mL with other proprietary excipients. One part of Reagent A was slowly added to four parts of Reagent B under very controlled processing conditions. The resulting COD precipitate was centrifuged and the supernatant was decanted. The pellet was re-suspended in water then spiked into the Quantimetrix Dip&Spin® and QuanTscopics® urine sediment control formulations for evaluation under standard brightfield microscopy and on the IRIS IQ®200 automated urine sediment analyzer.

Results: Brightfield microscopy at 400x magnification showed a majority of COD crystals with near perfect octahedral, bi-pyramidal morphology at various sizes ranging from 5-20 µM in diameter in a single preparation. Calcium oxalate monohydrate (COM) crystals in an ovoid morphology were also apparent to a lesser degree in the same preparation. Different preparations made under non-optimal processing conditions resulted in very large COD crystals with complex four-armed cruciform, stellate, or cloverleaf morphologies. The optimized COD crystal preparation in both the Dip&Spin® and QuanTscopics® formulations were accurately identified and characterized by the IRIS IQ®200 analyzer.

Conclusion: This novel method for synthesizing COD crystals with the native octahedral morphology identical to the most commonly observed crystal type found in human urine makes for an ideal urine sediment control. Automated urine sediment systems like the IRIS IQ®200 and 77 Elektronika UriSed® analyzers have been particularly challenging as they do not always recognize the presence of crystals in some third party control formulations. The new Quantimetrix Dip&Spin® and QuanTscopics® formulations with the improved COD crystals are easily characterized by both standard microscopy and automated microscopy methodologies making for an excellent control solution for the clinical lab.

B-182

Reference Intervals for Random Urinary Calcium and Magnesium

V. Gounden, C. Y. Park, T. Prabhala, K. Spaid, S. J. Soldin. *National Institutes of Health (Clinical Center), Bethesda, MD*

Background: To estimate the rate of excretion of urinary constituents, a 24 hour sample is required. However these are cumbersome for the patient and often are not collected accurately. The use of the analyte ratio to urine creatinine accurately reflects the 24 hour excretion as creatinine is excreted at a constant rate throughout the day. At the Department of Laboratory Medicine, NIH Clinical Center reference intervals for urine calcium:creatinine and urine magnesium: creatinine ratios based on the hospital population were previously unavailable. Thus it was difficult to interpret random urine results for calcium and magnesium and this required that a timed or 24 hour specimen be collected for accurate interpretation.

Objectives: To determine the reference intervals for urinary calcium:creatinine and urinary magnesium : creatinine ratios using the Hoffmann method (Hoffmann RG Statistics in the practice of medicine JAMA 1963;185:864-73) at the National Institutes of Health Clinical Center

Methods: Data was collected for a total of 159 individuals between the ages of 4-73 years. This study population consisted of 131 healthy outpatients seen at the NIH Clinical Center and 28 normal volunteers. Data analysis was performed on urine samples collected between May and July 2013. Outpatient data for urine calcium, magnesium and creatinine results as well as demographic details were obtained from the laboratory information system (SoftLab®, SCC Soft Computer ,FL). The Dimension XPand chemistry analyzer (Siemens Diagnostics,Tarrytown NJ) was used to measure the concentrations of urine creatinine, calcium and magnesium following the manufacturer's guidelines. All analytes are measured by spectrophotometric, bichromatic rate technique.

Urine creatinine, magnesium and calcium values were converted to SI units (mmol/l) before further data analysis. Statistical analyses were performed on Microsoft Excel. The ratios were ranked from smallest to largest values. Then, we developed a percent cumulative frequency chart and plotted the cumulative percent frequencies against the log of these ratios/values. Using the Hoffmann approach, we analyzed the linear portions of the curve and calculated the line of best fit. We calculated the 2.5th and 97.5th percentile values as the new reference intervals of these analytes.

Results: The following reference intervals were found employing the Hoffman approach. Urine calcium:creatinine ratio was 0.11 - 1.03 mmol/mmol and for urine magnesium: creatinine the ratio was 0.20-0.76 mmol/mmol.

Conclusions:The availability of reference intervals for urine calcium: creatinine and urine magnesium:creatinine ratio allow for random specimens to be better utilized for clinical and diagnostic purposes. This is also more convenient for the patient and health care providers. An interesting question for future studies revolves around possible gender and racial disparity of these reference intervals and the influence of seasonal changes on these ranges.

B-183

Reference range for sodium, potassium and chloride in single spot urine samples

A. C. C. S. Botelho[1], W. Pedrosa[1], L. S. Vasconcellos[2], S. M. Eloi-Santos[2]. *[1]Hermes Pardini, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: The determination of urinary electrolytes is of great importance in the investigation of systemic metabolic disorders. Since renal excretion daily rate is not uniform during the day, the 24-hour urine is considered the gold standard biological sample. However, its replacement by a fresh urine sample could reduce patient discomfort and the possibility of pre-analytical interferences. Nevertheless the findings in the literature are conflicting. This study aimed to estimate the pattern of excretion of sodium, chloride and potassium over 24h and define reference ranges for the periods presenting good correlation with the 24-hour urine.

Method: Timed sequential urine samples were collected from 41 healthy adults, aged 18-60 years in the following periods: 6am-9am (after breakfast), 9am-12pm, 12pm-3pm, 3pm-6pm, 6pm-9pm and 9pm-6am. Sodium, potassium and chloride were measured by Ion Selective Electrode method and creatinine by Kinetic Colorimetric method (Roche P- Modular®). The values of each sample were correlated with those obtained from the 24-hour urine. The urine samples from the best correlated periods were used to establish the reference ranges, following Clinical and Laboratory Standards Institute (CLSI). For that, samples of 120 healthy subjects were used and confidence interval was 95% and the significance level of 0.05.

Results: For sodium and chloride, high positive correlation was seen in all period samples. However, the best correlation was obtained with samples collected between 6am-9am, after breakfast (sodium: r=0.6185, p=0.0028; chloride: r=0.5787, p = 0.0060). For potassium measurement, the best correlation was with urine collected between 6pm-9pm (r=0.5824, p=0.0001). The reference ranges are shown in Table 1.

Conclusion: It is possible to replace the 24-hour urine sample by spot urine collected in predetermined periods: 6am-9am for sodium and chloride and 6pm-9pm for potassium.

Reference range for sodium, potassium and chloride in single spot urine samples

Electrolytes	Time of collection	Lower limit (mEq/g creatinine)	Higher limit (mEq/g creatinine)
Na	6am-9am	24 (90% CI 15.0 to 33.7)	300 (90% CI 229.8 to 309.8)
Cl	6am-9am	19 (90% CI 12.8 to 36.6)	287 (90% CI 275.2 to 337.3)
K	6pm-9pm	11 (90% CI 10.1 to 14.1)	91 (90% CI 74.2 to 108.6)

B-184

A Comparison of Creatinine Measurement by the Jaffe and Enzymatic Methods in an Outpatient Population

A. H. Adams, J. A. Straseski, C. M. Lehman, R. L. Schmidt. *University of Utah and ARUP Laboratories, Salt Lake City, UT*

Background: Serum creatinine (SCr) concentrations and estimated glomerular filtration rates (eGFR) are widely used for the evaluation of renal function. The Jaffe and enzymatic methods are the most common methods for creatinine measurement. The Jaffe method is generally less expensive than the enzymatic method but is more susceptible to interferences. Significant savings could be obtained if populations could be identified where the interference rate of the Jaffe method is acceptably low. Most studies on Jaffe interferences have used spiked samples and the rate of interferences in defined patient populations has not been well characterized. The interference rate is likely to vary by patient population. The objective of this study was to compare the creatinine and eGFR results from Jaffe and enzymatic creatinine methods in an outpatient population.

Methods: This study analyzed 545 unique, randomly selected, outpatient samples over a period of 45 days. Samples were analyzed using both the Jaffe (kinetic alkaline picrate, Abbott Laboratories) and enzymatic (Creatininase, Abbott Laboratories) methods using an Abbott Architect c8000. eGFRs were calculated using the CKD-EPI equation. A 20 day precision study, following CLSI guidelines, was also performed that evaluated both creatinine methods at concentrations of 0.28, 0.79, 1.21, 2.73, and 5.08 mg/dL.

Results: Orthogonal (Deming) regression showed no significant difference between the Jaffe and enzymatic methods. The slope was 1.006 (95% CI: 0.998, 1.103) and the intercept was -0.005 (95% CI: -0.015, 0.006). The average difference (bias) between the methods was -0.007 mg/dL. The Bland-Altman (BA) limits of agreement (LOA) for the creatinine difference were -0.139 and 0.136 mg/dL. The Bland-Altman limits of agreement for the CKD-EPI eGFR were -10.3 and 10.4 mL/min. 3.1% (17 of 543) of CKD-EPI eGFR discrepancies resulted in a change of classification with respect to the 60 mL/min decision limit. The difference between the predicted and actual CKD-EPI discordance at the 60 mL/min decision limit was not statistically significant (chi sq= 0.03, p=0.86). The Jaffe method performed with greater precision at four of the five concentrations in the 20 day precision profile. At concentrations of 0.28, 0.79, 1.21, 2.73, and 5.08 mg/dL, the CVs of the Jaffe method were 3.0%, 1.4%, 0.8%, 0.8% and 0.8%, respectively. The corresponding CVs of the enzymatic method were 2.9%, 1.7%, 1.7%, 1.3%, and 1.2%.

Conclusion: At our institution the Jaffe method generally had greater precision than the enzymatic method. Discrepancies in the CKD-EPI eGFR based on the Jaffe method did not result in a statistically significant increase in disease reclassifications at the 60 ml/min decision limit in an outpatient population. Studies are needed to characterize the relative rate of interference in additional populations.

Wednesday, July 30, 2014

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

Molecular Pathology/Probes

B-185

Case Report: Molecular and Cytogenetic characterization of a 46,XX male

V. D. T. Niewiadonski[1], D. C. Abreu[2], A. F. Cobacho[1], C. S. Rodrigues[1], O. P. Denardin[1], N. Gaburo Jr.[1]. *[1]DASA, Sao Paulo, Brazil, [2]DASA, Rio de Janeiro, Brazil*

Background: The Y chromosome evolves from an auto chromosome and accumulates male-related genes including sex-determining region of Y-chromosome (SRY) and several spermatogenesis-related genes. 46,XX subjects carrying the determining SRY gene usually have a completely male phenotype.

Objective: To identify Y chromosome material in an azoospermic male with a 46, XX karyotype.

Method: A 39 years old male was tested to micro deletions of Y chromosome in order to investigate the origin of an infertility characterized by azoospermia. He did not report any previous familiar history of infertility and was never submitted to any infertility treatment before. The isolated DNA was submitted to a PCR reaction to amplify the following regions of Y chromosome according to the protocol previous described by Simoni et al (2004). The patient was also submitted to a karyotype analysis which shown presence of two X chromosomes. To confirm the presence of Y or part of chromosome, fluorescence in situ hybridization (FISH) was performed using LSI SRY/CEP X probe set (Vysis®).

Results: PCR amplification of DNA was detected using QIAxcel DNA Screening Kit (Qiagen, Hilden Germany) and showed the presence only of the sex-determining region of the Y chromosome (SRY) and the absence of others target regions of Y chromosome (AZFa, AZFb and AZFc). FISH analysis showed an X chromosome containing SRY gene sequence on the top of the short arm. This Y chromosome gene was not visible by conventional cytogenetic analysis which shown presence of two X chromosomes.

Conclusion: Molecular and FISH techniques were very useful for detecting and locating Y sequences in this particular case, allowing an accurate diagnosis and correct management of the patient. Testing new Y chromosome markers in XX males will make it possible to narrow the breakpoints further in each case and to establish correlations with the clinical features, identifying the Y regions implicated in the definition of the phenotype.

B-188

Validation of molecular testing of the Y chromosome microdeletions in DNA analyser: A case of laboratory automation

F. S. V. Malta, R. L. A. Bottrel, M. S. Goncalves, G. C. Lopes, A. G. Assini, E. C. C. Mateo, A. C. S. Ferreira. *Instituto Hermes Pardini, Vepasiano, Brazil*

BACKGROUND: Infertility is a medical condition that affects 10-15 % of couples seeking to have children.Large proportion of cases of idiopathic male infertility is due to the presence of microdeletions in the Y chromosome genes related to spermatogenesis. Located on the long arm of the chromosome Y, the 3 regions known as “azoospermia factors” (AZFa , AZFb and AZFc) are found fully or partially deleted in azoospermic patients or patients with severe oligozoospermia. Our laboratory offers the test for Y chromosome microdeletions by multiplex PCR followed by electrophoresis on 2% agarose gel stained with ethidium bromide. Once it is a manual method occasional errors may occur in prepare the gel or pipetting the samples. Furthermore, ethidium bromide is a mutagenic reagent and therefore the represents a risk to both the operator and the environment.

OBJECTIVE: The objective of this study is to standardize the diagnosis of Y chromosome microdeletions through a more efficient and secure methodology.

METHODS: For this study, the capillary electrophoresis was chosen to substitute the agarosegel electrophoresis. Modifications were made in the PCR reactions, the primers were labeled with different dyes and the settings in the analysis software GeneMapper ID-X v. 1.1.1 were modified to optimize theanalysis of this genetic testing in different fluorescence channels in the 3730 DNA Analyzer.In order to evaluate such changes, the results of 35 patients who carried out themicrodeletions of the Y chromosome were both analyzed on agarose gel and incapillary electrophoresis. The Kappa statistic was used to compare the results.

RESULTS: Results accordance obtained between the two techniques fromthe 35 samples using Kappa statistics was perfect 1,0 (0,669 to 1,0 CI 95%). There were no statistically relevant difference ($p < 0,001$) among compared methodologies.

CONCLUSIONS:We concluded that capillary electrophoresis could be used as an alternative method for the study of Y chromosome microdeletions, providing a more efficient and secure assay.

B-189

Integrative analyses of Hippo pathway components in human cancer genome

T. Yu, Y. Chang, J. Bachman, Z. Lai. *Pennsylvania State University, University Park, PA*

Background: The Hippo signaling pathway regulates cell proliferation and apoptosis to control tumor growth and organ size. Cancer genome projects found more somatic mutations in Hippo pathway than candidate approach. However, it is elusive for their damaging degree and their association with other mutated genes in the signal transduction network. In here we have analyzed the somatic non-synonymous mutations of the Hippo pathway components from cancer genome database.

Methods: Hippo pathway components mutation data are downloaded from COSMIC (Catalogue Of Somatic Mutations In Cancer) and TCGA (The Cancer Genome Atlas). The conservation of the mutated residues and the 3D structure remodeling are evaluated by ClustalW2 and CPH models 3.2. All the mutation pathogenicity are determined by PolyPhen2, Mutation Assessor, SIFT and Provean. We also integrated knowledge of the mutated sites from literature using candidate mutagenesis, or large scale phosphoproteomics approaches. The carcinoma genome of ten tissues with top mutated frequencies of Hippo Pathway components are downloaded from TCGA data portal. Significant concurrent and mutually-exclusive relationships ($p\text{-value}<0.01$) were revealed among the mutations of the Hippo components, its interactome, and cancer census genes. The unbiased screening selected a list of genes with strong associations with Hippo components. The significantly mutated genes were identified from 57, 57, 28 and 25 patients carried predicted damaging mutations of LATS1, LATS2, STK3 and STK4, and compared with the ones found in 19, 33, 32, and 27 patients with synonymous or predicted neutral mutations. Survival analyses were also performed for the patients with Hippo pathway alterations.

Results: The mutation spectrum of Hippo Pathway components has been explored. Core Tumor suppressors(LATS1/2 and STK3/4) (5%) have higher damaging mutation frequency than core oncogenes (YAP1/WWTR1 and TEAD2/4) (1%) in the Hippo pathway. 67% out of total 652 mutations and 36 predicted damaging mutations sit in the domain and residues, respectively, reported by literature. Hippo interactome test found that PSMD2, WWP1, NEDD4 involved in the degradation of tumor suppressors in Hippo pathway are found concurrent mutated with those tumor suppressors. Cancer census gene comparison testing found that XPO1, TCEA1 and SUZ12 are concurrent with Hippo pathway components, while PIK3CA, TP53 and STK11 are mutually exclusive. The unbiased screening identified CENPC1, ORC are concurrent with Hippo pathway components, while CDKN2A and RHOA are mututully exclusive. Patients with up-regulation of oncogenes has worse survival rate than the patients without.

Conclusions: The analyses for Hippo Pathway mutations in cancer patients provided comprehensive reference for physicians and researchers to further investigate the mutations. The genomic analyses shed light on elucidating mechanisms of Hippo pathway and the concurrence and mutually exclusivity with its associated genes in cancer, which could aid in the development of a cancer biomarker panels for prognoses and treatments.

B-191

Comparative Genomic Hybridization arrays (aCGH) Technique as a diagnostic tool for 22q11.2 microduplication syndrome.

E. C. Mateo, C. B. Campos, C. P. Melo, A. C. S. Ferreira. *Instituto Hermes Pardini, Vepasiano, Brazil*

Background: At the moment, it`s uncertain whether the 22q11.2 microduplication is a natural genetic variant-we are all different-or whether it`s a real syndrome whose effects can be highly variable. The 22q11.2 syndrome is related to development delay, intellectual disability, slow growth leading to short stature and poor muscle tone. All patients underwent examinations of karyotype and in all cases were normal. Most people have a 22q11.2 microduplication that is about <3 Mb in size. Most of the 30-40

genes in the <3Mb stretch of duplication have not been fully characterized. However, researchers believe that the duplication of one particular gene, known as *TBX1*, is responsible for many of the typical symptoms of the syndrome. aCGH is a molecular cytogentics technique, able to identify unbalanced chromosomal changes (gains or losses of genomic material) through the general analysis of the entire genome in a single experiment. Objective: The objective of this study was to show the use of the technique Comparative Genomic Hybridization arrays (aCGH) as a molecular tool for the diagnosis of microduplication 22q11.2. Methods: 3 samples of peripheral blood of pediatric patients referred to the Institute for study of loss or gain of genomic material were used. The samples were collected in tubes 4ml EDTA and stored at 4 ° C until processed. For DNA extraction was used *QiampDNA Blood Mini Kit* (*Qiagen*). After extraction the samples were quantified and evaluated reasons for A260/A280 and A260/A230 in NanoDrop2000/2000c. The samples had concentrations above 50 ng/uL and A260/A280 (1.8 to 1.9) and A260/A230 (1.5 to 1.9) were among the reasons for standard values. gDNA was digested with restriction enzyme labeled with the cyanines 3 (Cy3-reference) and 5 (Cy5-patient) and purified by *SureTag Complete DNA Labeling Kit* (*Agilen*t). After purification, the samples were again quantified to measure the incorporation of Cyanine 5 (Cy5). All samples had A260/A280 reason to 1.8, DNA concentration above 420.0 ng/uL, the incorporation of Cy5 was above 10.3 pmol/uL, the specific activity was above 24 pmol Cy5/µg DNA and yield ug DNA was above 8.9. The *OligoaCGH/ChIP-on-chip Hybridization Kit* (*Agilent*) and *Human Cot-1 DNA* (*Agilent*) kit was used to perform hybridization on the slide containing probes corresponding to 180,000 genes. After 24 hours of hybridization at 65 ° C the slides were washed with *AgilentOligoaCGH/ChIP-on-Chip 1* and *2 Wash Buffer Kit* (*Agilent*) and acetonitrile reagent (*Sigma*). After extraction of data through the Software *ScanControle Software Fecture Extraction*, these were analyzed in *Agilent CytoGenomics* Edition Software 2.7.8.0. Results: All samples met the standards of quality required for the analysis. The technical benchmark *DerivativeLR_Spread* (DLRS) was <0.30. Samples showed gain of genomic material (279 Kb) in the 22q11.2 region. Conclusions: This study shows the importance in examining the aCGH technique for the diagnosis of patients with 22q11.2 syndrome who had normal results in the karyotype.

B-192

Genotyping of rs12979860 and rs8099917 single nucleotide polymorphisms in HCV infected Brazilian patients.

V. D. T. Niewiadonski[1], P. G. C. Trojano[1], P. L. Oliveira[1], M. D. Freire[2], N. Gaburo Jr.[1]. *[1]DASA, Sao Paulo, Brazil, [2]DASA, Rio de Janeiro, Brazil*

Background: Recent studies have demonstrated the role of the interleukin 28B (IL28B) polymorphisms in predicting treatment response, spontaneous clearance and sustained virologic response for patients with Hepatitis C virus (HCV) infection. Two main single nucleotide polymorphism (SNP) were identified in proximity to interleukin IL28B: rs12979860 (homozygous for the major C allele are more likely to respond to treatment than those who were homozygous for the alternative nucleotide (T) and rs8099917 (strong predictor of sustained virologic response (SVR) to pegylated interferon and ribavirin for subjects with homozygous T/T genotype than subjects with G/G genotype).

Objective: To describe the frequency of the IL28B C/T SNP for rs12979860 and that of the T/G SNP for rs8099917 in a cohort of Brazilian patients with HCV infection.

Methods: Blood samples from 305 HCV infected Brazilian patients were analyzed from January 2013 to December 2013. DNA isolation was performed with the automated platform Qiasymphony SP (Qiagen, Hilden, Germany) according manufactured instructions. Genotyping for rs12979860 and rs8099917 were performed using TaqMan® SNP Genotyping Assays (Life Technologies, Foster City, CA) at Viia 7 Real Time PCR System (Life Tecnhologies). The results were analyzed using TaqMan® Genotyper Software version 1.0.1.

Results: Carriers of rs12979860 CT genotype predominated (160/305, 52.5%), homozygotes for allele C were 90/305 (29.5%) and the remaining homozygotes for IFN-resistant allele T were 55/305 (18.0%). As for the rs8099917 SNP, genotypes were distributed as follow: 175/305 (57.4%) carried the rs8099917 TT genotype, whereas 110/305 (36.0%) carried GT and 20/305 (6.6%) the GG genotype. The co-prevalence of genotypes is shown at Table 1.

Conclusion: The frequencies found were consistent with previous studies. Testing for rs12979860 and rs8099917 has become an important strategy to predict the patient treatment outcome.

Table 1- Combined genotype frequencies of the interleukin (IL)28B single nucleotide polymorphisms rs1297860 and rs8099917

	rs8099917			
	GT	**GG**	**TT**	**Total**
rs12979860	n (%)	n (%)	n (%)	
CT	81 (26.6)	2 (0.6)	77 (25.3)	160 (52.5)
CC	4 (1.3)	0 (0.0)	86 (28.2)	90 (29.5)
TT	25 (8.2)	18 (5.9)	12 (3.9)	55 (18.0)
Total	110 (36.0)	20 (6.6)	175 (57.4)	305 (100)

B-193

Validation of Hybridizing Genomic Comparative array (aCGH) technique to screen 180.000 genes in diseases Postnatal.

C. B. Campos, C. P. S. Melo, A. C. S. Ferreira, E. C. C. Mateo. *Instituto Hermes Pardini, Vepasiano, Brazil*

Background: aCGH technique has been widely used in postnatal diagnosis of patients with normal karyotyping and disturbs like autism, neuropsychomotordevelopmental delay, facial dimorphism, mentaldevelopment delay, low weight and low height, among others. Because of its capacity to detect microdeletions and microduplications, aCGH is an important tool to investigate such clinical cases. Objective: The objective of study was validation and implements the Hybridization Genomic Comparative array (aCGH) technique for postnatal diagnosis of normal karyotyping patients. Methods: Samples from 12 patients were analyzed in duplicate by Hermes Pardini Institute and a reference laboratory. The samples were collected in tubes 4ml EDTA and stored at 4 ° C until processed. For DNA extraction was used *QiampDNA Blood Mini Kit* (*Qiagen*). After extraction the samples were quantified and evaluated reasons for A260/A280 and A260/A230 in NanoDrop2000/2000c. The samples had concentrations above 50 ng/uL and A260/A280 (1.8 to 1.9) and A260/A230 (1.5 to 1.9) were among the reasons for standard values. gDNA was digested with restriction enzyme labeled with the cyanines 3 (Cy3-reference) and 5 (Cy5-patient) and purified by *SureTag Complete DNA Labeling Kit* (*Agilen*t). After purification, the samples were again quantified to measure the incorporation of Cyanine 5 (Cy5). All samples had A260/A280 reason to 1.8, DNA concentration above 420.0 ng/uL, the incorporation of Cy5 was above 10.3 pmol/uL, the specific activity was above 24 pmol Cy5/µg DNA and yield ug DNA was above 8.9. The *OligoaCGH/ChIP-on-chip Hybridization Kit* (*Agilent*) and *Human Cot-1 DNA* (*Agilent*) kit was used to perform hybridization on the slide containing probes corresponding to 180,000 genes. After 24 hours of hybridization at 65 ° C the slides were washed with *AgilentOligoaCGH/ChIP-on-Chip 1* and *2 Wash Buffer Kit* (*Agilent*) and acetonitrile reagent (*Sigma*). After extraction of data through the Software *ScanControle Software Fecture Extraction*, these were analyzed in *Agilent CytoGenomics* Edition Software 2.7.8.0. Results: All samples met the standards of quality required for the analysis. The technical benchmark *DerivativeLR_Spread* (DLRS) was <0.30. Results for the same patient were compared between both laboratories and all of them had the same sensibility, specificity and reproducibility. Four patients had at least one pathogenic alteration detected by aCGH. One had many LOH regions detected showing that this patient's parents came from the same ancestor. The other seven had alterations considered non-pathogenic. Pathogenic alterations detected were a deletion on the chromosome 4 short arm (4p16.3p16.2), that overlaps the Wolf-Hirschorn syndrome region; a duplication on the chromosome 9 short arm (9p24.3p24.2), related to developmental delay; a deletion on the chromosome X long arm (Xq27.2), related with mental development delay and obesity; a duplication on the chromosome 7 long arm (7q36.3), also related with mental development delay; and a deletion on the chromosome 15 long arm (15q11.2-q13.1), that overlaps Prader-Willi and Angelman syndromes region. Conclusions: Concluding, almost half of the patients presented a pathogenic alteration that was not detected by conventional karyotyping proving the importance of aCGH as a complementary diagnosis tool.

B-194

Comparison of Invivoscribe's T Cell Receptor Gamma Gene Rearrangement Assay 2.0 vs TCRG Gene Clonality Assay (developed by the Euroclonality, previously BIOMED-2 Group)

B. Peh, J. Iqbal, L. Oon. *Singapore General Hospital, Singapore, Singapore*

Background Histology or cytology, supplemented with immunohistology or flow cytometric immunophenotyping has been used to discriminate between malignant and reactive lymphoproliferations. However, in some of the cases, the diagnosis is difficult. The diagnosis of lymphoid malignancies can be supported by clonality assessment as all cells of a malignancy have a common clonal origin. Gene rearrangement analysis used to be performed by Southern Blot-based techniques which is very reliable, but is increasingly replaced by PCR techniques because of

the greater efficiency and sensitivity of PCR. These gene rearrangements generate products that are unique in length and sequence for each cell. Therefore, PCR assays can be used to identify lymphocyte populations derived from a single cell by detecting the unique V-J gene rearrangements present within these antigen receptor loci. We evaluated the T cell receptor gamma gene clonality assay (Euroclonality's primer) and T cell receptor gamma gene rearrangement assay 2.0, both from Invivoscribe, for clonality assessment of T cells lymphoproliferative disorders.

Methods 22 archived formalin-fixed, paraffin-embedded (FFPE) clinical samples were extracted with QIAamp DNA FFPE tissue kit, following manufacturer's recommendation. 2 proficiency panel samples were included as well. The extracted genomic DNA was quantified and amplified using the specimen control size ladder master mix in the Invivoscribe kits and AmpliTaq Gold DNA polymerase. All samples were tested in duplicates of 50ng and 100ng of DNA to check for presence of inhibitors and DNA quality. Next, PCR was performed with TCRG tube A, TCRG tube B and TCRG 2.0 with samples in duplicate, polyclonal, monoclonal and negative control. The PCR products were denatured with Hi-Di formamide and GeneScan 600 Liz size standards and anlayzed on ABI 3500 capillary electrophoresis instrument. Data are automatically displayed as size and color specific peaks.

Results Majority of the samples were concordant with the in-house developed test for TCRG, TCRG clonality assay and TCRG 2.0. However, 2 of the samples showed discrepant results. TCRG 2.0 requires shorter hands-on time to perform and is easier to analyze as it is single tube and single color (blue). TCRG clonality assay has 2 tubes (tube A and B) and each tube is dual-color (green and blue). The dual-color can be quite confusing to interpret. The clone size detected is different for both assays as the PCR primers are different. Hence, for different labs using these 2 assays, it is hard to correlate if the clone detected is the same.

Conclusion The TCRG clonality assay and TCRG gene rearrangement assay 2.0 from Invivoscribe generated similar results for clonality assessment of T cells lymphoproliferative disorders. We had 2 cases with discrepant results and will emphasize that the results of molecular clonality tests must always be interpreted in the context of clinical, histological and immunophenotypic data. TCRG clonality assay adopts the Euroclonality's primers and is more widely used in laboratories. Even though TCRG gene rearrangement assay 2.0 is easier to perform and interpret, more data should be generated for the better comparison of these 2 assays.

B-195

HPV E6/E7 mRNA RT-qPCR Assay for Detecting High Grade of Cervical Lesion with ThinPrep Pap Samples

G. Kim[1], J. Munkhdelger[2], H. Wang[3], D. Lee[4], S. Kim[1], Y. Choi[5], S. Park[1], Y. Kim[1], H. Kim[1], S. Ahn[1], J. Kim[1], H. Jin[6], S. Park[7], K. Park[2], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]Department of Pathology, Wonju College of Medicine, Yonsei University, Wonju, Korea, Republic of, [3]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [4]Department of Clinical Laboratory Science, Hyejeon College, Hongseong, Korea, Republic of, [5]Department of Biomedical Laboratory Science, Songho College, Hoengseong, Korea, Republic of, [6]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [7]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of*

Background: Human cervical cancer is the second most common cancer among women worldwide. Several decades ago, human papillomaviruses (HPV) were found out to be a major factor of cervical cancer. HPV DNA genotyping assay has been the method of the choice, since it has shown high analytical sensitivity. The latest results show oncogenic HPV DNA appeared not only in cancerous tissues, but also in the normal tissues according to cytological diagnosis. For this reason, HPV test targeting E6 and E7 mRNA of 5 oncogenic HPVs (HPV genotype 16, 18, 31, 33, and 45) which are known to be responsible for oncogenesis of cervical cancer has been commercialized using real-time nucleic acid sequence based amplification (NASBA) assay. The previous data showed that the real-time NASBA assay has higher clinical specificity than HPV DNA testing (97.1 % vs. 53.7 %). However, the sensitivity of real-time NASBA assay was lower than that of the HPV DNA testing (41.1 % vs. 100 %).

Methods: In the present study, therefore, HPV E6/E7 mRNA targeting RT-qPCR assay was designed to detect 16 oncogenic HPV genotypes (HPV genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, and 69), it was performed with RNA prepared from ThinPrep® Pap (Hologic Inc., Bedford, MA, USA) samples, and the results were compared to real-time NASBA data.

Results: For the detection of $CIN2^+$ high grade cervical lesions, the sensitivity and specificity of RT-qPCR assay were 92 % and 98.6 %, respectively. Therefore, HPV E6/E7 mRNA RT-qPCR assay showed the significantly higher sensitivity (91.1 %) compared to real-time NASBA assay (41.1 %). In normal cytology cases, the specificity was 98.6 % and 53.7 % by HPV E6/E7 mRNA RT-qPCR assay and HPV DNA testing. These results revealed that HPV E6/E7 mRNA RT-qPCR assay better reflects cytological diagnosis.

Conclusion: It is suggested that Real-HPV E6/7 mRNA® assay (M&D, Wonju, Republic of Korea) could overcome the shortcoming of lower specificity in DNA assay as well as the lower sensitivity of commercialized HPV mRNA real-time NASBA assay, NucliSENS EasyQ HPV v1.1 (bioMérieux, Marcy, France), with ThinPrep® Pap (Hologic Inc.) samples.

B-196

Donated organ genomic signature in circulating DNA of the liver transplant recipient to monitorization the transplanted liver health

H. C. Macher, G. Suarez-Artacho, J. M. Guerrero, S. Alvarez-Gomez, P. Molinero, M. A. Gomez-Bravo, P. Jimenez-Arriscado, A. Rubio. *Virgen del Rocío University Hospital, SevillE, Spain*

Background:Health assessment of the transplanted organ is very important due to the relation of long-term survival of organ transplant recipient and the maintaining of organ health. It has been described that transplanted organ cells suffering damage liberate DNA. Thus, during organ rejection, apoptosis cell death deals to the release of specific transplanted organ DNA to the host plasma. In this context organ cell free DNA may give us a differential genomic marker of the donated organ health. In a first approximation the quantification of DNA from Chromosome Y in women host under male organ transplantation may be a useful tool. The objective of this work was to validate the usefulness of quantifying specific organ circulating DNA (cDNA) in serum of transplanted patients as noninvasive diagnostic genomic marker, in the diagnosis of graft injury. With this purpose we monitorized serum cDNA from chromosome Y in eight women after male liver transplantation.

Methods: cDNA quantization of the SRY gene was performed by real-time quantitative PCR before, at the moment of transplantation (day 0) and during the stay at the intensive care unit. Beta-globine cDNA levels, a general cellular damage marker, were also quantified. Patients were grouped based on clinical outcome. Group A, were patients that accepted liver transplantation without any complication (Patients 1-3); group B were patients that accepted the male organ but suffer complication not related with the transplantation (patients 4-6); patient 7 suffering an autoimmune hepatitis rejected the first transplanted organ but accepted the second one, and finally patient 8 underwent a liver transplantation from a male liver donor suffering a sepsis by colangitis that developed to general organ failure and died.

Results: All patients showed an increase of cDNA levels at the moment of transplantation that decreased until patient stabilization. Group A, showed the early peak at day 0 that immediately disappear. Patients from group B showed an increase of beta-globine gene levels but not of SRY gen ones at the moment of any clinical complication. Patient 7 showed high levels of of beta-globine gene levels and SRY gen after the first transplantation which was rejected and decreased after the second one accepted. Liver transplantation was succeful for patient 8 showing low levels of SRY gen during most the first weeks after surgery. However the beta-globine levels persisted elevated due to a colangitis that ended in a sepsis, multiorganic failure and death. At the moment of multiorganic failure SRY gen levels were also increased.

Conclusion: Our results shows that donor-derived cDNA may be quantified in the serum of organ transplant recipients, and that high levels of donor DNA might be used as an indication of graft injury vs other pathologies of the patients

B-197

Genetic variants of glucose-6-phosphate dehydrogenase (G6PD) in Brazilian children with positive neonatal screening for G6PD deficiency, and correlation with neonatal jaundice

S. F. FONSECA[1], C. S. NOBRE[2], V. B. A. S. EIRA[1], P. L. M. BELMONTE[1], D. O. ALENCAR[3], J. V. THOMAS[4], R. H. JACOMO[5]. *[1]Universidade de Brasilia, Brasilia, Brazil, [2]UniCEUB, Brasilia, Brazil, [3]DLE Medicina Diagnostica, Rio de Janeiro, Brazil, [4]Secretaria de Estado Saúde do Distrito Federal-GDF, Brasilia, Brazil, [5]Laboratório Sabin LTDA, Brasilia, Brazil*

Background: G6PD deficiency, the most common human enzymopathies throughout the world, causes a spectrum of phenotypes including neonatal hyperbilirubinemia and acute and chronic hemolysis. Genetically, G6PD deficiency is a heterogeneous

condition, with approximately 150 mutations and 400 variants identified. Molecular studies seek to define the origin of the enzymopathy in a determined population and correlate the G6PD variants with the clinical course of the disease, as well as identifying the G6PD deficiency in heterozygous females. Previous reports have shown that the prevalence of G6PD deficiency, in several regions of Brazil, is around 10% among males of African origin and between 1-6% on euro-descendent males. The data regarding the types of G6PD variants in Brazilian population are fragmented and scarce. The capital of Brazil, which lies in the Federal District, has a mixed population representing the different regions of Brazil. The Neonatal Screening Program (NSP) in Federal District indicates a prevalence of 4.5% for G6PD deficiency. The objective of the current study was identify the types of variants in the G6PD gene in a group of children screened through the NSP in the Federal District, and correlate these data with the presence of neonatal jaundice. Methods: Oral mucosa samples were collected from eighty boys and four girls diagnosed with G6PD deficiency through the NSP in January and February of 2014, whose parents signed an informed consent form. The majority of the newborns presented with residual enzyme activity of around 50% (moderate deficiency). All representatives of the children filled out a questionnaire with relevant details regarding family history, history of neonatal jaundice and therapy. Molecular analysis was carried out using real-time PCR (allelic discrimination). The G202A and C563T mutations in the G6PD gene were analyzed using specific primers and probes. Results: Seventy of the 84 families were unable to provide information regarding ethnic origin of the child, 13 claimed indigenous descent, and one claimed Portuguese and Spanish descent. 60.7% of the children presented with neonatal jaundice, 76.5% presented at 48 hours post-natal, and 29% required phototherapy. Molecular analysis identified a high proportion (98.8%) of neonates positive for the G202A mutation (variant G6PD A-): 79 boys were hemizygous and 4 girls were homozygous for this mutation. Only one boy presented the Mediterranean C563T mutation. Analysis of the correlation between genotype and presence of neonatal jaundice was compromised by the intense predominance of the G202A mutation in the sample group. Conclusions: This is the first study carried out in the population of individuals with G6PD deficiency in the Federal District of Brazil. Although the sample group studied was relatively small, the high prevalence of a single mutation suggests that G6PD deficiency in the population of the Federal District is principally due to the G202A mutation. Neonatal jaundice was frequent among G6PD deficient children. The absence of cases of heterozygous females in the sample group may reflect the inability of neonatal enzyme screening to detect G6PD deficiency in these cases.

B-199

Validation of a quantitative CMV Simplexa kit for clinical use in a private hospital in Sao Paulo, Brazil

N. H. Muto, L. Oyakawa, J. F. Melo, J. R. Pinho, R. Sitnik. *Hospital Albert Einstein, São Paulo, Brazil*

Background: Human Cytomegalovirus (CMV) is a member of the Herpesviridae family. Primary CMV infection in healthy individuals is asymptomatic or results in a mild, non-specific illness. After acute infection, CMV establishes latent infection. Reactivation can occur in immunocompromised patients with an important morbidity and mortality in this group. Early diagnosis and CMV viral load monitoring in high risk patients are critical for an efficient infection management. To improve molecular diagnostic of CMV in our Clinical Laboratory, we validated the quantitative FOCUS CMV Simplexa kit in plasma samples.

Methods: Nucleic acids from plasma samples were extracted using automated EasyMag system and submitted to Real Time PCR with Focus Simplexa CMV kit, using the 3M Integrated Cycler equipment which is a nucleic acid amplification system based on a centrifugal micro fluidic platform. Validation was conducted according to CAP guidelines. We evaluated accuracy, linearity, precision (intra and inter-assay) and sensitivity. Accuracy was tested comparing obtained results with previous data. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using 4 pools of samples (high, medium, low-medium and low) and a negative plasma obtained from the blood bank. Sensitivity was established using samples with viral loads close to the detection limit of the kit.

Results: For accuracy, we compared the results of 53 samples and obtained a mean log difference of 0.21 Log copies/mL. Analytical linearity was evaluated in quadruplicates using the calibration CMV standard from FOCUS, ranging from 2,010,000,000 copies/mL (9.3 Log copies/mL) to 750 copies/mL (2.8 Log copies/mL). The correlation curve obtained had R^2=0.9993. Intra and inter-assay variation of all 4 pools of samples were lower than 10%, as expected. Finally, Detection limitwas tested with samples with 330, 153 and78 copies/mL. The test was able to detect viral load of 330 copies/mL with 100% confidence and 153 copies/mL with 90% confidence.

Conclusion: The results of validation demonstrated that the kit is reliable and useful for quantification of CMV in plasma samples, and combined with its fast turnaround time and decreased hands-on time, make this assay highly suitable for the rapid diagnostics of CMV infections in the clinical laboratory.

B-200

Sodium citrate at 8% is equivalent to EDTA as anticoagulant of choice for circulating cell-free DNA analysis: low contamination by blood cells genomic DNA and inhibition of blood nuclease activity.

G. B. Barra, V. M. Silva, R. H. Jácomo, J. A. R. Vaz, T. H. Santa Rita. *Laboratorio Sabin, Brasilia, Brazil*

Background: Despite the intensive research, few circulating cell-free DNA (cfDNA) analysis have been translated to clinical practice. The lack of preanalytical consensus is a major obstacle. Traditionally, the EDTA is the anticoagulant of choice for studding cfDNA. Moreover, because of the lack of cell protection, the cfDNA is susceptible to blood nucleases, but the impact of these enzymes has long been neglected. Here, we studied the initial amount of cfDNA, it's stability and the blood nucleases activity in plasmas (EDTA, citrate, heparin) and serum samples.

Methods: Fresh blood from 20 health donors was collected simultaneously in K3EDTA, sodium citrate 3.2%, sodium heparin, and Z serum clot activator tubes (all from Greiner-bio-one). The citrate 8% samples were obtained by transferring fresh blood sequentially to 3 citrate 3.2% tubes. Serum or plasmas were generated within 10-15 minutes after the venipuncture. DNA extraction was performed by using Nuclisens easyMAG (Biomerieux). RNAse P was the target used for cfDNA quantification in a StepOne qPCR System (Life technologies) by using hydrolysis probe chemistry and absolute quantification. The results were shown as median in Genomic Equivalents/mL. Statistical analysis was Friedman's test. The cfDNA stability was evaluated treating (or not) the samples with 25U of DNAse I for 1h at 37°C before RNAse P assay. To investigate sample's nuclease activity a hydrolyze probe and a passive reference (ROX) were added to the crude samples and the fluorescence increase were measured for 24h at 37°C in the qPCR system. For nucleases inhibition assay a serial dilution of citrate (0.4 to 14%) was used.

Results: The cfDNA amounts in EDTA (158.7 GE/mL) and in citrate (130 GE/mL) were similar (p=0.27) and lower than the levels found in heparin (413 GE/mL; p=0.031-EDTA, p<0.0001-citrate) and in serum (815 GE/mL; p=0.0012-EDTA, p<0.0001-citrate). The nuclease activity was higher in heparin (arbitrary considered 100%), 90% in serum, 66% in citrate and not detected in EDTA. The nuclease activity curve in citrate was different from serum and heparin suggesting an inhibitory effect. The treatment with DNAse I reduced the cfDNA amount in EDTA by 1.1-fold, in serum by 1300-fold, in heparin by 242-fold and in citrate by 1.3-fold. In the citrate serial dilution experiment, no nucleases activity was detected from 7%. Increasing the citrate concentration to 8% did not change the initial cfDNA amount (96.86 GE/mL) compared to EDTA (129.2 GE/mL; p=0.12) and citrate (89.57 GE/mL; p=0.99). The nuclease activity was not detected in citrate 8% and treatment with DNAse I did not alter its cfDNA amount, reduction of 1.01-fold.

Conclusion: The citrate 3.2%, citrate 8% and EDTA have similar initial cfDNA, although lower when compared to heparin and serum. The nuclease activity was higher in heparin and serum, partially inhibited in citrate 3.2% and completely blocked in EDTA and citrate 8%. The divalent ions chelators citrate 8% and EDTA share a common mechanism of both avoid blood cells genomic DNA contamination to cfDNA and inhibit blood nucleases. The high-levels of cfDNA in serum and heparin should be attributed to the coagulation and direct lyses of blood-nucleated cells, respectively.

B-201

Development of a real-time PCR genotyping assay to detect HLA-B*5701 allele associated with abacavir hypersensitivity reaction

H. S. Yang, A. Smith, K. L. Lynch, A. H. Wu. *University of California, San Francisco, CA*

Background: Abacavir sulfate is a nucleoside reverse-transcriptase inhibitor with potent antiviral activity against HIV. 5-10% of individuals being treated with abacavir develop a potentially life-threatening hypersensitivity reaction (ABC-HSR). Human leukocyte antigen (HLA) B*5701 allele strongly predicts ABC-HSR. Therefore, Pharmacogenetic screening for the HLA-B5701 allele is recommended prior to initiation of abacavir therapy. In San Francisco General Hospital, a two-color B57-specific immunofluorescence assay with FacsCanto flow cytometry was utilized to detect all HLA-B57 subgroups. Preliminary positive samples are sent to a reference

lab to identify true HLA-B*5701 positive patients. Recent studies have shown that a HCP5 single-nucleotide polymorphism (SNP), rs2395029, is in perfect linkage disequilibrium with the HLA-B*5701 allele: the sensitivity of the HCP5 SNP for carriage of the HLA-B*5701 allele was 100% and specificity was 99%. **Objective:** Develop an accurate in-house assay utilizing real-time polymerase chain reaction (PCR) and fluorescence monitoring. **Methods:** DNA extraction from blood samples was performed with a Qiagen DNA mini kit, and DNA concentration was measured using a NanoDrop ND2000. A rapid-cycle PCR was developed using the Rotor-Gene Q 2plex HRM system. Forward primer: GAGTGCCCATTGAACTACACA, reverse primer: GCTGGTCTCTGGACACATACTG, wild-type probe: FAM - AGCTGCCACAGGG - BHQ1 plus, mutant probe: CAL Fluor Orange 560 - AGCTGCCCCAGGG - BHQ1 plus. Thermocycling conditions were 20 sec at 95□C, followed by 40 cycles at 95□C for 3 sec and 60□C for 30 sec. PCR was performed in a 25-ul volume in the presence of 1X Taqman GTXpress master mix, 900 nmol/L of each primer, 250 nmol/L of each probe, 2 ul DNA, and DEPC H2O. 2-fold serial dilutions of a wild-type (T/T) sample and a mutant sample (G/G), with each dilution amplified in triplicates, were tested to evaluate the linearity and repeatability, as well as the limit of detection of the genotyping assay. A standard curve was constructed for each sample on the basis of DNA serial dilution, on which Ct values were plotted against the log value of the target DNA amount. Blood samples of 49 patients who were diagnosed of HIV were included in the patient comparison study between the new RT-PCR assay and PCR-SSOP method of the reference laboratory. **Results**: Ct values were obtained from amplification of serial dilutions of a wild-type sample from100 ug/ul to 3.125 ug/ul and a homozygous mutant samples from 10 ug/ul to 0.625 ug/ul, respectively. The regression equation of the wild-type sample was y=-3.4317x + 30.912, with a R^2 of 0.9985. The intra-assay coefficient of variation (CV) for all dilutions ranged from 0.037% to 0.73%. The regression equation of the homozygous mutant was y=-3.2123x+31.021 with a R^2 of 0.9907. CV for all dilutions ranged from 0.13% to 0.31%. Patient comparison study revealed that this real-time PCR assay demonstrated 100% sensitivity and 100% specificity when validated with 10 positive and 39 negative samples previously confirmed by the reference lab. **Conclusion:** A real-time genotyping assay was developed to identify positive and negative HLA-B*5701 alleles. This approach offers a sensitive, rapid and cost-effective screening assay prior to abacavir prescription. The genotyping assay has a wide dynamic range of reliable amplification linearity.

B-202

Development and Validation of a Clinical Sequencing Assay Using RNA-Seq to Direct Treatment of Relapsed Pediatric Cancers

M. Meyers-Needham, C. Chiang, M. S. Mahadevan, L. A. Silverman, R. Legallo, I. M. Hall. *University of Virginia, Charlottesville, VA*

Background: Cancer continues to be the leading cause of death in children in the US. The aim of this study was to develop a clinically-validated, RNA-sequencing (RNA-Seq) assay of formalin-fixed, paraffin-embedded (FFPE) tumor tissue to detect actionable mutations and/or pathway activation in pediatric cancer relapse patients who have a <20% chance of event-free survival.

Methods: We studied FFPE tissue from 12 tumors previously identified to have gene amplification and/or over-expression. Purified RNA was quantified and evaluated by use of Nanodrop (2 - 3,000 ng/uL RNA), Invitrogen Quant-IT Qubit RNA Broad-Range Assay Kit (1 ng/uL - 1 ug/uL) and Agilent Bioanalyzer RNA Pico 6000 Kit (AMR 0.05 - 5ng/uL). RNA Integrity Numbers ranged from 2 - 4.3; 28S/18S values were 0 for the majority of samples. All purified samples were sonicated to ensure sufficient fragmentation of RNA. The first 4 samples were sequenced in multiplex on the Illumina MiSeq platform, and manual analysis of overexpression was performed. Within-sample normalization of genes of interest was accomplished by selecting 9 housekeeping genes expressed in all samples. When matched normal tissue was unavailable, data from The Cancer Genome Atlas (TCGA) database was used for comparison with our results.

Results: At abstract submission, 4/12 specimens have been fully analyzed: dedifferentiated liposarcoma of kidney, invasive ductal carcinoma of breast, B-cell lymphoma metastasized to tonsil, and lung adenocarcinoma metastasized to lymph node. Specimens contained known gene amplification of MDM2, HER2 (ERBB2), or MYC, or an EGFR mutation, respectively. RNA transcript over-expressions of MDM2 (177-fold), HER2 (20-fold) and EGFR (7-fold) were detected by manual analysis in tumors with matching gene amplification or mutation. Number of reads ranged from 3.5 - 7.3 million per sample, and thus coverage was insufficient to produce reads in exons 19-21 of the EGFR gene in order to detect intragenic mutations or deletions. Therefore only one specimen per flow-cell was sequenced in subsequent runs, achieving approximately 25 million reads per sample. At time of abstract submission, the TCGA database had no normal control data of the tissue type for the MYC sample analysis; MYC data will be analyzed with a bioinformatics pipeline in development that will define approximately 100 suitable housekeeping genes for normalization.

Conclusions: We describe a proof-of-principle for a clinically validated whole-transcriptome RNA-Seq assay from archival FFPE tumor tissue in order to detect overexpression of clinically relevant genes in cancer patients.

B-203

Diagnositc yield of chromosomal microarray for individuals with developmental disabilities or congenital anomalies.

A. Repáraz-Andrade, C. Torreira Banzas, M. Pombar Pérez, M. Blanco Pérez, O. Blanco Barca, M. A. Andrade-Olivié, J. R. Fernandez Lorenzo. *Complexo Hospitalario Universitario de Vigo, Vigo, Spain*

Chromosomal microarray (CMA) is increasingly utilized for genetic testing of individuals with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA). Guidelines recommend the use of CMA as first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies.

OBJECTIVES To implement an algorithm for array comparative genomic hybridization (aCGH) testing in patients with unexplained unexplained DD/ID, ASD, or MCA. To assess the diagnostic yield of aCGH for individuals with developmental disabilities or congenital anomalies and compare it to the literature data.

METHODOLOGY We performed, since 06/2009, aCGH to 161 patients referred from the Neuropediatrics department of our hospital with unexplained DD/ID, ASD, or MCA. DNA was extracted with an automated method, QIAamp DNA Blood Mini Kit in a QIACube instrument (QIAgen). aCGH was performed in the beginning with Nimblegen CGH ISCA Plus 6x630K arrays (Roche Diagnostics) and since its exit from the microarray business with the Signature Genomics CGX-HD 4x180K arrays (Perkin Elmer). Both probes design follow the ISCA consortium guidelines (International Standards for Cytogenomic Arrays). Results were reported following ISCN 2013 recommendations. If a CMA variant was observed, parental samples were analyzed to assess whether it is a de novo or an inherited alteration. Copy number variations are assigned the following interpretations: Abnormal (well established syndromes, de novo variants and large changes); VOUS (variants of unknown significance) and likely benign (not previously reported but inherited from a healthy parent). Diagnostic yield was defined as the number of patients with abnormal variants divided by the total number of patients tested.

RESULTS 161 patients and 42 parents were studied. 110 of the 161 patients (68,32%) had a normal aCGH result. 51 patients (31,68%) showed an abnormal result. After analyzing every single case and performed parental tests, we classified the alterations as follows: 21 abnormal (13,04%), 7 likely benign (4,35%), 1 VOUS (0,62%) and 9 are still pending parental aCGH results (5,59%). Abnormal variants deletion or duplication size varied from 200 Kb to 8 Mb. The diagnostic yield, calculated as 21 abnormal patients divided 161 patients, is 13,04%.

CONCLUSIONS aCGH showed a much higher diagnostic yield than conventional cytogenetics techniques for the diagnosis of unexplained unexplained DD/ID, ASD, or MCA. These figures are according to the literature. Our results could be even higher as there are still 9 cases pending parental aCGH results, so these variants can be reclassified as abnormal or likely benign. The use of CMA as a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies has proven to surpass the classical approach with conventional cytogenetics. The use of CMA is cost effective in a child with DD/ID, ASD, or MCA. CMA is not inexpensive, but the cost is less than the cost of a G-banded karyotype plus subtelomeric FISH plus other techniques such as MLPA, and the yield is greater.

B-205

A retrospective analysis of Western Blot findings and subsequent Nucleic Acid Amplification Testing of samples with Immunoassay Screening positive for Human Immunodeficiency Virus

A. Omar, S. D. S. Cosio, W. B. Y. Lee, M. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

Background: With the first clinical observation of Acquired Immune Deficiency Syndrome (AIDS) in 1981, the entire medical landscape pertaining to the diagnosis, treatment and monitoring of an infection by the Human Immunodeficiency Virus (HIV) has progressed continuously. The western blot assay is the current method of choice for confirming HIV results positive by immunoassays. We compared Western Blot outcomes against viral load quantification on immunoassay-positive samples. A total of 70 anonymised samples that were screening-positive on the Roche HIV

Combi or Combi PT immunoassay (Roche Diagnostics, Switzerland) were sent for western blot confirmation and also tested for viral load using the improved Roche Cobas Taqman HIV1 Monitor version 2 (Roche Diagnostics, Switzerland).

Samples are determined to be western blot positive if any 2 bands for p24, gp41, gp120/160 or 2 of 3 envelope bands with or without Group Antigen and/or polymerase bands are present, as defined by Centres for Disease Control and Prevention and World Health Organisation criteria respectively. Indeterminate results are defined as the presence of bands that do not meet positive criteria while inconclusive findings are those that do not fit negative, positive or indeterminate criteria.

Methods: A total of 84 anonymised samples were screened for infection by the Human Immunodeficiency Virus, using the Roche Diagnostics immunoassay HIV Combi or Combi PT. Seventy samples were determined to be at the grey zone cut-off index of 0.9 or greater, indicating presumptive positivity. These samples were subsequently sent for western blot analysis at the National Reference HIV Laboratory with split samples tested using the HIV Monitor v2 on the Roche Taqman 48.

Results: Of 84 samples tested, 14 samples were determined to be screening-negative with no viral RNA detected by nucleic acid testing. Thirty-four samples were screening-positive and subsequently confirmed by western with viral loads of log 1.53 to 6.00 copies per milliliter. Two samples were defined as western blot indeterminate, with no virus detected, while the remaining 7 indeterminate samples and 1 inconclusive sample had viral loads of log 5.33 to 6.48 copies per milliliter. The mean and standard deviation of viral loads for western blot non-positive and non-negative samples were 6.17 and 0.44 respectively. Western blot positive samples yielded a lower average value of log 4.62 copies per milliliter with a standard deviation of 0.73.

Conclusion: While there is a robust concordance between definitive western blot outcomes and nucleic acid testing, the majority of results classified as 'inconclusive' and 'indeterminate' were associated with significant viral loads. This is not unexpected as the western blot is an antibody assay. Re-testing is usually recommended at least 4 weeks after the first result for these patients. Thus, from a clinical perspective, treatment with anti-retroviral prophylaxis in these patients may potentially be delayed until such time when the western blot turns positive with full seroconversion, possibly leading to a sub-optimal outcome for these patients.

B-206

Development and evaluation of a new molecular diagnostic method for HER2 testing

W. Chen[1], K. Chiu[2], R. Yu[2], Y. Liu[3], H. Ko[4], H. Wu[1], W. Hsieh[1], Z. Lee[5], P. Zhang[5], M. Liu[4], K. Chang[1], I. Su[1], Y. Yeh[4]. *[1]National Cheng Kung University Hospital, Tainan 704, Taiwan, [2]Genetics Development Corporation, Lake Bluff, IL 60044, USA, IL, [3]Curiemed Corporation, Hsinchu 30076, Taiwan, [4]General Biologicals Corporation, Hsinchu 30076, Taiwan, [5]Shanghai GenePharma Co. Ltd,, Shanghai 201203, China*

Background: HER2 PCR Testing results have been published for decades with favorable concordance with IHC and FISH testing results. However, on the ground of "insufficient evidence", current guidelines still exclude PCR-based method from determining HER2 status. To find a remedy, we surveyed and found that the incompatible and insufficient evidence was primarily due to the lack of consistency in PCR assay design. Here, we propose a standardized HER2 RT-PCR assay with proven reproducibility for adoption with the hope that a standardize HER2 RT-PCR testing will gain the traction in creating more compatible data toward achieving the goal of having ASCO/CAP to include HER2 RT-PCR assay in HER2 testing guideline.

Methods: One-step RT-PCR with external calibrators was utilized to quantify HER2 RNA copies in samples. To evaluate the performance and concordance between RT-PCR and FISH test, breast invasive ductal carcinoma (IDC) or adjacent normal specimen was collected and processed into FFPE or OCT sample immediately after biopsy or surgery. RNA samples were determined to obtain optimal final concentration of 25μg/mL. HER2 positive cutoff value was established by conducting a statistical population study on tumor versus adjacent normal samples.

Results: The intra- and inter-run coefficient of variations of the assay were <5% (in terms of concentration in $\log_{10}$). The dynamic range of the assay was between $5.4x10^3$-$5.4x10^{12}$ copies/mL (R^2=0.99). Moreover, around 85% overall percentage agreement (OPA) was obtained when compared with FISH test with FFPE or OCT samples.

Conclusion: A standardized RT-PCR assay and user procedure presented here can produce consistent test results regardless of the types of patient samples and has high potential to be included in the ASCO/CAP HER2 testing guideline.

HER2 RT-PCR versus FISH test in NCKU hospital			FISH test			Agreement Score		
			Positive	Negative	Sum	PPA	PNA	OPA
HER2 RT-PCR test	FFPE samples	Positive	16	3	19	80%	89%	85%
		Negative	4	24	28			
		Sum	20	27	47			
	OCT samples	Positive	8	2	10			
		Negative	2	16	18			
		Sum	10	18	28			
PPA: Positive Percentage Agreement; PNA: Negative Percentage Agreement; OPA: Overall Percentage Agreement								

B-207

Real-time NASBA Targeting HPV E6/E7 mRNA Overcomes Low Specificity of HPV DNA Test

G. Kim[1], Y. Choi[2], J. Munkhdelger[3], D. Lee[4], S. Kim[1], Y. Kim[1], H. Kim[1], S. Park[1], S. Ahn[1], J. Kim[1], H. Jin[5], S. Park[6], K. Park[3], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]Department of Biomedical Laboratory Science, Songho College, Hoengseong, Korea, Republic of, [3]Department of Pathology, Wonju College of Medicine, Yonsei University, Wonju, Korea, Republic of, [4]Department of Clinical Laboratory Science, Hyejeon College, Hongseong, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of*

Background: Cervical cancer is one of the most common cancers in women, and ranks second by female cancer mortality rate. About 40 of human papillomaviruses (HPV) can infect the cervix and are divided into high- and low-risk groups based on their frequent detection in carcinoma or low-grade lesions, respectively. Persistent infection with these types is the major risk factor for the development of cervical cancer. So it was recommended HPV genotyping test as complementary diagnosis. But, HPV DNA was detected both in cancerous tissues and in normal tissues according to cytological diagnosis. Several studies have suggested that detection of E6/E7 oncogene transcripts of high-risk HPV types would provide higher specificity in screening for the risk of development of high-grade of cervical samples. The synergistic effects of E6 and E7 proteins results in disturbance of cell cycle regulation, prevention of apoptosis, and, ultimately, transformation and survival of neoplastic and dysplastic cells.

Methods: This study aims to evaluate the clinical performance of the commercial kit targeting HPV E6/E7 mRNA and compare it with HPV DNA genotyping for the detection of high-grade squamous intraepithelial lesions (HSIL) and cancer in a Korean population. NucliSENS EasyQ HPV kit (bioMérieux, Marcy, France) using a nucleic acid sequence-based amplification (NASBA) technique is chosen. This assay utilizes molecular beacon probes for real-time detection and typing of E6/E7 mRNA from HPV genotype 16, 18, 31, 33 and 45.

Results: HPV DNA tests were positive in 100% and 53% of abnormal (SCC, HSIL, ASC-H, LSIL, and ASC-US) and normal cytology cases, respectively. Positivity rates of HPV E6/E7 mRNA assay were 75%, 74%, 60 %, 56%, 29% and 9% for SCC, HSIL, ASC-H, LSIL, ASC-US, and normal cases, respectively. The data from this study seems clearly show that the real-time NASBA for diagnosis of cervical cancer has higher clinical specificity than HPV DNA genotyping test, since the positivity rate detected by the real-time NASBA in normal samples was significantly lower than that detected by the HPV DNA genotyping test. However, the positive rate detected by the real-time NASBA in cancerous samples was much lower than that detected by the HPV DNA genotyping test. In this respect, the sensitivity of the real-time NASBA for cervical cancer diagnosis seems to be lower than current HPV genotyping test. The main reason of this low sensitivity was primarily due to the different prevalence of the high-risk cervical cancer causing HPV genotypes of Korea.

Conclusion: Data from the current study suggests that the NucliSENS EasyQ HPV E6/E7 assay (bioMérieux) has higher specificity than DNA assays, and can overcome DNA assays' shortcoming of low specificity in clinical detection of high-grade cervical lesions and ability to predict the risk of their development in HPV-infected women. Expanding the types of HPV targeted by mRNA assays may increase sensitivity as well as specificity of detection of high-grade cervical lesions.

B-208

Bioinformatics analysis to determine prognostic mutations of 72 de novo acute myeloid leukemia cases from The Cancer Genome Atlas (TCGA) with 23 most common mutations and no abnormal cytogenetics

K. J. Welsh, E. Nedelcu, A. Wahed, Y. Bai, A. Dasgupta, A. N. D. Nguyen. *University of Texas Health Science Center at Houston, Houston, TX*

Objective: Acute myeloid leukemia (AML) is a heterogeneous malignancy with many described karyotypic and molecular abnormalities. Recurrent karyotypic abnormalities in AML have been well established for treatment. However, approximately half of AML patients have no karyotype abnormality (CN-AML). An important issue in the treatment of AML is how gene mutation patterns may help physicians guide the management of patients in daily practice. The Cancer Genome Atlas (TCGA) database has available data from 200 cases of de novo AML including cytogenetics, 260 gene mutations, and survival duration for each case. As previously reported, in this database a total of 23 genes were significantly mutated and another 237 were mutated in two or more samples. We utilize clustering analysis on this database to correlate the presence of 23 common mutations with the prognosis of de novo CN-AML cases.

Methods: Cases with positivity for the most common 23 mutations and no cytogenetic abnormalities were selected from the TCGA. Unsupervised neural network analysis with NeuroXL Clusterizer (OLSOFT LLC, Moscow) was performed on these cases to group them into clusters according to their pattern of mutations and survival duration. The next step was to find interaction between wild-type genes, gene mutations (with their associated proteins) and common chemotherapy to gain more insight into response of CN-AML patients to treatment, which was achieved with Ingenuity IPA software (Ingenuity Systems, Inc., Redwood City, CA).

Results: 121 cases with positivity for the 23 most common mutations were obtained from the original set of 200 AML cases. Subsequently, 72 cases with no cytogenetics abnormalities (CN-AML) were obtained from these 121 cases. Within the 72 CN-AML cases, the following mutations were not present: TP53, NRAS, KIT, EZH2, and HNRNPK, leaving 18 mutations in this subset of patients. Using appropriate threshold for mutation frequency (75%), clustering was found to be based on only 4 mutations: NPM1, FLT3-ITD, RUNX, and DNMT3A. The following prognostic groups were found: (a) good: NPM1, CEBPA, or TET2, (b) intermediate: NPM1/DNMT3A, or other mutations, (c) poor: RUNX1, FLT3-ITD/NPM1, FLT3-ITD/CEBPA, or FLT3-ITD. Pathway analysis revealed significant causality between the mutations FLT3-ITD, NPM1, DNMT3A, IDH2, RUNX1, TP53, KIT, and CEBPA and the chemotherapy agents cytarabine, idarubicin, fludarabine, topotecan, etoposide, hydroxyurea, dexamethasone, methotrexate, and decitabine.

Conclusions: Combinations of mutations appear to dictate the clinical behavior of AML in terms of prognosis. This study provides further molecular characterization and prognostic data for the heterogeneous group of CN-AML patients.

B-209

Validation of a genetic test for lactose tolerance in a Brazilian hospital

N. H. Muto, L. Oyakawa, J. R. Pinho, R. Sitnik. *Hospital Albert Einstein, São Paulo, Brazil*

Background: Lactose, the predominant carbohydrate in breast milk, is hydrolyzed in the intestinal mucosa by lactase-phlorizin hydrolase (LPH). Lactase is produced during the weaning and undergoes a physiological decline in adult, which leads to lactose intolerance symptoms. In some populations LPH activity persists throughout life, a condition known as adult lactase persistence. The conventional diagnostic test is performed challenging the patient with lactose, which can cause diarrhea and abdominal pain in intolerant patients. Several studies have shown that two single nucleotide polymorphisms in the introns of a helicase (MCM6) upstream the lactase gene [rs4988235 (LCT-13910C/T) and rs182549 (LCT-22018G>A)] correlate with lactose intolerance through differential transcriptional activation of the lactase promoter. Intolerance genotype is a recessive trait in both SNPs: CC-13910 and GG-22018.

Methods: In order to offer a molecular test that will not need lactose consumption, we developed two Real Time PCRs reactions to target those SNPs. DNA was isolated from peripheral blood using EasyMag Extractor (Biomeriéux) and submitted to Real Time PCR using TaqMan® SNP Genotyping Assay kits (Applied Biosystems) with probes specific for each SNP.

Results: During validation, we tested 59 samples with previous conventional test results and found a 91.5% correlation and 100% reproducibility of all possible genotypes. Considering that lactose tolerance prevalence in different populations varies greatly, we determined the genotype frequency of 511 patients in our service and found a frequency of 51.86% CC, 41.49% CT and 6.65% TT for LCT-13910 and 51.66% GG, 40.70% GA and 7.63% AA for LCT-22018.

Conclusion: Molecular test was implemented in our Clinical Laboratory in November, 2012 and since then has been an excellent option for patients instead of the conventional test. Approximately, half of tested patients were intolerant to lactose. Those data are in accordance with previous Brazilian studies, and may reflect the great admixture in Brazilian population.

B-210

Genotyping of drug-metabolizing enzymes CYP2D6, CYP2C19, CYP2C9, CYP3A4 and CYP3A5 in patients prescribed pain medications

Z. Su, G. McIntire, S. Holt, F. Wallace, J. Putnam, J. Zimmerman. *Ameritox Ltd., Greensboro, NC*

Background: The cytochrome P450 (CYP450) superfamily represents a group of enzymes which are associated with the metabolism of more than 90% of human drugs. To date, a large number of allelic variants as well as copy number variations (CNV) have been reported and the number of alleles is still growing. Many genetic polymorphisms within the CYP450 superfamily result in altered enzyme expression or activities, significantly effecting drug metabolism. Based on the genotypes of the CYP450 enzymes, patients may be categorized as ultra-rapid metabolizer (UM), extensive metabolizer/normal metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM). Even though the value of routine pharmacogenetic testing is still debated, accumulated evidence indicates that genotyping drug-metabolism enzymes will help physicians to tailor pain treatment to individual patients.

Methods and Materials: DNAs were extracted from buccal swabs collected from 200 patients prescribed pain medications. Five genes in CYP450 superfamily: CYP2D6, CYP2C19, CYP2C9, CYP3A4, and CYP3A5, were genotyped. Genotypes of the 5 genes were reported using the star (*) allele nomenclature. 16 alleles (*2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *14, *15, *17, *29, *41) and copy number of CYP2D6; 10 alleles (*2, *3, *4, *5, *6, *7, *8, *9, *10, *17) of CYP2C19, 2 alleles (*2, *3) of CYP2C9, 6 alleles (*1B, *2, *3, *12, *17, *22) of CYP3A4, and 8 alleles (*1D, **2, *3, *3B, *6, *7, *8, *9) of CYP3A5 were tested. Allele *1 is assigned by default when no other listed alleles are detected. Phenotypes were assigned based on the guidelines from the clinical pharmacogenetics implementation consortium (CPIC) and/or published general rules: 1) UM: more than 2 copies of functional alleles; 2) EM: 2 copies of functional alleles, or 1 copy of functional allele and 1 copy of decreased functional allele; 3) IM: 2 copies of decreased functional alleles, or 1 copy of functional and 1 copy of non-functional alleles, or 1 copy of non-functional and 1 copy of decreased functional alleles; 4) PM: 2 copies of non-functional alleles.

Results: Among the 200 specimens, 11 allelic variants (*2, *3, *4, *5, *6, *9, *10, *11, *17, *41) in CYP2D6, 3 allelic variants (*2, *9, *17) in CYP2C19, 2 allelic variants (*2, *3) in CYP2C9, 3 allelic variants (*1B, *3, *22) in CYP3A4, and 4 allelic variants (*1D, *2, *3, *6) in CYP3A5 were detected. The most common genotypes and phenotypes are: 1) for CYP2D6: *1/*2 (EM, 20%), *1/*4 (EM, 13.5%), and *1/*41 (EM, 10.5%); 2) for CYP2C19: *1/*1 (EM, 38.5%), *1/*17 (UM, 27%), and *1/*2 (IM, 19%); 3) for CYP2C9: *1/*1 (EM, 67.5%), *1/*2 (EM, 22.5%) , and *1/*3 (IM, 8%); 4) for CYP3A4: *1/*1 (EM, 80.5%), *1/*1B (EM, 10%); and *1/*22 (EM, 4.5%); and 5) for CYP3A5: *3/*3 (PM, 76.5%), *1/*3 (IM, 16%), and *3/*6 (PM, 1.5%).

Conclusions: The frequencies of allelic variants and genotypes we found in the five genes are in accordance with preciously published results. In our targeted population, the most common allelic variant found was the non-functional *3 allele in CYP3A5, with a frequency of 86%.

B-211

Development of an LNA-Blocker enhanced, allele-specific, loop-mediated isothermal amplification (AS-LAMP) method for detection of single base mutations in beta-thalassemia patients

T. Zhong, R. Hu, J. Ye, L. Jiang, X. Hu. *The First Affiliated Hospital of Gannan Medical University, China, Ganzhou, China*

Background: It was found that five mutations, namely 654M, 41/42M, 28M, 17M and 27/28M, are the most commonly occurring single base mutations in beta-thalassemia patients in China. Being able to detect and distinguish these mutations is important for patient screening and for diagnosis of the disease. The loop-mediated isothermal amplification (LAMP) method is known to be a rapid (complete in less than 45 min) and simple way for detection of gene deletion and insertion events, but is not specific

enough to distinguish single base difference. This study was aimed to improve the sensitivity of LAMP method on single base mutation detection in beta-thalassemia patients by using Locked nucleic Acid (LNA) modified primers in the reaction.

Methods: The LNA-Blocker enhanced, allele-specific, loop-mediated isothermal amplification (AS-LAMP) was carried out under isothermal condition. To enhance the specificity of AS-LAMP, an LNA modified allele-specific primer and an LNA modified wild type blocking primer were used in the reaction. LNA is a high affinity DNA analogue that has increased target specificity and high melting temperature. This method was used to detect and distinguish single base mutations in beta-thalassemia patients. This method was validated with positive and negative controls and with 145 clinical samples of patient genomic DNA (46 positive samples containing all 5 mutations and 99 negative samples).

Results: The LNA-Blocker enhanced AS-LAMP method showed high specificity with either plasmid or genomic DNA targets in reactions. The assay had a detection limit of approximately 100 copies of the target sequence (95.7% in sensitivity, 44/46), comparable to the traditional AS-LAMP. However, the specificity of LNA-Blocker enhanced AS-LAMP (96.0%, 95/99) was much higher than the traditional AS-LAMP (75.8%, 75/99). Multiplex detection of all 5 targets was also achieved with LNA-Blocker enhanced AS-LAMP.

Conclusion: LNA-blocker enhanced AS-LAMP provides a highly specific isothermal method for detection of single mutations and for screening of single mutations in beta-thalassemia patients.

B-212

Detection of fetal aneuploidies by quantitative fluorescent polymerase chain reaction in the Brazilian population

F. F. Coelho, M. S. Goncalves, G. Lopes, V. O. Almeida, N. Fonseca, A. C. S. Ferreira. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Aneuploidy occurs from non-disjuntion during gametogenesis and results in an abnormal number of chromosomal in the gametes. The gamete joined in fertilization contains an extra copy of one chromosome or one of the chromosomes is missing. These alterations can results in trisomy or monosomy in the fetus or embryo which are frequently associated with severe congenital abnormalities, mental retardation and shortened life expectancy. The majority of first trimester abortion cases is caused for aneuploidies. Quantitative fluorescent polymerase chain reaction (QF-PCR) is a method which has been regularly used for the diagnosis of common chromosomal abnormalities in recent years with low error rates. Objective: To determine the frequency of the most common chromosomal aneuploidies causing abortion in Brazilian population. Methods: The study is the retrospective statistical analyses of data registered on 70 women submitted to molecular studies of chromosome disorders at the Hermes Pardini Institute, Belo Horizonte, Minas Gerais state, Brazil in 2013. All patients had spontaneous abortion and the ovular remains, embryo tissue were analyzed after the DNA extraction. Was compared the genetic profile of the sample with the maternal blood, a differential that allows to detect maternal contamination in the sample, improving the reliability of the results. Contaminated samples were excluded from the study. After that all samples were tested using quantitative fluorescent polymerase chain reaction (QF-PCR) for detection or exclusion of aneuploidy in chromosomes 13, 15, 16, 18, 21, 22, X and Y (Chomo Quant ®QF-PCR Kits). Results: The age average of the patients was 34.4 ± 4.7 years old. We detected 44 subjects (62.9%) with aneuploidies or euploidies. Nine cases of trisomy 15 (12.9%), nine cases of trisomy 22 (12.9%), seven cases of monosomy X (10.0%), four cases of trisomy 13 (5.7%), four cases of trisomy 16 (5.7%), four cases of trisomy 21 (5.7%), five cases of triploidy (7.1%), a case of trisomy 18 (1.4%) and a case of paternal isodisomy (1.4%) were detected by QF-PCR. Conclusions: Quantittaive Fluorescent PCR is a robust and accurate method for rapid aneuploidy detection. One the first suspicions of spontaneous abortions is numerical abnormalities in 13, 18, 21 and sex chromosomes, representing 22.8% of our cases of aneuploidy. But the results show the importance of also being analyzed changes in chromosomes15, 16 and 22 which concentrated 31.5% of the aneuploidies detected. We are going to analyze a larger number of samples in order to confirm the importance of study chromossomes 15, 16 and 22 disorders.

B-214

Detection of Von Hippel - Lindau (VHL) Gene Copy Number Variations Using Digital Droplet PCR

D. Milosevic, S. K. Grebe, A. Algeciras-Schimnich. *Mayo Clinic and Foundation, Rochester, MN*

Background: Our current assay for detection of large deletions in the VHL gene uses a combination of MLPA based probes (MRC-Holland b.v) and Luminex® FlexMap technology. It is a two-day assay that requires 400ng of DNA input, and consists of overnight probe hybridization, amplification, bead hybridization and Luminex detection. Due to these complex workflows, assay failures are not rare. Digital droplet PCR (ddPCR) might represent a faster, more sensitive and more reliable alternative. DdPCR is based on traditional PCR amplification and fluorescent probe-based detection methods, but partitions each reaction into multiple nanodroplets. Quantitation is based on counting the proportion of droplets that show amplification. Poisson statistics are then applied to back calculate the copy number in the original sample. This allows for highly sensitive and reproducible absolute quantification of nucleic acids without the need for standard curves.

The objective of this study is to develop a method for detection of single or multiple exon deletions in VHL using digital droplet PCR (ddPCR).

Methods: Prior to PCR cycling, reactions are prepared as for traditional fluorescent qPCR methods. Fluorescent probes and two primer sets encompassing 80-120bp of each VHL exon are added to each reaction mixture, which also includes an additional reference gene. The final reaction mix is then partitioned into thousands of nano-droplets. Ideally, the starting DNA concentration is such that this will result in a mixture of droplets containing either zero or one template molecule per droplet. The droplets are pooled back into a PCR tube, and cycling is performed. At the conclusion of cycling, negative and positive droplets are counted in a flow cytometer like device. Total assay time is approximately three hours.

Various input concentrations of primers, probes and DNA were tested during validation. Intra- and inter-assay concordance of copy number assessment was tested on three different DNA specimens with and without deletions. Patient specimens containing deletions of one, two, or all three exons of the VHL gene were used for method comparison between MLPA and ddPCR. Finally, formalin fixed, paraffin embedded (FFPE) specimens, which habitually fail MPLA, were re-tested by ddPCR

Results: Primer and probe concentrations and DNA input were optimized and standardized for the method, with 10ng of DNA input required for successful copy number estimation. A direct comparison of TaqMan probes with 3'-MGB-labeled probes showed that MGB-labeling provided better specificity. Copy number assessment concordance was 100% between runs. The method comparison showed 100% concordance between MLPA and ddPCR. The FFPE specimens were successfully assayed by ddPCR while failed testing by MLPA.

Conclusion: DdPCR for copy number estimation of the VHL gene variants is fast, reliable and accurate. It requires minimal nucleic acid input, with a 40-fold reduction of input DNA compared to MLPA. Because of this advantage, difficult specimen types, including FFPE tissue, are now capable of being characterized. Additionally, same-day results are available with the ddPCR method, reducing total run-time from 48- to 3- hours.

B-215

Absolute Quantification of Graft derived cell-free DNA (GcfDNA) early after Liver Transplantation (LTx) using Droplet Digital PCR

J. Beck[1], J. Schmitz[1], P. Kanzow[2], O. Kollmar[3], M. Oellerich[2], E. Schütz[1]. *[1]Chronix Biomedical, Göttingen, Germany, [2]Dept. of Clinical Chemistry, University Medical Center, Göttingen, Germany, [3]Dept. of General, Visceral, and Pediatric Surgery, University Medical Center, Göttingen, Germany*

Background: The diagnostic value of GcfDNA as measure of graft integrity after LTx has been recently proven [1,2]. The yin and yang of using percentage values vs. absolute GcfDNA quantification is, nevertheless, under discussion [3]. Where the ratio of graft to host cfDNA has analytical advantages by eliminating disturbing variables, such as DNA extraction efficiency, variabilities in host cfDNA may obfuscate the view on the engrafted organ. The early phase after LTx was used as model to interrogate whether the percentage or absolute plasma concentration of GcfDNA is a more valuable graft integrity measure.

Methods: GcfDNA percentage was determined by droplet digital PCR (ddPCR - Bio-Rad) as described [1]. A synthetic sequence of non-human origin (average length

of cfDNA) was spiked into 1mL plasma, and quantified with ddPCR after DNA extraction in one fluorescent channel. The total cfDNA was quantified using two combined human genomic dPCRs in the second channel as copies/mL (cp/mL). Total cfDNA was calculated using the spike-in without being extracted to assess the DNA extraction efficiency in each batch. GcfDNA concentration was defined as of the total cfDNA(cp/mL) x GcfDNA%. Plasma obtained during the first 10 days after LTx from 15 patients (one split-LTx) was investigated

Results: Ten repeated extractions of the same plasma pool from healthy volunteers yielded an average of 1069 diploid genomic cp/mL plasma with a CV of 7.5%. Of 185 samples six showed a low (<50%) extraction efficiency; the remainders had an average of 67%+9%. The total cfDNA was highly variable peaking at 6hr after reperfusion ($3.9x10^5$±$2.0x10^5$cp/mL) weaning to $1.3x10^5$±$0.9x10^5$cp/mL at day 10. The respective GcfDNA was $3.1x10^5$±$1.8x10^5$cp/mL (6hr) and $1.5x10^3$±$0.9x10^3$cp/mL (day10). The correlation between GcfDNA% and GcfDNA(cp/mL) values was weak (r=0.61;p<0.05). A comparison of the AUC (day1-day5) of AST with GcfDNA percentage and concentration showed a better association with absolute GcfDNA (r=0.65;p<0.05) compared to percentages (r=0.31;p=0.27). The initial half life was 1.3±0.6days for GcfDNA(cp/mL) and 2.9±1.6days GcfDNA(%), compared to 2.6±1.2days for AST.

Conclusion: A robust and precise ddPCR method for absolute quantification of GcfDNA, was developed, combining the analytical advantages of graft/host ratio (e.g. eliminating possible bias from interferences), with a robust quantification of total cfDNA. The GcfDNA concentration seems better associated with AST-values early after LTx and showed more rapid dynamics than GcfDNA percentage. Even though the initial post Tx phase, with highly variable amounts of total cfDNA, is particularly complicated, this method may also provide a better view on graft integrity in other situations, where the host cfDNA is increased due to non-transplantation related causes. As to whether the clinical utility is improved compared to percentage values for stable patients as well, is subject to further investigations.

1. Beck J. et al. (2013) Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients(...). Clin.Chem. 59:1732-1741.

2. Oellerich M. et al. (2014) Use of Graft-Derived Cell-Free DNA as an Organ Integrity Biomarker to Reexamine Effective Tacrolimus Trough Concentration(...). Ther.Drug.Monit. Epub

3. Lo YM (2011) Transplantation monitoring by plasma DNA sequencing. Clin.Chem. 57:941-942.

B-220

Frequency of G1691A (FV), G20210A (FII), C677T, A1298C (MTHFR), C282Y, H63D E S65C (HFE) among Brazilian blood donors and evaluation of OpenaArray method for SNP detection.

V. D. T. Niewiadonski[1], J. Vieira[2], C. Almeida[3], N. Gaburo Jr.[1], E. C. Sabino[2]. *[1]DASA, Sao Paulo, Brazil, [2]Instituto de Medicina Tropical- USP, Sao Paulo, Brazil, [3]Fundação Pró-Sangue Hemocentro de São Paulo, Sao Paulo, Brazil*

Background: OpenArray® is a new PCR technology that uses a microscope plates and allow the detection of many SNPs at the same time.

Objective: We have used this technology to describe the genotypic frequencies of some SNPs related to venous thrombosis (G1691A and G20210A), to hyperhemocistinemia conditions (C677T, A1298C), and to hereditary hemochromatosis (C282Y, H63D and S65C), among Brazilian blood donors.

Methods: We tested 400 blood samples from Fundação Pró-Sangue Hemocentro of São Paulo, Brazil. The DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA concentration and quality was determined by spectrophotometry method using NanoDrop 2000 (Thermo Scientific, Wilmington, DE). The OpenArray platform (Life Technologies, Foster City, CA) was used to genotype the samples through TaqMan Genotyping detection system. The reproducibility of this method was evaluated running nine samples in triplicate in three different assays. The DNA samples were also tested using TibMol (TibMol, Berlim, Germany) reagents at Light Cycler 2.0 (Roche) plataform, with FRET as detection system, to compare the results and determine the accuracy of Open Array method. The genotypic frequency for each SNP was performed using by Taqman Genotyper v1.3 software (Life Technologies)

Results: Of 400 samples, 392 showed valid results. The results showed agreement of 100% for all assays tested, exception to HFE C282 (95.5% agreement). The only discordant sample result for HFE C282Y was confirmed by direct sequencing which showed that OpenArray result were correct. The calculated frequencies of each SNP found were FV G1691A 98,8% (G/G), 1,2% (G/A); FII G2021A 99,5% (G/G), 0,5% (G/A); MTHF C677T 45,5% (C/C), 44,8% (C/T), 9,8% (T/T); MTHF A1298C-60,3% (A/A), 33,6% (A/C), 6,1% (C/C); HFE C282Y 96,0%(G/G), 4,0%(G/A), HFE H63D 78,1%(C/C), 20,3%(C/G), 1,6% (G/G); HFE S65C 98,1% (A/A), 1,9% (A/T). The reproducibility test showed 100% of concordance among the replicates.

Conclusion: The Open Array genotyping method was used to detect simultaneously 7 different mutations in thrombophilia, folate and hemochromatosis related genes. Compared to other genotyping methods such as PCR-RFLP and sequencing, this method is, easy to perform and useful for high-throughput routines. The results found describe the frequency of SNPs related to diseases not well established by previous literature for Brazilian population. They are important to highlight the genetic profile of Brazilian blood donors.

Wednesday, July 30, 2014

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

Nutrition/Trace Metals/Vitamins

B-221

Serum IL-4 concentration in patients suffering from head and neck cancer

M. F. Godoy[1], E. Fabios[1], O. Casbarien[2], M. S. Feliu[1], A. Navigante[2], N. Slobodianik[1]. *[1]School of Pharmacy and Biochemistry, Buenos Aires, Argentina, [2]Angel Roffo Institute ., Buenos Aires, Argentina*

Interleukin 4 (IL-4) , is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class switching to IgE, and up-regulates MHC class II production. IL-4 decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12.It presents an anti-inflammatory role (1). Previous results in a group of adult patients suffering from head and neck cancer(H&N) showed an altered nutritional and inflammatory status (2). The aim is to analyze the serum IL-4 concentration in adult patients(n=17) suffering from H&N ,at the beginning of the specific treatment. The study was approved by the Ethics Committee of the University of Buenos Aires and met the recommendations stated in the Helsinki Declaration. All participants gave written consent before recruitment. Reference values were obtained from a healthy adult group (n=41, R)(3). Blood samples were collected from fasting patients. Serum IL-4 concentration was determined by Elisa' method (Human IL-4 ELISA set, BD OptEIATM).Results (pg/mL) expressed as Mean ±SD were compared to reference values; H&N showed statistical decreased concentration compared with R at a level of $p < 0.01$: 5.5±2.1 vs 8.2±3.9 with a range between 2.5-9.5 pg/mL This finding point out to and confirm an inflammatory status in the studied group.

References 1) Margni R. La respuesta inmune. En: Inmunología e Inmu¬noquímica. Fundamentos, 5ª Edición, Buenos Aires: Ed. Médica Panamericana; 1996, p. 14-32.

2)M S Feliu , C Silva, P Cresta, O Casbarien, A Ross, A Navigante, N Slobodianik. Biochemical parameters in patients suffering from head and neck cancer. . 5th International Immunonutrition Workshop .Puerto Vallarta, México 6-8 abril 2011. Oral Presentation

3)M Godoy , I Fernandez , M S Feliu , E M Insani , N H Slobodianik. Valores séricos de interleuquina-4 en un grupo de adultos sanos. Acta Bioquím Clín Latinoam 2013; 47 (2): 419-20

Supported by University of Buenos Aires (Code:20020100100044)

B-223

New HPLC method for determination of vitamin K_1 and K_2 in human serum

J. Cepova, E. Klapkova, R. Prusa. *Faculty Hospital Motol, Prague 5, Czech Republic*

Objective: Vitamin K_1 is an essential cofactor in the synthesis of activ blood-clothing factors, vitamin K_2 prevents bone loss and fractures. Objective of this study was to evaluate new HPLC metod for determination of vitamin K_1 and vitamin K_2 in human serum.

Methods: We developed a new HPLC method for the determination of vitamin K_1 and vitamin K_2 in human serum with fluorescence detection after post-column zinc reduction. Vitamin K_1 and K_2 were purchased from Sigma-Aldrich, The internal standard was obtained from Immundiagnostik AG, Germany. 20 µl of internal standard were added to 500 µl of serum and two mL of ethanol were added to precipitate the proteins. The mixture was extracted with 4.0 mL of hexane for 10 min and than centrifuged at 3000 rpm for 5 min. The lower layer was additionally re-extracted with further 4 mL of hexane. The organic layers were than evaporated at 50 °C under a stream of nitrogen. The dry residue was reconstituted with 2 mL of hexane and solid phase extraction was than used (Sep-Pak, 500 mg, Waters). The cartridges were preconditioned with hexane, and vitamin K was eluted with diethylether in hexane (3:97, v/v). Eluates were evaporated under a stream nitrogen at 50 °C. The separation was accomplished on a BDS Hypersil C18, 3 µm column at 22 °C. The detection was performed at 246 nm (excitation) and 430 nm (emission). The mobile phase consisted of 880 mL methanol, 100 mL acetonitrile, 1.1 g zinc acetate, 10 mL acetic acid and 10 mL water, flow rate 1.0 mL/min. The retention times were 5.7 min, 9.2 min, 10.8 min for vitamin K_2, internal standard, vitamin K_1, respectively.

Results: A linear relationship between serum concentration and peak area was obtained for both substances with correlation coefficient r^2=0.9959 for vitamin K_1 and r^2=0.998 for vitamin K_2. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis and coefficients of variation were less than 8%. Mean recoveries of the corresponding compounds were 94.5% and 104%. No interference has been found between vitamin K_1 and vitamin K_2 or IS.

Conclusion: The analytical method developed to quantitate vitamin K_1 and vitamin K_2 in serum has been succesfully validated.

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic)

B-225

Performance Evaluation of LOCI® Vitamin B12 and Folate Assays on the Dimension® EXL™ integrated chemistry system with LOCI® module

C. Briggs, T. Johnson, S. A. Lewisch, L. Schiavoni, J. Thomas, C. Tyler. *Siemens Healthcare Diagnositics, Newark, DE*

Introduction: The objective of this study was to evaluate the performance of the fully automated LOCI® vitamin B12 (VB12) and folate (FOLA) assays for the Dimension® EXL™ integrated chemistry system with LOCI® module newly developed by Siemens Healthcare Diagnostics Inc. The vitamin B12 and folate assays are homogeneous, competitive immunoassays based on Luminescent Oxygen Channeling Immunoassay (LOCI) technology. LOCI reagents include two synthetic bead reagents, chemibead and sensibead, and a biotinylated analyte receptor. The sensibead is coated with streptavidin and contains a photosensitive dye. The chemibead is coated with an analyte analog and contains a chemiluminescent dye as the signal generation component. Before the immunological portion of the reaction is initiated, the patient sample is pretreated with sodium hydroxide and dithioerythritol to release analyte from endogenous binding proteins. The assays are calibrated with the five level multi-analyte LOCI Anemia Calibrator. B12 assay time is 32 minutes and folate is 21 minutes. Sample volume is 12 µL and 10 µL for the B12 and folate assays, respectively.

Methods: Precision estimates were obtained per CLSI EP05-A2 protocol (two replicates twice a day for twenty days) using quality control materials and human serum pools. Linearity was assessed through dilution of high and low analyte samples outside of the measuring interval. Specimen equivalence testing was performed with matched sets of serum and plasma samples. Method comparisons with patient samples were conducted versus vitamin B12 (VB12) and folate (FOL) assays on the Dimension Vista® system (X-axis). Accuracy was evaluated by recovery of the World Health Organization (WHO) Vitamin B12 and Folate International Standard 03/178.

Results: Linearity was demonstrated throughout the measuring interval for the B12 assay of 80 to 2000 pg/mL and 0.5 to 20.0 ng/mL for the folate assay (intervals span from the LoQ for B12 and LoD for folate to the upper calibration standard). With automated dilution, the measuring interval is extended to 6000 pg/mL for B12 and 100 ng/mL for folate. Equivalent results were obtained among serum, and lithium and sodium heparin plasma for both B12 and folate assays and EDTA plasma for the B12 assay. B12 levels were tested at 180, 498, and 978 pg/mL and resulted in repeatability of 5.6%, 2.3% and 2.5%, and within-laboratory precision of 6.5%, 3.7% and 2.8%, respectively. Folate levels were tested at 2.1, 6.6, and 16.7 ng/mL and resulted in repeatability of 4.3%, 4.1% and 2.2%, and within-laboratory precision of 7.6%, 5.5% and 4.0%, respectively. Passing-Bablok regression statistics for Dimension EXL B12 vs. Dimension Vista B12 were: slope: 1.00, intercept: -2.1, r: 0.999, n: 213, range: 62 to 1973 pg/mL. Simple linear regression statistics for Dimension EXL folate vs. Dimension Vista folate were: slope: 1.01, intercept: 0.05, r: 0.99, n: 138, range: 0.6 to 19.2 ng/mL. Recovery difference from the WHO Standard 03/178 was 2.1% (target 480 pg/mL) for B12 and -7.3% (target 5.33 ng/mL) for folate.

Conclusions: The study results demonstrate good performance of the fully automated vitamin B12 and folate assays on the Dimension EXL system.

B-227

Evaluation of Vitamin D Levels in Routine of a Private Laboratory

N. O. Lima, M. M. Magalhães, S. Sorato, A. G. L. Guimarães, J. P. D. Padilha, W. Pedrosa, A. C. S. Ferreira. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Humans get vitamin D from exposure to sunlight and from their diet, is metabolized in the liver to 25-hydroxyvitamin D, wish is used to determine a patients vitamin D status. Many studies considered major health problems from vitamin D deficiency resolved. But vitamin D deficiency is common in dark-skinned persons living in northern countries, or in Asian countries for example. This study tries to elucidate the prevalence of vitamin D deficiency and the geographic influencing factors in population of Brazil.

Objective: Compare serum concentrations of vitamin D (25-hydroxyvitamin D) in samples analysis in a large laboratory, with nationwide coverage in some periods of the year representing different sun exposes.

Methods: We use a cross-sectional study in a sample of patients who performed the dosage of 25OHD in a private laboratory in Brazil. We analyzed 19.185 samples of both genders aged ≥ 18 years. Two months were selected for the study: January and July 2012, representing the months of highest and lowest incident of sunlight, respectively.

The study was divided empirically into three geographic regions of different latitudes, and different exposures to sunlight, designated as Region (0): latitudes greater than -10°, Region (1): latitudes between -10° to -20°, and Region (2) latitudes lower than -20°.

Results: The samples collected in July showed concentrations of Vitamin D lower than those collected in January, with a higher proportion of sample with values considered deficient (≤20ng/mL). The difference was statistically significant.

The proportion of deficient patients in January and July respectively was 15,51% and 25,50% with statistically significant difference. The chance of been deficient was 1,87 times higher for samples collected in July (odds ratio; IC95%: 1,73-2,01- p<0,0001).

There were difference in the percentage of deficient patients in Region (2) between moths of January (17,42%) and July (30,25%). Other studies have find low levels of 25OHD in 42%of seniors in São Paulo and mean serum levels of 21,4ng/mL in Rio Grande do Sul, both states of Region (2).

Conclusion: The role of vitamin D deficiency in increasing the risk of many common and serious diseases, including some common cancers, type 1 diabetes, cardiovascular disease, and osteoporosis. The study confirms that the concentrations of Vitamin D are much lower when derived from regions of lower latitudes and during the month of July (compared to January) possibly due to a reduction in the incidence of sunlight. That conclusion should be taken in consideration and included in the clinical analysis of the patient and not the test result only itself.

B-228

Food-specific IgG antibodies in Brazilians: a descriptive, laboratory information management system-based study.

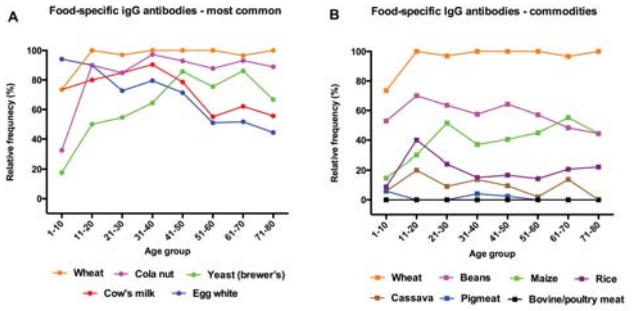
M. S. S. B. Caixeta, L. F. R. Velasco, L. F. A. Nery, S. S. S. Costa, G. B. Barra. *Laboratorio Sabin, Brasília, Brazil*

Background: The aim of this study was to identify the most common foods that trigger IgG immune responses in Brazilians of different age groups and describe the prevalence of this food hypersensitivity for the most common commodities available for consumption.

Methods: We retrieved the results from our food-specific IgG antibodies test performed between December 2012 and December 2013, 281 individuals (unique) were included and 185 (65.8%) were female. The five most common foods that trigger moderate (24-30 U/mL) or strong (>30 U/mL) IgG antibody responses were analyzed and presented by age group. The same was done with the commodities. The age groups were 1-10 (n=34), 11-20 (n=10), 21-30 (n=33), 31-40 (n=73), 41-50 (n=42), 51-60 (n=49), 61-70 (n=29), 71-80 (n=9). The food-specifics IgG assay was Genarrayt Microarray (Genesis Diagnostics), which detects total IgG against 221 foods in serum by ELISA.

Results: The five most commons foods that trigger IgG reaction in the patients were wheat (96.1%), cola nut (84.7%), cow's milk (75.4%), egg white (70.1%) and yeast (brewer's) (64.8%) and their age-dependent relative occurrence frequency can be found in figure 1A. Considering the commodities, their relative occurrence frequencies were as follows: wheat (described before), bean (57.7%), maize (40%), rice (17.1%), cassava (9.3%), pigmeat (2.1%), bovine meat (0%) and poultry meat (0%). Excepting the younger, each commodity elicits a constant and specific pattern of IgG hypersensitivity over age (figure 1B).

Conclusion: We identify the most common foods that trigger moderate/strong IgG response in Brazilians and also the hypersensitive frequency for the major commodities. Some foods (Cola nut, cow's milk, egg white and brewer's yeast) showed an age-dependent IgG serum levels that correlates with an expected age-dependent consumption. Conversely, the constant and specific patterns found for commodities suggest that these foods elicit specific immune response, especially when confronting the extremes (wheat *versus* meats).



B-229

Concentrations of some trace elements in hair of patients with prostate cancer

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

Backgrounds: Deficiency or excess of trace elements can induce metabolic disorders and cellular growth disturbance, even mutation and tumorigenesis. Many authors observed direct association between micronutrient deficiencies and the cancer mortalities. Prostate cancer ranked the sixth most common cancer among males in Saudi Arabia and there are few studies of the association between trace element levels and prostate cancer in Saudi Arabia. Objective: This study aimed to explore the association between concentration of selected hair trace elements including selenium, zinc, copper, manganese and iron as long-term biological markers with prostate cancer in Saudi Arabia. Patients and Methods: The study included 58 patients with prostate cancer, 64 benign prostate hyperplasia (BPH) patients and 52 healthy male subjects of matched age. Full history and clinical data were recorded for all subjects. Prostate cancer patients were undergo digital rectal examination (DRE), trans-rectal ultrasonography (TRUS) guided biopsy of the prostate, computed tomography (CT) scan of the pelvis, bone scan and histopathological examination, accordingly prostate cancer stages and metastatic disease were confirmed. Prostate cancer patients were classified into localized (n = 46) and metastatic prostate cancer (n = 12). Hair samples collected from the nape section of all subjects and hair trace elements Se, Zn, Cu, Mn and Fe levels were analysed by ICP-MS (Perkin Elmer 7300). Odd Ratio (OR) of trace elements levels in hair were adjusted for family history and smoking. Results: Mean Se and Zn levels in hair of the prostate cancer group were significantly lower as compared to BPH and healthy groups ($p<0.05$) whereas the mean levels of hair Cu, Mn and Fe were significantly higher in prostate cancer than BPH and healthy groups ($p<0.05$). Mean hair levels of Se and Zn were significantly differentiated between localized and metastatic prostate cancer ($p<0.05$) whereas mean hair levels of Cu, Mn, Ni and Fe failed to differentiate these groups ($p>0.05$). Conclusion: Prostate cancer may be associated with trace element metabolic disorders. Low levels of Se and Zn and high levels of Cu, Mn and Fe appear to be associated with the risk of prostate cancer in Saudi Arabia. Additional prospective studies are needed to confirm the inverse association between Se and Zn levels and prostate cancer.

B-230

A simple, fast, and sensitive high performance liquid chromatographic method for measuring vitamins A and E in human plasma

C. Yuan, M. Burgyan, D. R. Bunch, E. Reineks, R. Jackson, R. Steinle, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Sufficient levels of vitamins A and E are important in maintaining normal physiological functions in human. The objective of this work was to develop

a fast and sensitive high performance liquid chromatography (HPLC) method to measure the major forms of vitamin A (retinol) and vitamin E (α-tocopherol and γ-tocopherol) in human plasma. **Methods:** Two hundreds µL of plasma samples and 300 µL internal standard solution (5.0 mg/L of α-tocopherol acetate in ethanol) were mixed followed by addition of 1.0 mL of hexane. After vortex and centrifugation, the supernatant was removed, dried, and reconstituted in 200 µL of freshly made 3:1 mix of methanol: diethyl ether of which 25 µL was injected for HPLC analysis. Chromatographic separation was achieved using a C18 column (4.6x75 mm, 3.5 µm) with isocratic methanol elution at 1.8 mL/min. The chromatographic time between injections was 4.0 min. Retinol was detected by UV, whereas tocopherols were monitored by fluorescence. The six-point calibration was traceable to a NIST standard material (SRM 968e). Forty leftover patient specimens were used to compare this method with an independent HPLC-UV method. Plasma samples collected from 51 healthy donors were analyzed to establish the reference interval**. Results:** Intra-assay and total coefficient of variation were <6.0% at three levels tested. No interference was found from lipemic, hemolytic, icteric and uremic samples. Other performance validation data are listed in the Table. Agreeable results were obtained for retinol and α-tocopherol comparing to the HPLC-UV method. Discrepancy in the γ-tocopherol measurement was likely due to difference in the calibration methods as the independent HPLC-UV method used α-tocopherol calibration curve to quantify γ-tocopherol. **Conclusion:** This HPLC method offers rapid and sensitive quantification of vitamins A and E in human plasma.

Table. Assay Characteristics

Analyte	Linearity Range (mg/L)	Accuracy Range (%)	Reference Intervals (mg/L)	Method Comparison				
				Slope	Intercept (mg/L)	Correlation Coefficient	Standard Error of Estimate (mg/L)	Bias (%)
Retinol	0.03-5.14	88.5-114.6	0.30-1.20	1.040	0.00	0.979	0.04	3.9
α-tocopherol	0.32-36.02	87.8-96.7	6.0-23.0	1.115	-0.16	0.944	1.10	9.5
γ-tocopherol	0.10-9.99	96.2-100.2	0.30-3.20	1.358	-0.05	0.968	0.14	37.9

B-234

Dynamics of 3-epi-25-hydroxyvitamin D3 in Premature Infants During Neonatal Intensive Care Unit Hospitalization

A. Anderson-Berry[1], G. Jones[2], E. Lyden[1], M. Kaufmann[2], C. Hanson[1]. *[1]University of Nebraska Medical Center, Omaha, NE, [2]Queen's University, Kingston, ON, Canada*

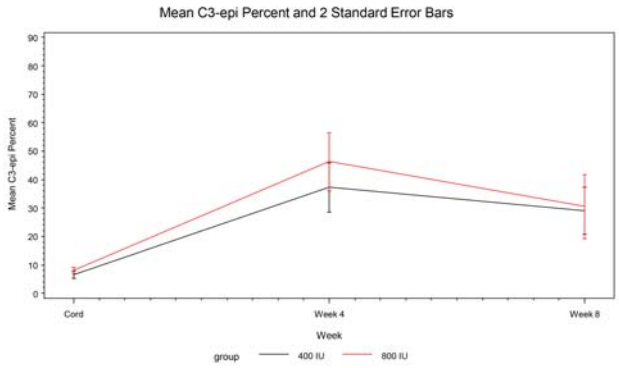
Objective: Evaluate concentrations of 25(OH)D3 and 3-epi-25(OH)D3 in premature infants over time.

Relevance: Availability of a valid biomarker to assess vitamin D status of infants is of great clinical importance. The interpretation of 25(OH)D3 in infants is complicated by the presence of a 3-epi-25(OH)D3 isomer that may falsely elevate the 25(OH)D3 concentration, while functional significance of this metabolite remains unclear.

Methods: 32 infants <32 weeks gestation were randomized to receive 400 or 800 IU/day of vitamin D3 orally. Serum samples were obtained monthly. Vitamin D metabolites were analyzed in triplicate using LC-MS/MS. Comparisons of categorical data was done using Fisher's exact test; continuous data was compared using the Wilcoxon Rank Sum test. Spearman correlation coefficients were used to assess the correlation of vitamin D metabolites. Measurements over time were fit with linear mixed effect models. P<0.05 was considered statistically significant.

Results Mean gestational age at birth was 30.5 weeks; mean birth weight was 1405 grams. Mean serum 25(OH)D3 concentrations in cord blood were 17.3 ng/mL; mean concentrations of 3-epi-25(OH)D were 1.3 ng/mL. Both 25(OH)D3 and 3-epi-25(OH) D3 increased over time (59.0 and 18.6 ng/mL, respectively), however the percent of the total 25(OH)D concentration that was 3-epi-25(OH)D3 increased significantly (p<0.0001 for cord blood vs. 8 weeks, figure 1). 25(OH)D3 and 3-epi-25(OH)D3 concentrations were highly correlated at each time point (r=0.86, 0.61, 0.73, and p= <0.0001, 0.0002, 0.0004 for cord, 4 week, and 8 weeks, respectively).

Conclusion 3-epi-25(OH)D3 concentrations were low in cord blood, but by 4 weeks gestational age, accounted for a significant proportion of the 25(OH)D3 level in this population. The high degree of correlation between total 25(OH)D3 levels and 3-epi-25(OH)D3 is consistent with 25(OH)D3 serving as the primary substrate for C3-epimerization. Vitamin D supplementation was effective in raising 25(OH)D3 levels, however significant increases in 3-epi-25(OH)D3 also occurred.



B-235

Determination of 25-(OH)-vitamin D_2 and D_3 in postmenopausal women and results comparison between immunochemical and chromatographic methods

J. Cepova, R. Prusa, E. Klapkova, M. Pechova. *Faculty Hospital Motol, Prague 5, Czech Republic*

Background: Objective of this study was to evaluate and validate HPLC metod for determination of 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2, and to measure 160 patient samples and compare the results obtained by immunochemical and chromatographic measurements.

Methods: For determination of 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2 was used high performance liquid chromatography (HPLC) with UV detection (Agilent 1200). The samples were measured by kit (Recipe, Germany). The separation was accomplished at 40 °C, samples were detected at 264 nm. Chemiluminescent immunoanalysis was performed on Abbott Architect *i*4000SR analyzer (Abbott Laboratories, Germany). We measured 160 patient samples from postmenopausal women with osteoporosis and postmenopausal women without osteoporosis. For statistical evaluation we used GraphPad Prism 6.0.

Results: A linear relationship between serum concentration and peak area was obtained for both substances with correlation coefficient r^2=0.9989 for 25-(OH)-vitamin D_3 and r^2=0.9986 for 25-(OH)-vitamin D_2. The limit of detection for 25-(OH)-vitamin D_3 was 4.9 nmol/L and for 25-(OH)-vitamin D_2 13.8 nmol/L. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis. Intraday CV for 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2 were 5.7% and 2.8%. Intraday CV were 3.7% and 6.4%. Within-day accuracy expressed by the calculated bias between observed and theoretical concentrations for 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2 were 4.6% and 6.7%. Mean recoveries of the corresponding compounds were 94.5% and 104%. No interference has been found between 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2 or internal standard. We also compared the levels of 25-(OH)-vitamin D_3 measured by HPLC with the levels of 25-(OH)-vitamin D measured by immunochemical method. Furthemore, we compared the sum of 25-(OH)-vitamin D_3 and 25-(OH)-vitamin D_2 with 25-(OH)-vitamin D. The first data were tested by Mann Whitney test, the second part by unpared t test. The data showed significantly differences, p = 0.0061 for comparison of 25-(OH) vit D_3 with 25-(OH)-vit D, and p < 0.0001 for the sum of 25-(OH)-vit D_3 and 25-(OH)-vit D_2 with 25-(OH)-vit D.

Conclusion: We assumed that the data measured by immunochemical method will be higher than the data measured by HPLC due to the large number of cross-reactions, but the results (expressed as median ± SEM) were contrary: 75.3 ± 38.8 nmol/L measured by HPLC vs. 64.7 ± 23.3 nmol/L measured by immunochemical method.

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic)

B-236

Comparison of the Roche Cobas Electrochemiluminescent Vitamin D Assay to the DiaSorin Chemiluminescent Method

R. Rosecrans, J. Greene. *NorthShore University HealthSystem, Highland Park, IL*

Background: Low vitamin D has been implicated in cancer, cardiovascular disease, diabetes, multiple sclerosis, autoimmune diseases, even autism, and headaches resulting in an exponential increase in vitamin D testing. Clinical diagnostic manufacturers have responded in meeting the vitamin D testing demand. Immunoassay manufacturers of vitamin D assays claim their methods correlate to LC/MS/MS, the reference method, but the literature has been varied. This study compares the Roche Cobas (Roche Diagnostics, Indianapolis, IN) electrochemiluminescence binding assay to the DiaSorin (Stillwater, MN) chemiluminescent assay for vitamin D.

Methods: The primary method used by the laboratory is the DiaSorin Total Vitamin D assay. 200 patient samples were chosen for the comparison study. To insure an adequate concentration distribution, patient samples were grouped as follows: less than 10.0ng/mL, 10.1 to 20.0ng/mL, 20.1 to 40.0ng/mL, 40.1 to 60.0ng/mL, and greater than 60ng/mL. After determination on the DiaSorin Liaison, the samples were frozen and held until transport to a laboratory performing the Roche Cobas assay.

Results: Linear regression analysis between the Roche Cobas and the DiaSorin Liaison demonstrated a correlation coefficient of 0.7272. Bland-Altman plots demonstrate value differences ranging from (-)44 ng/mL to (+)54 ng/mL. Patient subgroups were divided and analyzed separately. One patient group (n =91) had values between 5 to 20ng/mL as determined by the DiaSorin method. Linear regression analysis of this subgroup demonstrates a correlation coefficient of 0.6749. The second population subgroup (n=172) had DiaSorin vitamin D results between 20.1 to 40.0ng/mL and a correlation coefficient of 0.1742 when compared to the Roche Cobas assay. Roche Cobas had an overall positive bias compared to the DiaSorin that resulted in 48%,15.8%, and 14.8% fewer patients being classified as deficient (<10ng/mL), insufficient (10.1-31ng/mL) and sufficient (> 32ng/mL), respectively.

Conclusion: The Roche Cobas vitamin D assay has a positive bias compared to the DiaSorin Liaison for patients in the range of 4.0 to 40.0 ng/mL. 48%fewer patients were classified as deficient by the Roche assay, 15.6% fewer classified as insufficient, and 14.8% fewer as optimal. After vitamin D dissociation from its binding protein, the DiaSorin Total vitamin D assay adds an isoluminol labeled vitamin D that competes with an anti-vitamin D Ab bound to magnetic particles in a competitive binding assay. The Roche assay is different, after separation of vitamin D from its bind protein the vitamin D is incubated with ruthenium labeled vitamin D binding protein. Next, biotinylated labeled vitamin D is added which binds to the unoccupied vitamin D binding protein sites. The biotinylated vitamin D is bound to a solid phase which interacts with the streptavidin. Potential sources of error may be heterophile antibody interference for each assay or in the Roche assay the presence of exogenous biotin. Biotin is present in some foods, cosmetics, hair and nail products, and OTC supplements. This study further demonstrates the need for vitamin D standardization and a more vigorous approach by all manufacturing companies on possible assay interferences.

B-237

Measurement of serum total 25-hydroxy vitamin D and its metabolites by liquid chromatography - tandem mass spectrometry: Agreement with the NIST traceable Chromsystems method

S. Vanavanan[1], A. Chittamma[1], P. Meemaew[1], S. Promnuch[1], B. Intachak[1], M. Rochanawutanon[2]. *[1]Clinical Chemistry Division, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [2]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Background: Serum 25-hydroxy vitamin D (25OH-D) is considered to be a reliable indicator of vitamin D status. Liquid chromatography - tandem mass spectrometry (LC-MS/MS) was recently proposed as a reference method. We compared our in-house LC-MS/MS method for total 25OH-D and its metabolites (25OH-D_2 and D_3) measurement with the NIST traceable Chromsystems. Agreement of vitamin D status between both LC-MS/MS systems was assessed based on clinical cut-off value for total 25OH-D.

Methods: Seventy eight serum patient samples were randomly selected from Ramathibodi Hospital. Determination of total 25OH-D, 25OH-D_2 and 25OH-D_3 were performed by two LC-MS/MS methods using the Chromsystems as a reference method. The concentration of each analyte was divided into low and high levels for data analysis. Comparison study and between-assay agreement were examined using Bland-Altman analysis, Passing-Bablok regression and inter-rather agreement analysis (Kappa).

Results: A negative bias was found for all total 25OH-D, 25OH-D_2 and 25OH-D_3 (ranged from -4.47 to -0.04 ng/mL). The Bland-Altman analysis demonstrated slight concentration-dependent bias. Overall, Passing-Bablok fit showed that the in-house LC-MS/MS method was statistically equivalent to Chromsystems (p value > 0.05) with a high to very high correlation coefficient (r = 0.876 to 0.986), **Table 1**. The ability to properly classify patients according to their vitamin D status was very satisfactory for the tested method which the Kappa values were 0.864, 1.00 and 0.944 for total 25OH-D, 25OH-D_2 and 25OH-D_3 (concordance >90%), respectively.

Conclusion: The in-house LC-MS/MS method for total 25OH-D, 25OH-D_2 and 25OH-D_3 determination correlated very well with the NIST traceable method. Strength of agreement for classifying patients into the low or optimal vitamin D levels with the reference assay was very good. The observed bias had little impact on clinical decision therefor is clinical acceptable. We conclude that our LC-MS/MS method met the minimum requirements for the assessment of vitamin D status in clinical laboratories.

Table 1 Passing-Bablok Regression Analysis

	Passing-Bablok regression								
Analytes	Min	Max	SD	Intercept	95% CI	Slope	95% CI	*p*-value	*r*
25OH-D_2 (ng/mL)	0	63.01	18.05	0.000	0.000	0.968	0.938 – 1.001	*0.89*	0.959
- <10 (n=31)	0	7.56	1.82	0.000	0.000	0.965	0.000	*0.99*	0.986
- ≥10 (n=47)	11.64	63.01	11.11	0.559	-3.747 – 5.976	0.948	0.758 – 1.092	*0.86*	0.854
25OH-D_3 (ng/mL)	0	54.23	11.82	-1.679	-2.382 – -0.676	0.968	0.917 – 1.018	*0.73*	0.978
- <20 (n=51)	0	18.59	5.29	-1.557	-3.532 – -0.370	1.006	0.887 – 1.171	*0.68*	0.909
- ≥20 (n=27)	20.97	54.23	8.89	-0.951	-4.785 – 4.099	0.930	0.777 – 1.050	*0.86*	0.948
Total 25OH-D (ng/mL)	0	82.36	18.20	-0.874	-2.383 – 1.647	0.931	0.861 – 0.995	*0.23*	0.952
- <30 (n=31)	0	28.91	7.60	-0.480	-2.091 – 1.600	0.885	0.806 – 0.959	*1.00*	0.973
- ≥30 (n=47)	30.17	82.36	11.97	7.328	0.273 – 14.32	0.779	0.646 – 0.950	*0.62*	0.876

B-238

Evaluation of a Random Access Total 25-Hydroxy Vitamin D (THVD) Immunoassay (IA): Patient Correlation with HPLC-Mass Spectrometry (MS)

N. Yadak[1], S. Freeman[2], C. Hoang[3], C. Finch Cruz[3], E. S. Pearlman[3]. *[1]University of Tennessee Health Sciences Center, Memphis, TN, [2]Veterans Administration Medical Center, Memphis, TN, [3]Veterans Affairs Medical Center, Memphis, TN*

Background: The VAMC evaluated a THVD assay to be employed on our Vitros-5600 platform [OCD; Raritan, NJ]. Specimen results with compared to HPLC-mass spectrometry (MS) taken to be the reference method.

Methods: Specimens (n=42) received frozen from a reference lab [(RL)/ARUP; Salt Lake City, UT] had been assayed for THVD using MS. These were thawed shortly before IA and run in random order according to manufacturer's directions. Two of the specimens had IA values below the analytical measurement range and were excluded from the regression analysis. There were 13 samples with adequate residual volume to be returned as blinded liquid samples and re-assayed with MS. In a separate study 40 in-house specimens were run twice using IA with the specimens being stored at 4 degrees overnight between assays. Regression analysis used Table Curve-2D software (Systat; San Jose, CA).

***Results*:** The relationship between IA (Y) and MS (X) data was fit reasonably well by the linear equation [with 95% CIs]:

Y = 1.015 [0.88, 1.15] • X + 1.25 [-8.30, 5.80] (n=40, r-sq = 0.856).

Using a THVD concentration of 30 mcg/L as the threshold for optimality there were six specimens with MS values >30 mcg/L that were < 30 mcg/L by IA and 2 specimens with the reverse situation. The median (range) within-pair precision for IA (n=40) was 2.56% (0-11.83%) with the CV being <10% in 39/40 instances. A plot of CV (Y) vs. Mean (X) suggests a non-linear relationship with a CV that decreases with increasing mean concentration but the 95% CI on the slope includes zero. The median (range) within pair precision (n=13) using MS was 10.5% (2-30.9%) with 4 specimens having a CV >20%. All MS specimens with initial THVD i concentrations of >30 mcg/L (n=8)] however remained optimal and likewise for those initially with sub-optimal THVD.

***Conclusions*:** The overall performance of the IA from OCD appears satisfactory but there may be some increase in the number of patients with suboptimal concentrations with IA compared to MS. IA reproducibility after 24 hours of refrigeration was very good. Although the number of specimens retested by MS was small there is the suggestion that a freeze thaw cycle and transportation time may result in significant imprecision although clinical reclassification was not observed.

B-240

High prevalence of Vitamin D Deficiency in Korean pregnant women and association between maternal 25-hydroxyvitamin D level in pregnancy and neonatal outcomes

R. Choi[1], S. Oh[2], H. Yoo[3], Y. Cho[4], S. Kim[4], J. Chung[4], S. Lee[1]. *[1]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [2]Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [3]Biostatistics Team, Samsung Biomedical Research Institute, Seoul, Korea, Republic of, [4]Division of Endocrinology and Metabolism, Department of Medicine, Thyroid Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of*

Background: There is growing concern about functional impacts of maternal vitamin D status on multiple adverse health outcomes in mothers and on their offspring and low maternal levels of 25-hydroxyvitamin D [25(OH)D] has been suggested to be associated with some adverse obstetrical and neonatal outcomes. However, there were no reliable data based estimation of vitamin D status using LC-MS/MS in Korean pregnant women. **Objective**: The aim of this study was to carry out the first population-based survey on vitamin D status in Korean pregnant women to assess vitamin D status during pregnancy and the effect of vitamin D deficiency on pregnancy outcomes; premature rupture of membrane, preterm birth, and child born small for gestational age. **Method**: Korean pregnant women (n=220) were recruited prospectively and tested for 25(OH)D levels in serum using liquid chromatography-tandem mass spectrometry with assessment of maternal characteristics. Their 25(OH)D levels were compared with those of 500 healthy nonpregnant women. We analyzed vitamin D status according to demographics, seasons, and obstetrical characteristics together with the assessment of obstetrical and neonatal outcomes. **Results**: The median concentrations of 25(OH)D in Korean pregnant women (n=220) and healthy nonpregnant women (n=500) were 12.6 ng/mL and 15.4 ng/mL, respectively. The overall prevalence of vitamin D deficiency [25(OH)D < 20 ng/mL] in pregnant women and healthy nonpregnant women were 77.3% (170/220) and 79.2% (396/500), and the prevalence of severe vitamin D deficiency [25(OH)D < 10 ng/mL] were 28.6% (63/220) and 7.2% (36/500), respectively. The prevalence of vitamin D deficiency was higher in winter (100%) than in summer (45.5%) in Korean pregnant women. The 1st trimester had a higher risk of vitamin D deficiency than the 3rd trimester (adjusted OR 4.3; 95% CI 1.2-15.2; $P < 0.05$). No associations were observed between vitamin D deficiency and pregnancy or birth outcomes including premature rupture of membrane, preterm birth, and child born small for gestational age. **Conclusions:** The prevalence of vitamin D deficiency was high in pregnant women in Korea and showed the highest during the 1st trimester of pregnancy. Although there was no association between vitamin D deficiency and pregnancy outcome, further research about the long term consequences of vitamin D deficiency during pregnancy on the mother and the offspring is warranted.

B-242

Serum biomarkers that predict clinical outcomes in an immobilized population: Predictors of lean body mass loss

G. J. Davis[1], S. H. Gawel[2], M. Luo[2], N. K. Edens[2], N. E. Deutz[3], R. R. Wolfe[4], S. L. Pereira[2]. *[1]Companion Diagnostics R&D, Abbott Laboratories, Abbott Park, IL, [2]Abbott Nutrition, Columbus, OH, [3]Texas A&M Univ, College Station, TX, [4]Univ. Arkansas Medical Sciences, Little Rock, AR*

Background: Loss of LBM during extended bed rest (BR) (i.e. hospitalization) is a major contributor to functional decline and loss of mobility, especially in older adults. This problem is generally under-recognized due to lack of practical diagnostic tools to measure lean body mass (LBM) over hospitalization. Identifying blood biomarkers that predict a hospitalized individual's risk of losing LBM could provide a practical alternative to expensive and tedious existing methods for LBM measurement (MRI, DXA, CT). Ease of identification of susceptible populations will increase awareness of the problem, allow for timely intervention, and have a huge impact on the health economics of hospitalization.

Methods: Eighteen healthy subjects (age 60-76 y, 3 male, 15 female) were confined to 10 days of complete BR and received either placebo (n=8) or treatment (Ca-β-hydroxy-β-methylbutyrate-HMB) (n=10) over BR. Fasting serum samples were obtained prior to the start of BR (D_1) and analyzed using multiplexed immunoassay array Human DiscoveryMap ® 1.0 (RBM-Myriad). LBM was assessed by Dual energy X-ray absorptiometry (DXA) before and at the end of BR (D_{10}). Baseline biomarker data from both groups were merged, and multiple-hypotheses testing and partition analysis (with 5-fold cross validation) were used to identify baseline markers that predict LBM loss over BR.

Results: Over the 10 day BR period, change in total LBM varied between individuals (-4.47 kg loss to 0.82 kg gain) indicating some subjects were more predisposed to LBM loss over others. Of the 187 markers analyzed at baseline, 63 were excluded due to low detection levels in ≥30% subjects. One pair of markers was found to correlate with percent change in LBM over BR: Tissue inhibitor of metalloprotease-1 (TIMP1) and Tenascin C (TNC) [R^2=0.71, all subjects; R^2= 0.76, females]. Subjects with TIMP1≥ 141 ng/ml at D_1 had larger losses of total LBM at D_{10} whereas subjects with TIMP1< 141 and TNC≥ 461 ng/ml at D_1 did not lose total LBM over BR. Two additional markers were found to correlate with percent change in leg lean mass over BR: Matrix metalloprotease-3 (MMP3) and Apolipoprotein A2 (APOA2) [R^2=0.59, females]. Females with MMP3< 6.93 ng/ml at D_1 were more likely to lose leg lean mass at D_{10} compared with females with MMP3≥ 6.93 and ApoA2< 276 ng/ml at D_1 who did not lose muscle at D_{10}.

Conclusion: Panels of blood biomarkers may be useful in predicting key clinical outcomes such as LBM loss over immobilization (e.g. hospitalization). Validation of these markers in large clinical studies is needed. Sponsored by Abbott Laboratories

B-244

Performance Characteristics of the ARCHITECT Active-B12 (Holotranscobalamin) Assay: A Marker of Vitamin-B12 Deficiency

S. D. Merrigan, W. E. Owen, J. A. Straseski. *ARUP Laboratories, Salt Lake City, UT*

Background Vitamin B12 (cobalamin) is a necessary cofactor in methionine and succinyl-CoA metabolism. Some studies estimate the prevalence of deficiency may be as high as 30% in the elderly population. Cobalamin deficiency is a serious health risk due to its role in carbon metabolism, cell division, DNA synthesis and the clinically important outcomes of anemia and progressive and irreversible neurologic dysfunction. Ten to thirty percent of circulating cobalamin is complexed with transcobalamin and is called holotranscobalamin (holoTC). HoloTC can readily enter cells and is therefore considered the bioactive form. The objective of our study was to evaluate the analytical performance of the ARCHITECT $i2000_{SR}$ Active-B12 (Holotranscobalamin) assay and compare results to manual and automated immunoassays.

Methods Manufacturer-specified limits of blank (LoB), detection (LoD), and quantitation (LoQ), imprecision, interference and linearity were evaluated for the ARCHITECT $i2000_{SR}$ Active-B12 (Holotranscobalamin) assay (Abbott Diagnostics, Abbott Park, IL) per CLSI guidelines. Residual de-identified serum samples were used to compare results from the ARCHITECT HoloTC assay with the results from the automated Abbott AxSYM Active-B12 (Holotranscobalamin) assay (Abbott Diagnostics) and the manual Active-B12 (Holotranscobalamin) Enzyme Immunoassay (EIA) (Axis-Shield Diagnostics, Dundee, Scotland, United Kingdom).

Results Manufacturer's claims of <0.4, <1.9 and <5.0 pmol/L for LoB, LoD, and LoQ, respectively, were verified for the ARCHITECT HoloTC assay. Total within-assay imprecision was 5.7% at a mean concentration of 16.2 pmol/L (SD 0.9 pmol/L), and 4.9% at a mean concentration of 46.7 pmol/L (SD 2.3 pmol/L), verifying the manufacturer's imprecision claims. Interference studies demonstrated <10% deviation in recovery for hemolysis, icterus, and lipemia up to concentrations of 200 mg/dL, 20 mg/dL, and 850 mg/dL, respectively. The ARCHITECT HoloTC assay was linear up to the highest concentration measured (113.4 pmol/L). The largest mean deviation from the calculated recovery was 8.5% at an expected holoTC concentration of 26.2 pmol/L. Method comparison of the ARCHITECT HoloTC assay to the AxSYM HoloTC assay for samples with results within the AMR gave the following Deming regression statistics with 95% confidence intervals: ($ARCHITECT_{HoloTC}$) = 0.941 ± 0.062($AxSYM_{HoloTC}$) + 1.2 ± 2.5 pmol/L, Sy/x = 6.4, r = 0.947 (n=98). Average bias between the methods was -0.9 pmol/L (-3%). Method comparison of the ARCHITECT HoloTC assay to the Active-B12 EIA gave the following Deming regression statistics with 95% confidence intervals: ($ARCHITECT_{HoloTC}$) = 1.105 ± 0.046($EIA_{Active\text{-}B12}$) – 6.8 ± 2.7 pmol/L, Sy/x = 11.0, r = 0.950 (n=221). Average bias between methods was -1.7 pmol/L (-3%). A medical decision point analysis at 35.0 pmol/L was calculated using Deming statistics. The AxSYM HoloTC assay agreed within the 95% confidence intervals of the ARCHITECT assay (33.7 – 35.7 pmol/L), while the Active-B12 EIA comparison was slightly below (31.7 – 34.2 pmol/L). Concordance between the assays at 35 pmol/L was 93% (AxSYM) and 94% (EIA).

Conclusions This assay performed acceptably for LoB, LoD, LoQ, imprecision, interference, linearity and method comparison to the predicate device (AxSYM).

An additional comparison to a manual Active-B12 EIA method performed similarly, with minor exceptions. This study determined that the ARCHITECT HoloTC assay is suitable for routine clinical use.

B-245

Achieving 25(OH)vitamin D_2 and 25(OH)vitamin D_3 Equimolarity for the Dimension® EXL™ Vitamin D Total Assay[1,2]

E. Garcia, W. Bedzyk, J. Li, S. Manoj, T. Q. Wei. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: The Dimension EXL Vitamin D Total (VitD) assay is a homogenous, competitive immunoassay based on LOCI® technology. The VitD assay utilizes 4 reagents: a releasing reagent, two latex bead reagents and a biotinylated monoclonal antibody reagent. The releasing reagent releases 25(OH)vitamin D_2 and 25(OH) vitamin D_3 from the endogenous vitamin D binding proteins (DBP). The released 25(OH)vitamin D_2 and 25(OH)vitamin D_3 are then bound by the biotinylated assay antibody to produce the assay signal. The assay is intended for the equimolar determination of 25(OH)vitamin D_2 and 25(OH)vitamin D_3.

Methods: Recovery of 25(OH)vitamin D_2 or 25(OH)vitamin D_3 was assessed using the 25(OH)vitamin D_2 or 25(OH)vitamin D_3 spiked human serum samples. Available anti-25(OH)vitamin D monoclonal antibodies were screened for anti-25(OH)vitamin D_2 specific binding using a prototype LOCI VitD assay. One anti-25(OH)vitamin D_2 antibody was selected and added to the biotinylated antibody reagent to bind the excess 25(OH)vitamin D_2, which led to 25(OH)vitamin D_2 /25(OH)vitamin D_3 3 equimolarity.

Results: A dose response curve showed the recovery ratio of 25(OH)vitamin D_2 /25(OH)vitamin D_3 changed from 118% to 84 % with the increase of anti-25(OH) vitamin D_2 antibody from 0 to 80 µg/mL. Adding 20 µg/mL of the antibody in the assay reagent achieved 100% recovery ratio or 25(OH)vitamin D_2 /25(OH)vitamin D_3.

Conclusion: By achieving 25(OH)vitamin D_2 /25(OH)vitamin D_3 equimolarity and employing highly sensitive LOCI technology, the fully automated Dimension EXL Vitamin D Total assay provides accurate and precise total 25(OH)vitamin D measurement on the Dimension EXL system.

1. Under development. Not available for sale. Due to local regulations, not all products will become available in all countries.

2.Patent pending.

B-246

Development of a Vitamin D Total Assay* with LOCI® Technology on the Dimension® EXL™ System

J. Li, Z. Teng, M. Drinan, R. Janzen, L. Larson, M. Stranz, D. Clark, B. Wessel, P. Singh, S. Manoj, T. Q. Wei. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: The Siemens Dimension EXL System incorporates multiple detection technologies, including LOCI technology, which enables high sensitivity immunoassay formats. Siemens is currently developing a Vitamin D Total assay utilizing LOCI technology on the Dimension EXL System.

Methods: The Dimension EXL Vitamin D Total assay (VitD) is a homogeneous competitive chemiluminescent immunoassay based on LOCI technology. It measures the total 25(OH)vitamin D concentration (comprised of 25(OH)vitamin D_2 and 25(OH)vitamin D_3) in both serum and plasma. The VitD LOCI components include a releasing reagent, two synthetic bead reagents and a biotinylated monoclonal antibody. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a 25(OH) vitamin D_3 analog and contains chemiluminescent dye. The sample is incubated with the releasing reagent to release 25(OH)vitamin D molecules from the vitamin D binding proteins. The reaction mixture is then incubated with biotinylated antibody to form a 25(OH)vitamin D/biotinylated antibody complex. Chemibeads are added to remove the excess free biotinylated antibody, and then Sensibeads are added to bind to the biotinylated antibody. Aggregates of the Chemibead-analog/antibody-biotin/streptavidin-Sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH)vitamin D in the sample.

Results: The method requires 8 µL of serum or plasma and is linear from 4 to 150 ng/mL. Time to first result is 32 minutes with a stable calibration for 7 days. Repeatability and within lab CVs were less than or equal to 2.4% and 3.2% CVs respectively between 10-100 ng/mL. A patient sample correlation (n=215) showed a Passing-Bablok regression: Dimension EXL Vitamin D Total assay = 1.02× ADVIA Centaur Vitamin D Total assay, Reference Measurement Procedure (RMP).** + 1.84 ng/mL, r=0.94, range = 3 - 131 ng/mL. Less than 10% cross-reactivity was observed at 500 pg/mL for 1,25$(OH)_2$vitamin D_2 and 1,25$(OH)_2$vitamin D_3, at 100 ng/mL for 3-epi-25(OH)vitamin D_3, and at 1000 ng/mL for Vitamin D_2 and Vitamin D_3. This assay is equimolar and aligned to the ID-LC/MS/MS 25(OH)vitamin D Reference Measurement Procedure (RMP).

Conclusion: The Dimension EXL Vitamin D Total assay demonstrates acceptable precision, accuracy and turnaround time for the total 25(OH)vitamin D measurement on the Dimension EXL System.

*Under development. Not available for sale. Due to local regulations, not all products will become available in all countries.

**Under FDA review. Not available for sale in U.S. Due to local regulations, not all products will become available in all countries.

B-247

Development of Candidate Standard Reference Material® 3949 Folate Vitamers in Frozen Human Serum

J. E. Camara, M. S. Lowenthal, J. S. Pritchett, K. W. Phinney, L. C. Sander. *NIST, Gaithersburg, MD*

The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials (SRMs) for the analysis of nutrient levels in clinical matrices. NIST currently offers SRM 1955 Homocysteine and Folate in Human Serum, which possesses certified values for homocysteine and 5-methyltetrahydrofolate (5-mTHF) and reference values for folic acid, also known as pteroyl-glutamic acid (PGA), over three concentration levels of material. SRM 1955 Level 1 required dilution and SRM 1955 Level 3 required spiking of a serum pool to achieve the target concentrations. Once out of stock, this SRM will be replaced with a new candidate material, SRM 3949 Folate Vitamers in Frozen Human Serum. Input from The National Institutes of Health Office of Dietary Supplements (NIH-ODS) and the Centers for Disease Control and Prevention (CDC) indicates interest in an updated material with folate levels reflecting those currently observed in the population.

This new SRM will have three concentration levels with low, medium, and high certified values for both 5-mTHF and PGA. NIST also intends to assign reference values for the additional minor folate metabolites tetrahydrofolate (THF), 5-formyltetrahydrofolate (5-fTHF), 5,10-methenyltetrahydrofolate (5,10-methenylTHF), and the oxidation product of methyl folinate (MeFox). The goal levels for 5-mTHF and PGA, respectively, are: Level 1, 10 nmol/L and 1 nmol/L; Level 2, 50 nmol/L and 10 nmol/L; Level 3, 30 nmol/L and 5 nmol/L. In addition, Level 3 has goal levels of 5 nmol/L, 5 nmol/L, 5 nmol/L, and 3 nmol/L for THF, 5-fTHF, 5,10-methenylTHF, and MeFox, respectively.

To produce SRM 3949, pilot sera were collected from 15 individual donors, five of which were given a 400 µg folic acid supplement one hour prior to blood draw in an attempt to increase serum levels of 5-mTHF and PGA for the high level material without the requirement for additional spiking. To stabilize the folates, 0.5 % (w/v) ascorbic acid was added as soon as possible after collection of serum. These pilot sera were screened for five folates plus the oxidation product, MeFox, at the CDC by ID-LC-MS/MS. Screening results ranged from 5 nmol/L-72 nmol/L for 5-mTHF, 0.4 nmol/L-32 nmol/L for PGA, 0.25 nmol/L-2.2 nmol/L for THF, and 0.13 nmol/L-3.2 nmol/L for MeFox. Both 5-fTHF and 5,10-methenylTHF were below the limits of detection for all sera. Four pilot sera from donors administered a folic acid supplement displayed significantly elevated levels of both 5-mTHF and PGA. Based on these results, a blending protocol was specified to obtain the desired folate concentrations in each of the three SRM 3949 concentration levels. The endogenous levels of 5-mTHF and PGA in all three concentration levels and enhanced folate stability via ascorbic acid addition are improvements over the original SRM 1955 that should better serve the end users.

The candidate material has been blended and packaged and will undergo additional analyses by ID-LC-MS/MS at both NIST and the CDC. NIST is also investigating updates to its current ID-LC-MS/MS method for folates in serum based on the current CDC method, which may be applied to the certification measurements of SRM 3949.

B-248

The Frequency of Vitamin B12 Deficiency in Metformin-treated Brazilian Type 2 Diabetes Patients.

C. B. Damião[1], G. F. Taboada[1], A. O. Rodrigues[1], M. F. M. C. Pinheiro[2], M. C. Freire[2], R. A. Cruz Filho[1], G. P. Cardoso[1], G. A. B. Lima[1]. *[1]Divison of Endocrinology, Universidade Federal Fluminense, Rio de Janeiro, Brazil, [2]DASA, Rio de Janeiro, Brazil*

Background: Vitamin B12 is an essential micronutrient required for optimal hematopoietic, neurocognitive and cardiovascular function. Metformin is a biguanide recommended as initial medical therapy for type 2 diabetes mellitus (T2DM). Despite the known effectiveness, there are disadvantages in its use. Studies indicate a prevalence of 14% to 30% of vitamin B12 deficiency among patients undergoing long-term treatment with metformin, with a still controversial mechanism. The objective of this study was to evaluate the frequency of vitamin B12 deficiency and the factors associated with serum vitamin B12 levels in metformin-treated Brazilian type 2 diabetes patients.

Methods: Cross-sectional study that included 231 T2DM patients in metformin therapy. All the patients were followed at the Endocrinology Division of the University Hospital. The serum B12 levels were measured by chemiluminescence method (Access, Beckman Coulter, CA, USA). Vitamin B12 deficiency was defined by a serum level below 180 pg/mL. SPSS 13.0 was used for statistical analysis. The Mann Whitney test was used to compare numerical variables between groups, the McNemar test to assess the association between binary variables, p value <0.05 was considered to be statistically significant.

Results: Median age was 61 (34 to 79) years and 72% were women. The median time of T2DM was 12 (3 to 41) years, duration of metformin use was 8 (3 to 30) years and dose of metformin was 1,700 (500 to 2,550) mg/day. The vitamin B12 mean levels were 272 (68 to 1,000) pg/mL. The frequency of cobalamin deficiency was 26.8%. The patients with vitamin B12 deficiency had longer disease duration (14 vs 10 years; p= 0.042) and longer metformin use (10 vs 8 years; p= 0.016). Vitamin B12 levels were significantly lower in patients that were using H2 antagonists or proton pump inhibitors (210 vs 292 pg/mL; p= 0.002). After multiple regression analysis, only duration of metformin treatment and the use of H2 antagonists / proton pump inhibitors were significantly correlated to cobalamin levels.

Conclusion: Our study confirmed that the frequency of vitamin B12 deficiency is high in metformin-treated T2DM patients. The patients with vitamin B12 deficiency were using metformin for a longer time, suggesting a cumulative effect of the drug. The use of H2 antagonists or proton pump inhibitors negatively influenced the serum levels of vitamin B12, demonstrating the role of acid gastric reduction as a predisposing factor to vitamin B12 deficiency. Finally, as vitamin B12 deficiency is a cause of peripheral neuropathy, it should be considered in the differential diagnosis of diabetic neuropathy.

B-249

Performance of Roche Elecsys Vitamin D Assay in Different Patient Populations and in Patients with Vitamin D2 supplement.

X. Yi, N. Babic, M. H. Varnamkhasti, E. K. Y. Leung, K. T. J. Yeo. *The University of Chicago, Chicago, IL*

Background: Demand for vitamin D (vit D) testing has increased worldwide, partially due to mounting evidence linking vit D status to overall health and well-being. Currently available methodologies include immunoassays and liquid chromatography tandem mass spectrometry (LC-MS/MS). It has been reported that the accuracy of some immunoassays is dependent on the concentration of vitamin D binding protein (VDBP); excess VDBP amounts may interfere with antibody binding, leading to vit D underestimation (e.g. in pregnant subjects). In addition some immunoassays are optimized for vitamin D3 (D3) and may not detect vitamin D2 (D2). We studied the performance of Elecsys vit D assay (Roche, IN) in different patient populations with varying VDBP concentration, and in patients taking D2 supplement. Method: A total of 211 patient specimens from 4 clinical areas: intensive care unit (ICU), obstetrics (OB), gastroenterology (GAST) and primary care (PCG) were collected and analyzed by in-house developed LC-MS/MS assay, Roche Elecsys assay and DiaSorin (Stillwater, MN) radioimmunoassay (RIA), respectively. Unlike immunoassays, our validated LC-MS/MS method can quantitate 25-OH vit D2 and D3 concentrations individually. The results were analyzed by Passing-Bablok regressions and Bland-Altman plots. Results: Comparison studies showed the following for the entire patient cohort (n=211): [RIA vit D] = 0.93 [LC-MS/MS vit D] +1.80, mean bias =1.2 ng/ml; [Elecsys vit D]= 0.63 [LC-MS/MS vit D] + 3.60, mean bias = -6.7 ng/ml. For patients with only D3 detected (n=153), the correlation showed [Elecsys vit D] = 0.79 [LC-MS/MS vit D] + 3.04, mean bias = -2.4 ng/ml. In 58 patients found to have detectable D2: [Elecsys vit D] = 0.47 [LC-MS/MS vit D] + 2.72, mean bias = -15.2 ng/ml; when D2 concentration is >50% of total vit D (n=27), [Elecsys vit D] = 0.30 [LC-MS/MS vit D] + 5.18, mean bias = -17.8 ng/ml. There was lesser underestimation of D2 by the RIA method when compared to LC-MS/MS method. Regression analyses revealed significant differences between the various patient populations (patients with detectable D2 were excluded): PCG, [Elecsys vit D] = 0.90 [LC-MS/MS vit D] + 2.54, mean bias = 0.45 (n=33); ICU, [Elecsys vit D] = 0.74 [LC-MS/MS vit D] + 3.92, mean bias = -1.17 (n=31); OB, [Elecsys vit D] = 0.71 [LC-MS/MS vit D] +4.64, mean bias = -2.62 (n=31); GAST, [Elecsys vit D] = 0.73 [LC-MS/MS vit D] - 1.54, mean bias = -8.15 (n=21), respectively. Conclusion: The Roche Elecsys vitamin D assay underestimates measurement of vitamin D concentrations in patients who have higher concentrations of D2 and in OB and GAST groups. There was good agreement between Roche Elecsys vitamin D assay with LC-MS/MS assay for the PGC and ICU groups when patients with D2 were excluded.

B-250

An Improved Cleanup Strategy for Patient Samples using Anion Exchange Solid Phase Extraction for the Analysis of Vitamin B6 Status

M. Boraski[1], S. Baek[2], N. Bhavsar[1], M. Pikulski[1]. *[1]Clinical Pathology Laboratories, Austin, TX, [2]Biotage, Charlotte, NC*

Background: Low levels of vitamin B6 are implicated in health problems involving the nerves, skin, mucous membranes, and circulatory system. In children, vitamin B6 deficiency is related to cases of anemia and seizures. Vitamin B6 dependent seizure disorders are an important and treatable cause of childhood epilepsy. Pyridoxal 5'-phosphate (PLP), the primary biologically active form of vitamin B6, is the preferred method for assessing vitamin B6 status. Most of the published procedures for PLP involve extraction via protein precipitation from plasma followed by derivatization to enhance the fluorescence signal. In this work, we present a more selective sample extraction by using anion exchange solid phase extraction (SPE). This procedure is also followed up with derivatization. Our objectives were to automate the procedure by using SPE, decrease the noise and increase the resolution in the chromatography, improve the HPLC column lifetime and create a more robust method with cleaner sample extracts.

Method: To 200µL of plasma, 30µL semicarbazide/glycine derivatizing reagent was added. The samples were then vortexed and incubated. The samples were then processed using strong anion exchange SPE cartridges (EVOLUTE AX from Biotage). The eluent was then evaporated and reconstituted in HPLC grade water. After vortexing, 60µL of the sample was injected onto a HPLC-FLD equipped with a pre-column (KrudKatcher, Phenomenex) and a 4.6x100mm, 3µ, Gemini-NX C18 HPLC column (Phenomenex). HPLC mobile phase A was 10mM sodium phosphate dibasic in 0.3% acetic acid and mobile phase B was acetonitrile/methanol (70:30) in a 5 minute gradient (1mL/min). The excitation and emission wavelengths were optimized at 370 and 470nm, respectively.

Results: The improvements in our method using the SPE cleanup included increased column lifetime (3X), improved resolution and chromatographic robustness and improvements in the run failure rate. The recovery was increased from 73% to 91%. Three levels of plasma quality controls (QCs) were tested at 33, 92, and 360nmol/L. The first two QCs were commercially available (Chromsystems) and the third was prepared in-house from pooled patient samples. The coefficient of variation percent (CV%) for intra-assay precision for the QCs were 5.1%, 4.6%, and 6.9% and inter-assay precision were 10.3%, 5.3%, and 7.3%. The linear regression analysis data for six calibration curve points spanning 2-400nmol/L yielded an R2=0.9994. The lower limit of quantitation (LLOQ) is 2nmol/L. A method comparison was performed with an outside laboratory utilizing 50 patient samples. The Deming regression analysis resulted in correlation R=0.96 for PLP using HPLC-FLD.

Conclusion: An improved method for the analysis of pyridoxal 5'-phosphate has been developed and has proven to be accurate, precise and robust.

B-251

Reference intervals for intestinal disaccharidase activity determined from a non-reference population

S. A. Hackenmueller, D. G. Grenache. *University of Utah, Salt Lake City, UT*

Background: Dietary disaccharides are important exogenous sources of glucose. Because the small intestine is normally impermeable to disaccharides, the activities of intestinal disaccharidases are required for hydrolysis into component monosaccharides that are subsequently absorbed. Decreased or absent activities of one or more disaccharidases can result in carbohydrate maldigestion. The measurement of disaccharidase activities in small intestine mucosa is considered the gold standard test for diagnosis of disaccharidase deficiency. Due to the inability to obtain intestinal biopsies from a healthy reference population, laboratories that perform disaccharidase testing have adopted historical cutoff activities that are used to identify a disaccharidase deficiency (lactase, <15; maltase, <100; palatinase, <5; and sucrase, <25 U/g protein). The objectives of this study were to validate these historical activity cutoffs using the Hoffman method for reference interval determination and to evaluate disaccharidase activities and demographics of individuals for whom intestinal disaccharidase testing was performed.

Methods: 14,827 samples for which all four disaccharidase test results were available were extracted from the laboratory information system. For cutoff validation, results of 0 U/g protein were excluded. Enzyme activities were log transformed and the Hoffman method was used to calculate a reference interval. This method involved determining the cumulative frequency distribution for each enzyme and performing linear regression over the linear portion of the distribution. The reference interval (RI) was calculated as RI_{lower}=2.5(m)+b and RI_{upper}=97.5m+b (m=slope; b=intercept). The frequencies and patient demographics of all possible disaccharidase activity phenotypes were determined from the entire population.

Results: The reference intervals for each enzyme were calculated to be 5-55 for lactase, 105-380 for maltase, 9-32 for palatinase, and 26-110 U/g protein for sucrase. The ratios of historical cutoffs to the calculated lower reference limits were 0.95, 0.56 and 0.96 for maltase, palatinase and sucrase, respectively, indicating the historical cutoffs were less than the lower reference limit for each enzyme. The ratio for lactase activity was 3, indicating the historical cutoff was within the calculated reference interval. Examination of the frequency distribution of the activities of each enzyme revealed that maltase, palatinase, and sucrase were unimodal while lactase showed a bimodal distribution. The intersection of the two lactase populations corresponded to a lactase activity of 10 U/g protein, which produced a ratio of 1.5 if taken to be the lower reference limit. The median patient age of the entire data set was 13 years (range, <1-88 years) and 45% were male. Using the historical cutoffs, 52% of samples had no enzyme deficiencies. Deficiencies of lactase, maltase, palatinase, and sucrase were present in 47, 11, 3, and 13% of the samples, respectively. 35% of samples were deficient only in lactase. 3% of samples were deficient for all four enzymes.

Conclusion: The historical cutoffs for maltase and sucrase were validated. To align with calculated reference intervals, the palatinase cutoff should increase to 9 U/g protein, and the lactase cutoff should decrease to 10 U/g protein. Disaccharidase testing is most commonly performed in patients <18 years. Lactase deficiency is the most frequently observed single-disaccharidase deficiency. Pandisaccharidase deficiency is rare.

B-252

Validation of a 25-OH Vitamin D (total) ELISA on the DRG:HYBRiD-XL, a Fully Automated Analyzer for Immunoassays and Clinical Chemistry

M. Herkert[1], C. Lauf[1], B. Uelker[1], T. Dudek[1], T. Zeller[1], C. E. Geacintov[2]. *[1]DRG Instruments, Marburg, Germany, [2]DRG International, Springfield, NJ*

Background: Vitamin D plays an important role in regulating body levels of calcium and phosphorus and bone mineralization. Physiological Vitamin D levels result from dietary intake and production in the skin after sun exposure. 25-hydroxyvitamin D (25-OH-D), the major metabolite of Vitamin D circulates bound to Vitamin D binding protein (VDBP). Determination of 25-OH D in serum or plasma supports the diagnosis and therapy control of postmenopausal osteoporosis, rickets in children, osteomalacia, renal osteodystrophy, neonatal hypocalcemia and hyperparathyroidism.

Objective: To validate the 25-OH D Elisa on the DRG:HYBRiD-XL.

Methods: 25-OH-D was validated on the DRG:HYBRiD-XL, a fully automated analyzer for immunoassays and clinical chemistry parameters. Assay Procedure: 25 µl of human serum are incubated for 30 min with 50 µl denaturation buffer to release the Vitamin D bound to VDBP. Thereafter, 200 µl neutralization buffer, 50 µl Enzyme Conjugate (biotinylated Vitamin D) and 50 µl Enzyme Complex (Streptavidin-HRP) are added. The reaction volume is mixed and transferred to a well coated with VDBP. After incubation for 60 min, the well is washed with wash buffer. Then 200 µl of TMB substrate are added to the well. After incubation for 30 min, 150 µl of the blue TMB substrate is transferred to a cuvette and measured at 645 nm (450 nm reference wave length). Quantification is done based on a master standard curve that is barcoded on the kit box.

Results: 25-OH-D can be quantified from serum and plasma (EDTA, heparin, citrate) on the DRG:HYBRiD-XL. The dynamic range of the assay is between 2.3-130 ng/mL. The limit of detection (according to CLSI guideline EP-17A) is 5.6 ng/mL, the limit of quantification is 11.4 ng/mL. The mean within-run precision (EP-5A) is 8.9%, the mean between-run precision is 12.9%. Recoveries of 6 samples were found from 85.0-114% (mean 96.5%). The linearity (EP6-A) of 6 samples ranges from 85.4-114.5% (mean 93.7%). Cross-reactivity (EP7-A2) was evaluated for 25-OH Vitamin D_3 (96.3%), 25-OH Vitamin D_2 (74.7%), Vitamin D_3 (3.8%), Vitamin D_2 (3.2%), and 1,25 $(OH)_2$ Vitamin D_3 (0.9%). Bilirubin and Hemoglobin (up to 0.5 mg/mL) and Triglycerides (up to 30 mg/mL) have no influence on the assay results. Method comparison (EP9-A2-IR) with the 25-OH-D manual Elisa (EIA-5396) from DRG Instruments gave a correlation coefficient of 0.958 (n=147; y=1.032x+0.679). A method comparison with the 25-OH-D Liaison (Diasorin) gave a correlation coefficient of 0.931 (n=147; y=1.028x+1.54). Median 25-OH-D values in the USA largely depend on collection month (winter: 19.1 ng/mL; summer 23.6 ng/mL), race (Afro-Americans 17.9 ng/mL; Hispanics 21.2 ng/mL; Caucasian 29.1 ng/mL), and latitude of collection (north 19.8 ng/ml; mid 19.1 ng/mL; south 28.8 ng/mL).

Conclusions: 25-OH-D can be determined on the DRG:HYBRiD-XL with good precision, and results show good correlation to DRG´s manual Elisa and to the Liaison from Diasorin. The concentration of 25-OH-D in serum decreases during winter time, with dark skin colour and with higher latitude.

B-253

Vitamin D Sufficiency Thresholds: Are Age-Specific Values Needed?

L. M. Soares[1], W. Pedrosa[1], S. M. E. Santos[2], L. S. Vasconcellos[2]. *[1]Hermes Pardini, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: Vitamin D insufficiency has become a global health problem that has been associated with metabolic bone disease and a great variety of chronic illnesses. Thresholds for vitamin D sufficiency have been based mainly on total 25-hydroxyvitamin D (25OHD) concentrations at which serum parathyroid hormone (PTH) increases, but there is great controversy surrounding the precise level at which these changes occur. Current guidelines mainly suggest values of 20 or 30 ng/mL. Although vitamin D requirements are thought to vary with age, there are no differences on the reported thresholds. The aims of this study were to analyze the relationship between 25OHD and PTH in different age groups and to evaluate the need of specific reference values for each one of them.

Methods: This was a cross-sectional analysis of 23,276 paired serum PTH and 25OHD levels measured from January 2012 to December 2012 in a large Brazilian reference laboratory. Data on laboratory tests and demographic variables were available from a computerized database. Serum 25OHD was measured through Chemiluminescence, using the Architect® 25-OH Vitamin D Assay (Abbott, Illinois, USA). Serum PTH was measured through Chemiluminescence, using the Access® Intact PTH Assay (Beckman Coulter, California, USA).

Results: Laboratory tests were equally distributed throughout the different seasons of the year. Eighty-one percent of the studied population was female and the mean age was 54.7±18.3 years. Serum 25OHD ranged between 8 and 160 ng/mL, with a mean of 27.7±11.4. Serum PTH levels were inversely correlated with serum 25OHD. The 23,276 patients were split by their 25OHD values into 50 groups and the median PTH of each was calculated, as well as the rate of results exceeding the upper limit of PTH. Next, the data were broken down into five age classes (0 to 20, 20 to 40, 40 to 60, 60 to 80, and older than 80 years-old). The median PTH and the rate of high results were plotted against the mean 25OHD and the graphs demonstrated that the PTH concentrations were consistently higher in the oldest adults at different 25OHD levels. The median PTH associated with 25OHD concentration of 20 ng/mL in each age group was: 23 (0 to 20 years-old), 38 (20 to 40 years-old), 40 (40 to 60 years-old), 48 (60 to 80 years-old), and 57 pg/mL (over 80 years-old). When 25OHD concentration of 30 ng/mL was considered, the median PTH of each group was, respectively, 23, 32, 34, 43 and 46 pg/mL.

Conclusion: We concluded that the serum concentrations of 25OHD required to overcome hyperparathyroidism are different depending on the age and that the cut-

off values obtained in older adults shouldn´t be applied to other groups. We assume that the development of specific reference values according to age will attenuate the overdiagnosis of vitamin D insufficiency and overtreatment of otherwise healthy young patients.

B-254

C-ing is Believing: Enhanced Specificity for Vitamin C using HPLC with Electrochemical Detection and Automatic Alternating Column Regeneration

Z. D. Clark[1], E. L. Frank[2]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT*

BACKGROUND: Vitamin C (L-ascorbic acid) is a water-soluble micronutrient that is essential for human health. Deficiency of vitamin C causes the fatal disease scurvy. Since the 1940's, vitamin C has been measured spectrophotometrically after derivatization to form colored products. These methods are subject to lack of specificity, limited sensitivity, and interference from other compounds. Newer, more specific techniques utilize chromatographic separation. While a high-specificity mass spectrometer may be an appealing detector choice, we found electrochemical detection (ECD) provided adequate selectivity and sensitivity at a fraction of the cost.

OBJECTIVE: The goal of this study was to develop a high-throughput HPLC-ECD method for the measurement of vitamin C in plasma.

METHOD: Protein is precipitated from plasma using 10% *meta*-phosphoric acid. An internal standard, 3,4-dihydroxybenzylamine, is added and the solution is incubated with dithiothreitol in phosphate buffer to reduce dehydroascorbic to ascorbic acid. Following re-acidification, a 5 µL aliquot is injected onto an Agilent HPLC system. The analytes exiting the analytical column undergo an electrochemical reaction in a coulometric cell. The current generated is proportional to analyte concentration and is measured by the Coulochem®III detector. The new assay was developed and validated using a single pump and a single analytical column. The injection-to-injection time was 13 min. Subsequently, to increase the method throughput and shorten turnaround time, a dual LC pump system with a 2-position/10-port switching valve capable of performing automatic alternating column regeneration was validated and implemented. The injection-to-injection time was reduced 2-fold.

RESULTS:

Parameter	**Results**	
LOQ	1.9 µmol/L	
AMR	5 - 5,000 µmol/L	
Linearity	y=0.977x-0.040; observed error 1.9%	
Imprecision	Low Control	High Control
Mean value	23.62 µmol/L	117.62 µmol/L
Within run CV	5.2 %	2.1 %
Btwn run CV	3.6 %	3.0 %
Total CV	6.3 %	3.7 %
Accuracy: Method Comparison	Discarded specimens (n=44): y=0.983x-8.93; $S_{y/x}$=7.42; R=0.9901 Fresh ref. interval specimens (n=41): y=0.834x-1.08; $S_{y/x}$=6.77; R=0.9167	
Carryover	Not detected after injection of sample with 14,200 µmol/L of ascorbic acid (AA).	
Analytical Specificity: Interference	33 common drugs and endogenous compounds tested. Only isoascorbic acid (erythorbic acid), a non-endogenous stereoisomer of AA, co-eluted. Gross hemolysis (166 µmol/L) reduced measured AA concentration by 12%.	

CONCLUSION: We have successfully developed and validated an HPLC-ECD method for the measurement of vitamin C in plasma. Advantages of this method include higher analytical specificity and significantly simplified sample preparation compared to the previously used spectrophotometric method. Additionally, much shorter injection-to-injection time compared to HPLC methods utilizing a single LC column was achieved by employing an automatic alternating column regeneration system.

Wednesday, July 30, 2014

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

Pediatric/Fetal Clinical Chemistry

B-255

Increased expression of aquaporin 9 in placenta from pregnant women with gestational diabetes

T. Vilariño-García[1], A. Pérez-Pérez[1], P. Guadix[1], V. Dietrich[2], A. Damiano[2], J. L. Dueñas[1], C. González-Rodríguz[1], V. SANCHEZ-MARGALET[1]. *[1]VIRGEN MACARENA UNIVERSITY HOSPITAL, SEVILLE, Spain, [2]University of Buenos Aires, Buenos Aires, Argentina*

Background: Leptin is expressed in human placenta acting as an autocrine signal for the trophoblast. In fact, leptin is now considered an important regulatory signal in fetoplacental physiology. Placenta leptin expression is increased in pathological pregnancies, such as gestational diabetes, and it may play a role in the overgrowth of placenta, which supplies a growing fetus with nutrients and water. This function of placenta is partly based on the expression of aquaporins/glyceroporins, such as aquaporin 9 (AQP9).

The aim of this study was to analyse the expression of AQP9 in placenta from gestational diabetes compared with that from normal pregnancy. In addition, we studied the in vitro effect of leptin on the AQP9 expression by trophoblast explants from control pregnancies, in order to check the possible leptin regulation of AQP9 expression in human placenta.

Methods: We collected 30 placentas (20 from control pregnancy and 20 from gestational diabetes), after caesarean delivery at term. The expression of AQP9 was determined by quantitative RT-PCR, immunoblot and immune histochemistry. In addition, explants from control placenta were incubated in vitro with increasing concentrations of leptin (0-100 nM) during 6 h and AQP9 expression was also measured. Mean values were analysed by ANOVA followed by Bonferroni´s post test.

Results: We have found that AQP9 expression was significantly increased by 30% in placenta from gestational diabetic pregnancy. Western blot confirmed the increased protein level of AQP9 in placenta from gestational diabetic women. Immune histochemistry demonstrated the localization of the overexpressed AQP9 in the sincitiotrophoblast. In vitro incubation of trophoblast explants with increasing concentrations of leptin demonstrated the dose-dependent effect of leptin on the expression of AQP9, with a maximal effect at 10 nM increasing three-fold the basal leptin expression.

Conclusions: Data show that AQP9 expression is increased in placenta from pregnant women with gestational diabetes and this may contribute to supply more nutrients to the fetus. The increased expression of AQP9 in gestational diabetes may be mediated at least in part by leptin, since leptin levels are known to be increased in gestational diabetes and we have found that leptin stimulates the expression of AQP9 in vitro. In conclusion, we have shown that leptin positively regulates the expression of AQP9 in the trophoblast and this effect may mediate the observed increase in AQP9 expression in trophoblast from pregnant women with gestational diabetes.

B-257

Absence of eosinopenia as predictor of procalcitonin < 2 ng/mL in pediatric patients

e. fernandez rodriguez, c. perez mendez, s. garcia alonso, e. fernandez garcia. *Hospital Cabuenes, Gijon, Spain*

BACKGROUND: The role of eosinopenia as a marker of sepsis has recently been evaluated. The aim of our study was to assess whether eosinophilia was a reliable criteria to predict procalcitonin (PCT) values under 2 ng/mL in pediatric patients presenting to the emergency department to rule out sepsis.

METHODS: From January 2012 to December 2013, a total of 605 consecutive children aged 3 to 36 months (median: 16.5 m; 46.6% male) were enrolled in the study. Procalcitonin levels and eosinophil counts were measured on admission. PCT was measured with a two-step two-site sandwich electrochemiluminescence immunoassay (ECLIA) on the ROCHE cobas e 411 system. An undetectable eosinophil count (<0.01 x 10(9) or <10/mm3) was considered as eosinopenia.

RESULTS: The absolute eosinophil count in the study population was 30/mm3 (median, range 0-1420). The average value of eosinophils in the group with PCT <2 ng/mL was 103/mm3, versus 28/mm3 in the group with PCT> 2 ng/mL ($p < 0.01$). At a cut-off value of <=0/mm3, the eosinophil count yielded a sensitivity of 51.4% and a specificity of 83.6%, a positive predicted value (PPV) of 17.2% and a negative predicted value (NPV) of 96.3%, with an area under the curve of 0.711 (95% CI: 0674-0747). At a cutoff value of 120/mm3, the NPV of the absolute eosinophil count was 98.6%, thus allowing to rule out a PCT>2 ng/mL value in 21.4% (141/568) of children.

CONCLUSION: The absence of eosinopenia can be considered as an inexpensive warning test for predicting PCT <2 ng/mL in pediatric patients. The presence of an absolute eosinophil count> 120/mm3 allows to rule out a PCT>2 ng/mL, thus avoiding PCT determination in 21.4% of children, which is of interest especially in the context of countries with limited resources

B-258

Neonatal umbilical cord blood cardiac troponin as reflecting fetal growth, age and well-being.

J. M. Potter[1], Z. Kesckes[1], F. Nouri-Girones[1], G. Koerbin[2], B. Jayasinghe[3], P. E. Hickman[1]. *[1]Australian National University, Canberra, Australia, [2]University of Canberra, Canberra, Australia, [3]Canberra Hospital, Canberra, Australia*

Background: The advent of highly sensitive assays for cardiac troponin have made possible investigations to better understand the biology and pathophysiology of these cardiac markers. The aim of the current study was to document the umbilical cord blood concentrations of troponins I (cTnI) and T (cTnT) using high sensitivity assays and correlate these with maternal and fetal clinical history.

Methods: Umbilical cord blood was collected immediately following delivery from 416 babies, including 12 sets of twins. Cardiac troponins were assayed using hs-cTnI on Abbott Architect and hs-cTnT on Roche E4111. Clinical history was obtained from clinical notes. Ethics permission was obtained from ACT Health Human Research and Ethics Committee for the study and consent was obtained from mothers for their participation.

Results: All results were above LoD for both assays (1.0 ng/L cTnI; 5.0 ng/L cTnT). Minimum cTnI concentration was 1.2 ng/L (median 6.9n/L; Q1 4.5, Q3 12.1), cTnT 7.0ng/L (Q1 27.1, Q3 51.0). Troponins were statistically significantly correlated [cTnT=21.45 ln(cTnI)-0.82; $R^2 = 0.3418$, $P<0.001$]. Babies who were born before 32 weeks gestation (n=17) had higher median cTnI of 14.7ng/L (Q1 6.7, Q3 22.7) and cTnT of 58.0 ng/L (Q1 56, Q3 102) compared with those born after 41 weeks gestation [n=24, median cTnI 6.0ng/L (Q1 4.5, Q3 10.43); cTnT of 31.2 ng/L (Q1 23.0, Q3 48.6). Babies with the highest cTnI (>48 ng/L, >95%ile) had markedly elevated cTnT (n=6, median 197 ng/L, range 151-297).

Conclusion:

The relationship between cTn and current objective measures of fetal well-being are assessed to determine whether cTn measurement in this clinical setting is of value.

B-259

Dynamic changes in circulating amino acids and acylcarnitines in children and adolescents: A CALIPER study of healthy community children and new pediatric reference intervals

T. Teodoro-Morrison[1], L. Kyriakopoulou[2], J. Raizman[1], V. Bevilacqua[1], A. Cohen[2], M. Chan[2], B. Wan[2], M. Yazdanpanah[2], Y. Chen[2], D. Colantonio[2], K. Adeli[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada*

Objective: An up-to-date and comprehensive database of pediatric reference intervals is essential for the accurate management of children with metabolic disease. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) program has established pediatric reference intervals from healthy community children for a comprehensive list of common biomarkers relevant to the diagnosis and management of inborn errors of metabolism (IEM).

Methods: Healthy children and adolescents from birth to 19 years were recruited based on informed parental consent. A cohort of over 500 healthy individual samples was used to calculate pediatric reference intervals for 36 acylcarnitines (LC-MS/MS) and 37 amino acids (Waters MassTrak amino acid analyzer). Over 100 healthy individual samples were focused in the 0 - 2 week age range to ensure reliable reference intervals were established in the newborn period. Reference intervals were calculated using non-parametric statistics according to CLSI-C28-A3 and partitioned based on age and sex.

Results: The majority of analytes demonstrated reference intervals requiring 2 - 4 age dependent partitions, with the most common being within the newborn period of 0 - 2 weeks. Also, several analytes displayed a unique reference interval during puberty, some of which demonstrated sex-based partitions. These were often related to energy metabolism and growth in the muscle. Finally, the minority of analytes demonstrated one reference interval for all ages and sexes.

Conclusions: The reference intervals established here for acylcarnitine and amino acid profiles will aid in the accurate diagnosis and monitoring of children suspected of IEM. Furthermore, reference intervals extending to 19 years of age will aid in the management of children and adolescents treated for metabolic disease. Importantly, these reference intervals are established for the indicated instrumentation and should be validated on other platforms and for local populations as recommended by CLSI.

Table 1. Pediatric reference intervals for biomarkers used in children with suspected metabolic disease. (BHB-beta-hydroxybutyrate).

Analyte	Age	Number of samples	Lower limit (95% CI)	Upper limit (95% CI)
Valine (umol/L)	0-<2wk	145	92 (73-96)	326 (299-349)
	2wk-<13yr	253	126 (123-139)	356 (339-364)
	13yr-<19yr	♂ : 46	166 (157-179)	301 (289-309)
		♀ : 51	155 (145-162)	259 (246-269)
BHB (mmol/L)	0-<1yr	91	0 (0-0)	1.73 (1.16-2.13)
	1yr-<19yr	219	0 (0-0)	0.12 (0.11-0.13)
Total carnitine (umol/L)	0-<1yr	67	21 (17-26)	78 (74-81)
	1yr-<12yr	136	28 (27-31)	61 (55-74)
	12yr-<19yr	♂ : 53	32 (30-33)	59 (57-61)
		♀ : 56	24 (21-27)	53 (50-54)

B-260

Closing the gaps in pediatric population reference values for cancer biomarkers: A CALIPER study of healthy community children

V. Bevilacqua[1], M. Chan[2], D. Armbruster[3], B. Shodin[3], K. Adeli[1]. *[1]University of Toronto, Toronto, ON, Canada, [2]The Hospital for Sick Children, Toronto, ON, Canada, [3]Abbott Diagnostics, Abbott Park, IL*

Background: The CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference value distributions for 11 key circulating tumor markers including those used in assessment of patients with childhood or adult cancers.

Methods: Healthy community children from birth to 18 years of age were recruited to participate in the CALIPER project with informed parental consent. Participants completed questionnaires and were assessed according to established inclusion and exclusion criteria. Serum samples from approximately 400-700 children were analyzed on the Abbott Architect ci4100 and reference intervals were established for Alpha-Fetoprotein (AFP), Ant-Thyroglobulin (Anti-Tg), Human Epididymis Protein 4 (HE4), Cancer Antigen 125 (CA125), Cancer Antigen 15-3 (CA15-3), Cancer Antigen 19-9 (CA19-9), Pro Gastrin-Releasing Peptide (ProGRP), Carcinoembryonic Antigen (CEA), Squamous Cell Carcinoma Antigen (SCC), as well as Total and Free Prostate Specific Antigen (PSA) according to CLSI C28-A3 statistical guidelines.

Results: Significant fluctuations of biomarker concentrations by age and/or gender were observed in 10 of 11 biomarkers investigated. Results for three of the markers examined (CA19-9, CA125 and SCC) are shown in Table 1. Age partitioning was required for CA15-3, CA125, CA19-9, CEA, SCC, ProGRP, Total & Free PSA, HE4 and AFP while gender partitioning was also required for CA125, CA19-9, Total & Free PSA.

Conclusion: The establishment of pediatric reference intervals for tumor biomarkers will not only aid in harnessing the full potential of tumor markers in a pediatric population but also in research aimed at determining the value of tumor marker use in various cancers.

Age- and Sex-Specific Pediatric Reference Intervals for 3 Tumor Biomarkers

		Male Reference Interval			Female Reference Interval		
Analyte	Age	Reference Interval	No. Of Samples	Confidence Interval	Reference Interval	No. Of Samples	Confidence Interval
CA19-9	0 - < 1 year	< 2.00, 54.40	59	< 2.00, (46.39, 58.90)	< 2.00, 37.6	56	< 2.00, (31.04, 42.63)
	1 - < 14 years	< 2.00, 27.40	172	< 2.00, (22.80, 32.60)	< 2.00, 27.40	172	< 2.00, (22.80, 32.60)
	14 - < 19 years	< 2.00, 12.02	66	< 2.00, (10.32, 13.13)	< 2.00, 12.02	66	< 2.00, (10.32, 13.13)
CA125	0 - < 4 months	2.4, 22.2	56	(2.0, 3.0), (20.2, 24.4)	2.4, 22.2	56	(2.0, 3.0), (20.2, 24.4)
	4 months - < 5 years	7.0, 32.6	191	(4.9, 8.0), (31.6, 35.3)	7.0, 32.6	191	(4.9, 8.0), (31.6, 35.3)
	5 - < 11 years	4.7, 28.5	174	(4.4, 5.8), (24.4, 29.4)	4.7, 28.5	174	(4.4, 5.8), (24.4, 29.4)
	11 - < 19 years	5.5, 27.7	122	(5.4, 6.5), (23.5, 30.2)	5.5, 39.1	128	(3.9, 6.7), (30.4, 41.0)
SCC	0 - < 1 week	> 70.0	44	> 70.0	> 70.0	44	> 70.0
	1 week - < 1 year	0.4, 6.7	114	(0.4, 0.5), (5.6, 7.9)	0.4, 6.7	114	(0.4, 0.5), (5.6, 7.9)
	1 - < 8 years	0.4, 1.7	81	(0.3, 0.5), (1.6, 1.8)	0.4, 1.7	81	(0.3, 0.5), (1.6, 1.8
	8 - < 19 years	0.5, 2.1	142	(0.4, 0.5), (2.0, 2.2)	0.5, 2.1	142	(0.4, 0.5), (2.0, 2.2)

B-261

Sex-based differences in gestational age at lung maturity as determined by lamellar body counts

C. Bookhout, C. Beamon, R. Strauss, F. Lin, M. Gearhart, C. Hammett-Stabler. *UNC Hospitals, Chapel Hill, NC*

Introduction: In late gestation, as fetal lungs prepare to transition to an air environment, alveolar epithelial cells called type II pneumocytes synthesize and store a mixture of phospholipids and proteins known as pulmonary surfactant. Around week 32 of gestation, surfactant release into the amniotic fluid begins in the form of structures called lamellar bodies (LBs). Adequate surfactant is an important predictor of fetal lung maturity, especially in cases of prematurity.

Lamellar bodies can be quantified in amniotic fluid using the platelet channel of automated hematology analyzers due to similarity in size. Inadequate surfactant production is associated with respiratory distress syndrome, which is more likely to occur in patients with lower lamellar body counts (LBCs).

Respiratory distress is more common in male late preterm infants than in females, possibly related to sex-based differences in lung maturity. Female fetuses have demonstrated earlier development of lung maturity than males in previous studies using other markers of lung maturity, such as the lecithin/sphingomyelin ratio, presence of phosphatidylglycerol, and loss of phosphatidylinositol; however, sex differences in lamellar body counts have not been assessed in the literature. This study aimed to determine if female fetuses demonstrate evidence of lung maturity using LBCs at an earlier gestational age than males.

Methods and Analysis: The population for this retrospective cohort study included all pregnant women who had amniocentesis with LBC analysis on the Advia Hematology System at our institution from 2003-2012 and subsequently delivered within 72 hours. Data were collected from our laboratory database of amniotic fluid LB counts. Gestational age at the time of the amniocentesis, fetal sex, and additional demographic and outcome data were collected from medical records. Lung maturity was defined as a LB count >35,000. Linear regression analysis of the data was done using Microsoft Excel, with statistical analysis performed by analysis of covariance (ANCOVA) to evaluate the relationship between gestational age, lamellar body count, and sex.

Results: 263 deliveries were included for analysis, with 128 female (49%) and 135 male (51%) infants. Gestational ages ranged from 30-41 weeks and lamellar body counts from 4332 to >200,000. Lung maturity with LBC>35,000 was demonstrated in 105/128 female (82%) and 107/135 male (79%) infants. The regression line of LBC versus gestational age for females was y=10188x-311669, with r=0.47, and for males it was y=10391x-317262, with r=0.47. The two lines did not differ significantly in either slope (p=0.93) or intercept (p=0.95).

Conclusion: Our study did not demonstrate a significant difference in gestational age at lung maturity by fetal sex as measured with LBCs. These results differ from prior data showing a higher degree of lung maturity in female than male infants using other indices, despite larger sample sizes in our study. Broad inclusion criteria and relatively wide scatter of data may have contributed to the non-significant results. Sex differences in LBCs were not found to explain increased prevalence of neonatal respiratory distress in males.

B-262

Pediatric reference intervals for 29 endocrine and special chemistry biomarkers on the Beckman Coulter DxI Immunoassay System: A CALIPER sudy of healthy community Children

M. Chan, V. Bevilacqua, Y. Chen, S. Bustos, C. O'Dwyer, K. Adeli. *CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada*

BACKGROUND: Appropriate interpretation of laboratory test results requires carefully established reference intervals based on a healthy population. Growth and development can markedly influence circulating concentrations of biomarkers, so accurate reference intervals established on the basis of a healthy pediatric population are essential for test result interpretation. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference intervals for endocrine and special chemistry markers on the Beckman Coulter DxI Immunoassay System.

DESIGN AND METHODS: Healthy children and adolescents were recruited as part of the CALIPER study. After informed parental consent was obtained, participants filled out a questionnaire including demographic information and provided a blood sample. We measured 29 proteins using the Beckman Coulter DxI Immunoassay System utilizing 443 - 636 samples per assay. The variance, age- and sex-specific concentrations of analytes were visually inspected from scatterplots of protein concentration as a function of age for both genders. Pediatric reference intervals were calculated according to Clinical Laboratory Standards Institute (CSLI) C28-A3 guidelines. Partitions based on age and/or sex were determined and statistically evaluated using the Harris and Boyd method. After removal of outliers, reference intervals were calculated using the non-parametric rank method, with values ranked and the 2.5th and 97.5th percentiles calculated.

RESULTS: We observed a complex pattern of change in most protein concentrations from the neonatal period to adolescence. The changes in concentration observed for each of the examined proteins were classified into 1 of 5 categories: (a) high variance and high concentration within the neonatal period that decreases abruptly shortly after birth: AFP, ferritin and prolactin; (b) high variance at birth that is significantly reduced around 1 year of age: free T4 and thyroglobulin (c) high variance and high concentration within the neonatal period that decrease gradually with age: SHBG and vitamin B12; (d) high variance at birth that decreases abruptly around 1 year of age and increases again in adolescence: cortisol, DHEAS, folate, testosterone (males), FSH (especially females) and LH (especially females); and (e) constant variance throughout life but variable concentration according to age: free T3 and total T4. Estradiol (and progesterone to a lesser extent) concentrations and variance were low from birth, then increased in females during adolescence. Insulin increased slightly with age for both genders. Ostase (bone-specific alkaline phosphatase) displayed constant variance and concentration, with a sharp decrease in adolescence, especially in females.

CONCLUSIONS: This study shows the complex expression profiles of several endocrine and special chemistry biomarkers as a function of age and gender. This allowed the calculation of age- and sex-specific reference intervals for 29 endocrine and special chemistry markers, specific to the Beckman Coulter DxI Immunoassay System. The completion of this study will aid in accurate diagnosis and laboratory assessment of children being monitored by Beckman Coulter immunoassays in healthcare institutions worldwide. It is however important that these reference intervals be validated for the local pediatric population as recommended by CLSI.

B-263

Using the clinical laboratory's database for indirect estimation of the reference intervals of serum creatinine.

A. C. Dias, A. L. Barbosa, S. F. Fonseca, S. S. S. Costa, L. F. A. Nery. *Sabin Laboratory of Clinical Analysis, Brasília, Brazil*

Background: Reference intervals (RI) age-specified of serum creatinine are important for triage, diagnostics and monitoring of chronic kidney disease. Ideally, RIs must be determined by sampling a healthy population. There are two methods of sampling: a direct one and an indirect one. The direct method, that follows the recommendations of CLSI and IFCC, involves the selection of reference individuals. The aim of this study is to estimate the RIs of serum creatinine through the indirect sampling method, suggested by Horn.

Methods: Transversal study conducted in 44.592 individuals, ages between 1 and 74, both genders, which performed serum creatinine (enzymatic method) in Advia 2400 equipment, in a private laboratory, between September 2013 and February 2014. The statistics included Box-Cox transformation, Tukey's test, Kolmogorov-Smirnov test and estimation of RI by non-parametric method of the percentiles. The confidence interval was determined as 90% for the reference limits (percentile 2.5 and 97.5). The statistics were calculated by MedCalc software.

Results: Tukey's test was used in the transformed data and it identified 2.39% of individuals as outliers. All results considered as outliers were excluded from the original data. The RIs were determined by non-parametric method of the percentiles of residual data (Table 1).

Table 1. Reference intervals for creatinine concentratios in serum (mg/dL) calculated with a nonparametric method.

Age group	n	Males (%)	outliers	Lower limit 2.5 percentil (90% CI)	Upper limit 97.5 percentil (90% CI)
1 to < 3 years	347	48.86	5	0.19 (0.18 to 0.20)	0.38 (0.36 to 0.40)
3 to < 5 years	417	51.86	13	0.26 (0.25 to 0.26)	0.46 (0.44 to 0.50)
5 to < 7 years	434	44.92	9	0.30 (0.29 to 0.31)	0.53 (0.51 to 0.55)
7 to < 9 years	496	40.76	7	0.33 (0.32 to 0.34)	0.58 (0.56 to 0.60)
9 to < 11 years	501	36.40	10	0.37 (0.35 to 0.37)	0.62 (0.61 to 0.62)
11 to < 13 years	482	37.37	13	0.41 (0.41 to 0.42)	0.71 (0.69 to 0.72)
13 to < 15 years	613	37.04	8	0.46 (0.44 to 0.47)	0.86 (0.83 to 0.89)
Women (18 to < 75 years)	25536	n.a.	600	0.50 (0.50 to 0.50)	0.94 (0.94 to 0.95)
Men (18 to < 75 years)	14700	n.a.	401	0.70 (0.69 to 0.70)	1.25 (1.24 to 1.25)

Conclusion: The direct method of sampling is preferred. However, the difficulty to obtain a representative number of reference individuals, especially for pediatrics' population, can be overcome by using the indirect method. This method demonstrated to be an excellent alternative method to determine the RIs, which for serum creatinine are compatible with the main description in the literature.

B-264

A Urine-based Immunoassay for Urocortin 3 and Diagnosis of Sleep Apnea

T. Pitcher[1], K. Goudy[2], M. Linder[3], R. Valdes[3]. *[1]University of Louisville, Louisville, KY, [2]PGxL Technologies, Louisville, KY, [3]University of Louisville and PGxL Technologies, Louisville, KY*

Introduction: Obstructive sleep apnea (OSA) affects 2-3% of children in the US and is characterized by obstruction of the upper airway during sleep resulting in disruption in ventilation, hypoxemia, and sleep fragmentation. Children suffering from OSA are more likely to develop behavior difficulties, learning disabilities, pulmonary/systemic hypertension and decreased growth. Currently, the gold standard for diagnosing OSA is an overnight sleep study which is labor intensive, limited by availability and expensive. Development of a non-invasive test using a urine biomarker would provide an inexpensive and more accessible test to diagnose OSA. In a previous study, proteomic analysis by mass-spectrometry revealed a 4 kilo Dalton peptide in urine which was significantly increased in children with OSA compared to controls (Pub Med #). The peptide was identified as the stress-coping peptide Urocortin 3 (UCN3); 38 amino acids in length and is expressed in kidney tubules, heart and brain. UCN3 is involved in modulating stress responses, osmoregulation, and in regulating the hypothalamus-pituitary-adrenal axis. The objective of this present study was to develop a urocortin 3 (UCN3) two-site immunometric assay for eventual use as a rapid non-invasive diagnostic screening tool for OSA in children

Methods: To develop monoclonal and polyclonal antibodies targeting the mature human peptide, we immunized mice and rabbits with synthetic UCN3 constructs conjugated with KLH. Following conventional prime and boost immunization strategies, the mice were sacrificed, the spleens were harvested and immortalized. ELISA was used to identify the clones with reactivity for unconjugated UCN3. Full length UCN3 and UCN3 fragments were coated in 96-well plates and assayed with hybridoma supernatants (neat and 1:10 dilution) to determine reactivity. The ideal clones were defined as those that yielded the highest signal to full length UCN3 and to either the N-terminal or C-terminal half of the peptide. All of the antibodies were affinity purified and used to develop a two-site immunometric assay. Antibody pairs were identified using checkerboard ELISA and affinity characterization using Biacore (GE life science). The highest affinity mAb was used for antigen capture and the polyclonal was used for detection.

Results: Analysis of the clones yielded twenty hybridomas reactive towards UCN3, with five clones exhibiting absorbance >1.0 to full length and either the N- or C-terminus of UCN3. Checkerboard and Biacore analysis using these five clones and an anti-UCN3 polyclonal antibody (pAb) identified that mAb 4D3 was the ideal capture antibody and the anti-UCN3 pAb as the detection antibody. These antibodies generated an assay with a linear range from 100 to 3100 ng/mL, an intra-assay CV of 2.7 and 3.7% (N=2) at 100ng/ml and 3160ng/ml, respectively. Recovery of UCN3 spiked into urine was 127% at 100 ng/mL and 100% at 3100 ng/mL (N=3).

Conclusions: The set of monoclonal and polyclonal antibodies developed provides a Urocortin 3 ELISA-based assay with analytical characteristics suitable for subsequent studies to test the clinical predictive value measuring UCN3 in urine for the diagnosis of OSA in pediatric subjects.

B-265

CLSI-based transference of the CALIPER database of pediatric reference intervals to Beckman Coulter Clinical Chemistry Assays

V. Bevilacqua[1], M. Chan[1], Y. Chan[1], S. Bustos[1], C. O'Dwyer[1], E. Randell[2], K. Adeli[1]. *[1]CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada, [2]Eastern Health Authority & Faculty of Medicine, Memorial University, St. Johns, NL, Canada*

OBJECTIVES: Reference intervals represent the range of results that are commonly observed in a population of healthy individuals. These intervals are defined as the range that encompasses the central 95% of the distribution of test results from reference individuals sampled from a healthy reference population. Comparison of a given test result to an appropriate reference interval enables proper clinical assessment. Accurate pediatric reference intervals obtained in healthy community children are essential for the accurate diagnosis and management of diseases in children. The CALIPER program has established a comprehensive database of age- and sex-stratified pediatric reference intervals for over 70 common biochemical markers, proteins, lipids, and enzymes, as well as endocrine markers and fertility hormones. However, this database was only directly applicable for assays performed on the Abbott ARCHITECT ci4100 system. We therefore sought to expand the scope of this database to biochemical assays performed on the Beckman Coulter Syndchron DxC800 chemistry platform, allowing for a much wider application of the CALIPER database.

DESIGN AND METHODS: Based on CLSI C28-A3 and EP9-A2 guidelines, CALIPER pediatric reference intervals were transferred to Beckman Coulter chemistry assays performed on the Synchron DxC800 instrument, using specific statistical criteria. First, 200 pediatric pooled patient serum specimens were analyzed on the Abbott ARCHITECT ci4100 and the Beckman Coulter DxC800, and the data was subjected to regression analysis, standardized residual, Bland-Altman, and quantile-quantile (Q-Q) plots. A total of 34 chemistry, enzyme, lipid/lipoprotein and protein markers were assessed. Regression analysis was performed to determine correlation of values between analytical systems, resulting in determination of R2 for each analyte. Analytes with R2 values equal to or greater than 0.95 were deemed transferable to the Beckman Coulter DxC800 platform. To assess the validity of the transferred reference intervals, 100 serum samples from the CALIPER cohort (healthy community children) were assayed on the Beckman Coulter DxC800 and validation was assessed based on CLSI C28-A3 criteria.

RESULTS: Most (22) of the analytes showed good correlation between the Abbot and Beckman systems with R2 values equal to or greater than 0.95. Ten analytes showed strong correlation, with R2 values between 0.77 and 0.94. Two analytes (carbon dioxide and calcium) showed poor correlation, and were not transferable to Beckman Coulter DxC800. Most transferred reference intervals determined using the Beckman system were validated through the analysis of CALIPER reference samples.

CONCLUSIONS: The current study allows successful transference of a large number of routine and special chemistry markers from the CALIPER database to the assays on the Beckman Coulter DxC800 analytical systems. This will greatly extend the utility of validated CALIPER reference intervals which will be directly applicable for assays performed on the Beckman Coulter DxC800 chemistry platform. Validation of the reference intervals should facilitate the broad application of CALIPER reference intervals at pediatric centers worldwide.

B-266

Development of a Pregnant Subject Biospecimen Bank

A. M. Gronowski, R. Colvin, C. Kramer, K. H. Moley. *Washington University, St. Louis, MO*

Background: A need exists in the research community for serum, urine, cord blood, placenta and other tissues from pregnant women in order to study disorders that affect both the mother and fetus and to establish gestational age-specific reference intervals.

Objective: To create an infrastructure to recruit pregnant subjects, collect biospecimens throughout pregnancy and make them available to researchers along with clinical information and outcomes.

Methods: In 2008, a system was put into place to consent subjects and track them across a major medical center throughout their pregnancy. Biospecimens, including serum/plasma, urine, vaginal swabs, follicular fluid, placenta, cord blood, semen, and infant heel stick blood are collected. Clinical data is gathered and stored in a database. A business plan was developed and a cost-recovery fee structure was implemented. Specimens are provided to researchers immediately or frozen and stored for short or long periods of time, at the researcher's discretion. Long term storage utilizes an already established university specimen repository.

Results: Funding has been obtained to support this structure for six years. The cost to launch a similar Biobank is ~$200,000 per year and to maintain this infrastructure has been ~ $250,000 per year and includes 3.5-5.0 FTE. Average enrollment is 12 women/week from 4 recruitment sites. Over 4,700 samples have been distributed to 11 researchers from 5 different university departments, and over 40,000 samples have been banked for future research. Major clinical outcomes are shown in the Table. The Biobank has helped university researchers receive grant funding including R01, SCOR, ICTS, and March of Dimes.

Conclusions: Here we describe the successful formation of a biorepository for specimens collected from subjects longitudinally throughout pregnancy. Over a six year period, we demonstrate that: funding can be obtained, enrollment is sufficient, accumulation of clinically significant outcomes can be achieved, and the Biobank allows researchers to fund and conduct research.

Table. Outcome data from subjects included in biobank

	Number	%
Pregnant	1669	84.9
Not pregnant	297	15.1
Status of Pregnant patients (if known)		
Delivered	1353	81.1
Loss	37	2.1
Unknown	279	16.7
Complications		
Preeclampsia	165	9.9
Pregnancy induced hypertension	139	8.3
Intrauterine growth restriction	101	7.5
Macrosomia	87	6.4
Fetal anomalies	84	6.2
Chorioamnionitis	102	6.1
Gestational diabetes	92	5.5

B-267

Evaluation of a Discriminatory Zone for Serum Beta-human chorionic gonadotropin (βhCG) in Early Pregnancy

M. A. V. Willrich, N. A. Baumann, N. V. Tolan, G. G. Klee, D. Brown, C. C. Coddington. *Mayo Clinic, Rochester, MN*

Background: The beta-human chorionic gonadotropin (hCG) discriminatory zone is the concentration of serum hCG at which a gestational sac should be visible on sonography in a normal intrauterine pregnancy (IUP) and has been used to aid in management of women presenting with pain and/or vaginal bleeding in early pregnancy. The reliability of an hCG discriminatory zone has been debated in the literature. In addition, large inter-individual variation of serum hCG concentrations and the lack of standardization between assays prohibits the use of a universal hCG cut-off. The aim of this project was to perform a retrospective study to determine a discriminatory zone for the serum hCG assay used in our institution.

Methods: Inclusion criteria included females with clinician-ordered ultrasound (US) performed and serum hCG measured within 48h between June 2010 and December 2012 (n=554). The Roche Cobas intact hCG+β assay was used to measure hCG on a Cobas e immunoassay analyzer (Roche Diagnostics). Chart review was performed on a subset of unique patients with serum hCG concentrations between 1,000-5,000 mIU/mL to determine pregnancy outcomes (n=106). Ultrasound reports were reviewed and presence of embryonic structures recorded. Serum hCG concentrations were correlated with presence or absence of gestational sac (GS) and pregnancies were categorized as ectopic or IUP. The IUP group was further categorized into viable or non-viable pregnancies. ROC curve analysis was performed.

Results: In the subset of chart-reviewed women, the median age was 31 years old (range:18-51) and serum hCG concentration was 2320±973 mIU/mL (mean±SD). Serum hCG concentrations were independent of estimated weeks of gestation (p=0.9611). GS was present in 66 cases (hCG 2412±1030 mIU/mL) and not visible by US in 39 cases (hCG 2180±870 mIU/mL). Of those cases with no GS present, chart review revealed that 15 were ectopic (hCG 2372±902 mIU/mL) and 24 were IUP (hCG 2060±846 mIU/mL). Serum hCG concentrations were not different between ectopic or IUP (2446±1128 mIU/mL versus 2287±933 mIU/mL, respectively, p=0.7056). Serum hCG concentrations between viable and non-viable IUPs were similar (2397±943 mIU/mL versus 2220±929 mIU/mL, respectively, p=0.3198). ROC curve analysis identified that in the absence of a GS, a serum hCG concentration of 1700 mIU/mL would be 87% sensitive and 63% specific in differentiating an ectopic pregnancy from an IUP (AUC=0.63, p=0.2783), and a cut-off of 3900 mIU/mL would provide a sensitivity of 13% and a specificity of 95%.

Conclusions: While defining an hCG discriminatory zone would prove valuable to clinicians, especially in the absence of visible gestational sac on sonography, we found a large overlap of serum hCG concentrations in females with ectopic and IUP. The use of a single measurement of serum hCG below or above a given threshold does not provide enough sensitivity or specificity to definitely diagnose ectopic pregnancy. However, when used in conjunction with clinical judgment and sonography, a higher hCG cut-off of 3900 mIU/mL provides increased specificity and may aid in the diagnosis and management of patients.

B-269

Quantitative amino acid analysis using liquid chromatography tandem mass spectrometry and aTRAQ reagents. Do we have a new gold standard?

A. Liu[1], J. Hobert[2], M. Erali[1], J. Pickering[1], N. Longo[3], M. Pasquali[2], I. De Biase[2]. *[1]ARUP Instute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, [3]Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT*

Background: Defects in the metabolism or the transport of a specific amino acid or a group of amino acids leads to disorders generally referred to as inherited disorders of amino acid metabolism. Accurately quantifying amino acids in biological fluids (plasma, urine, or cerebrospinal fluid) is essential for the diagnosis and follow up of aminoacidopathies, as well as useful for the nutritional assessment of patients with non-metabolic conditions. Amino acid analysis is conventionally performed on an ion-exchange chromatography (IEC) based amino acid analyzer, which provides excellent separation and reproducibility with minimal sample preparation. The IEC method has several disadvantages: long run time, large sample volume, and lack of analyte specificity due to interfering substances. To address our large clinical load and improve specificity, we have optimized the aTRAQ method (AB SCIEX) with ion-pairing reverse-phase liquid chromatography and tandem mass spectrometry, and transferred the assay in our clinical lab.

Methods: Samples were labeled with aTRAQ reagents prior to instrument analysis by API 4000 in conjunction with SHIMADZU HPLC. The chromatographic separation was performed using the ABSciex amino acid analysis column or the Phenomenex Gemini-NX column, C18, 4.6x150mm, 5um. Amino acid quantitation was obtained using 6-point external calibration with stable isotope dilution. MultiQuant software was used for data analysis.

Results: Our method allowed accurate quantification of 47 physiological amino acids and related compounds, including the isomers alloisoleucine and isoleucine, and four additional analytes: sulfocysteine, agininosuccinic anhydrides, formiminoglutamic acid, and glycylproline. The assay analytical performance was evaluated using standard solutions, spiked samples of varying concentrations, and de-identified clinical specimens. The assay was linear from 1 umol/L to 2500 umol/L. The total imprecision was less than 10% and the recoveries were between 90 and 110% for most amino acids. The assay was compared with IEC method with 115 plasma samples and 38 urine samples, run in parallel. The comparison yielded good correlation for most amino acids [slopes between 0.93 and 1.04, y-intercepts between -7.0 and +7.0, R^2 greater than 0.96]. In urine, as expected, correlation was poor for several amino acids due to interfering substances that cannot be separated by IEC. Reference intervals were established with a focus on the pediatric population. De-identified clinical samples from patients with known disorders of amino acids metabolism or transport were also analyzed with this method and were 100% concordant with IEC.

Conclusion: We have optimized the aTRAQ procedure for amino acid analysis to achieve lower imprecision and better batch to batch reproducibility. Compared with the IEC method, this assay has shorter instrument run time and increased specificity. We have made the assay robust and ready for implementation in the clinical lab. We are able to report 47 amino acids and related compounds from a single sample analysis. This represents a broad, all-inclusive panel for amino acids analysis that, in our opinion, could replace ion-exchange chromatography in the clinical lab.

B-270

Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma

T. H. Santa Rita, C. F. Chianca, L. F. R. Velasco, C. F. Sousa, L. F. A. Nery, S. S. S. Costa, G. B. Barra. *Laboratorio Sabin, Brasilia, Brazil*

Background: The paternity testing is increasingly becoming a clinical laboratory test and there are some situations that are necessary to perform fetal kinship analysis before the delivery. The prenatal paternity testing is invasive and most frequently done by testing of chorionic villi or amniotic fluid, which are associated with a stressful sampling that bears a small but existing risk for both mother and child. Actually, with the availability of SNP microarrays and next-generation sequencing, the prenatal paternity testing is been performed non-invasively by analysis of the cell-free fetal DNA in maternal plasma. However, such new methodologies are associated with extras know-how, equipment, and reactions costs. In this way, it is highly desirable to perform non-invasive fetal kinship analysis by using the legacy paternity testing technology. However, the higher maternal DNA background limits the detection of fetal DNA markers (STRs). So, it is necessary to explore the fetus and mother genetics differences (e.g. the Y chromosome in case of male fetus). Thus, the aim of this study is to determine the fetus Y-STR haplotype in maternal plasma during pregnancy and estimate, non-invasively, if the alleged father and fetus belong to the same male lineage.

Methods: The study enrolled couples with singleton pregnancies and known paternity. All participants signed informed consent and the local ethics committee approved the study. Fetal gender was determined by qPCR targeting DYS-14 in maternal plasma and it was confirmed after the delivery. The first consecutive 20 and 10 mothers bearing male and female fetuses, respectively, were selected for the Y-STR analysis. The median gestational age was 12 weeks (range 12-36). Peripheral blood was collected in EDTA tubes (mother) and in FTA paper (father). Maternal plasma DNA was extracted by NucliSens EasyMAG (Biomeriuex). All DNA samples were subjected to PCR amplification by ampFLSTR Yfiler (Life Technologies), PowerPlex Y23 (Promega) and an in-house multiplex, which together accounts for 27 different Y-STR. The PCR products were detected with 3500 Genetic Analyzer (Life Technologies) and they were analyzed using GeneMapper-IDX (Life Technologies). Fetuses' haplotypes (in Y-Chromosome Haplotype Reference Database standard format that consider only 15 of the 27 tested Y-STR) were compared to other 5328 Brazilian haplotypes available on www.yhrd.org.

Results: Between 22 and 27 loci were successfully amplified from maternal plasma in all 20 cases of male fetuses. None of the women bearing female fetuses had a falsely amplified Y-STR. The haplotype detected in maternal plasma matched the alleged father haplotype in all cases. One case showed a mutation in the DYS438 locus, which was confirmed after the delivery. Seventeen fetuses' haplotypes were not found in YHRD and three of them occurred twice, which corresponded to paternity probability of 99.981% and 99.944%, respectively.

Conclusion: High discriminatory fetal Y-STR haplotype could be determined from maternal plasma during pregnancy starting at 12 weeks of gestation. Moreover, all male fetuses could be attributed to the alleged father male lineage early in pregnancy. This strategy is an alternative for fetal kinship analysis before the delivery. The main limitation is that it's only applied for mothers bearing a male fetus.

B-271

Pediatric reference value distributions for vitamins A and E in healthy community children: Establishment of new age-stratified reference intervals from a CALIPER cohort

J. Raizman, A. H. Cohen, T. Theodoro-Morrison, B. Wan, M. Chen, C. Wilkenson, V. Bevilaqua, K. Adeli. *University of Toronto, Toronto, ON, Canada*

Objective: Vitamin A (retinol) and vitamin E (alpha tocopherol) are fat soluble micronutrients measured in the pediatric population to monitor deficiencies due to malabsorption secondary to gastrointestinal (GI) disorders. A major challenge of vitamin A and E testing is lack of reliable pediatric reference intervals (RI) which limits accurate interpretation of patient results. We report new pediatric RI for both vitamins as part of the Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER). Methods: Healthy community children were recruited with parental consent and whole blood samples collected from 342 healthy children 1 day to 19 years of age. Retinol and alpha tocopherol were extracted from serum using hexane before concentrations were measured with high performance liquid chromatography. Age and sex-specific RI were calculated using non-parametric and robust methods based on CLSI C28-A2 guidelines. Results: Comparison of vitamin A and E levels in males and females demonstrated a tight correlation and did not reveal any significant differences requiring no sex partitioning. Further analysis by age demonstrated distinct partitioning patterns. Both vitamin A and E showed age partitioning at 0 to <1 years with levels determined as early as the first day of life. Interestingly, vitamin A exhibited a complex pattern necessitating 4 distinct age partitions trending toward a general rise in levels over time. Vitamin E required 2 age partitions. Levels rose within the first year of life but were reduced slightly after this period requiring only one broad partition between 1 to <19 years. Ratios of vitamin E to cholesterol and triglyceride

were also calculated, correlating well to vitamin E levels. Conclusions: This study establishes pediatric RI for vitamin A and E in a healthy population from neonates to early adulthood. These values will be beneficial in assessing accurate vitamin status when monitoring children with GI disorders or malnutrition.

Analyte (micro mole/L	Age (years)	Lower Limit	Upper limit	Samples (n)	Higher confidence interval	Lower confidence interval
Vitamin A	1 - < 11	0.97	1.58	100	0.92,1.01	1.54,1.61
	11 - < 16	0.93	2	71	0.82,1.02	1.90,2.09
	16 - < 19	0.82	2.53	50	0.65,1.01	2.33,2.70
Vitamin E	0 - < 1	5.92	51.36	85	1.86,9.67	48.26,54.58
	1 - <19	14.5	33	245	14,3.78	6.45,6.92
Vitamin E/ cholesterol	1 - <19	3.7	6.7	82	3.53,3.78	6.45,6.92
Vitamin E/ triglyceride	1 - <19	8.53	44.48	83	7.51,9.52	40.37,47.76

B-272

Diagnosis of Primary Hyperoxaluria Type III, A Novel Hereditary Disorder of Hydroxyproline Metabolism, By Gas Chromatography-Mass Spectrometry

L. Hasadsri, P. Loken, D. S. Milliner, J. C. Lieske, P. Rinaldo, S. Tortorelli, D. Oglesbee. *Mayo Clinic, Rochester, MN*

Background and Objectives: Primary hyperoxaluria type III (PH3) is a newly discovered disorder caused by deficiency of mitochondrial 4-hydroxy-2-oxoglutarate (HOG) aldolase, which catalyzes the final step in the metabolism of hydroxyproline. The condition is characterized by the infant to childhood onset of recurrent nephrolithiasis and progressive nephrocalcinosis. Timely detection of primary hyperoxalurias, in particular PH3, remains a significant challenge. Patients have often reached end-stage renal disease by the time they are diagnosed. Here we describe a novel method for the detection of urinary metabolites in primary hyperoxaluria types I, II, and III by gas chromatography-mass spectrometry. We also summarize the clinical and laboratory features of 9 known (i.e., mutation confirmed by sequence analysis) and 2 novel cases of PH3 uncovered by our assay.

Methods: Patient samples and de-identified clinical information were obtained in collaboration with the Mayo Clinic Hyperoxaluria Center and Rare Kidney Stone Consortium. Samples analyzed included urine from unaffected controls, patients with PH1-3, and patients with hyperoxaluria of unknown etiology. Urine specimens were methoximated and extracted with 4:1 (v/v) ethyl acetate/propan-2-ol then evaporated to dryness and derivatized with BSTFA+TMCS in pyridine. Samples were re-extracted in isooctane and analyzed on an Agilent 5975 series, using hydrogen as the carrier gas. The following commercially available internal standards were used: glycolate-D_2, oxalate-$^{13}C_2$, and glycerate-D_3, in addition to custom-synthesized HOG-$^{13}C_2$.

Validation: The linear range of detection for HOG was 0.024 - 600 µg/mg creatinine (Cr). Pooled normal, intermediate, and elevated HOG urine samples (n=30 for each) were used to determine intra-assay precision (%CV = 2.83, 0.73, and 4.73, respectively) as well as inter-assay precision (%CV = 0.89, 0.48, and 2.60, respectively). In the absence of a comparison method for the analysis of HOG, the accuracy of this test was validating by correlation of test results with clinical findings. Blinded results from analysis of one hundred normal and 34 abnormal specimens were submitted for interpretation; 100% interpretive concordance was achieved for the 134 samples.

Results: The reference range of HOG concentrations in controls was 0.01 - 2.21 µg/mg Cr. In patients with PH1, the range was 0.01 - 0.96 µg/mg Cr. Patients with PH2: 0.01 - 3.10 µg/mg Cr. Testing of urine samples from patients with hyperoxaluria of unknown etiology (i.e., negative for PH1 and/or PH2 by molecular testing) uncovered two male infants with elevated levels of HOG, which were 116.86 and 261.78 µg/mg Cr, respectively. Mutation analysis of the HOGA gene in one of these patients revealed homozygosity for a known pathogenic mutation.

Conclusions: In contrast to previously published methods, our assay can rapidly detect all analytes of clinical utility for the diagnosis of primary hyperoxaluria types I-III. Patients with PH1 and PH2 do not have significantly increased excretion of HOG as compared to unaffected controls. Analysis of urine specimens from patients with hyperoxaluria of unknown etiology can potentially lead to a diagnosis of PH3.

B-273

Pediatric reference intervals for specialty endocrine and chemistry biomarkers on the Abbott Architect ci4100 System: A CALIPER study of healthy community children

V. Bevilacqua[1], M. Chan[1], Y. Chen[1], S. Bustos[1], C. O'Dwyer[1], F. Quinn[2], B. Shodin[2], D. Armbruster[2], K. Adeli[1]. *[1]CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada, [2]Abbott Diagnostics, Abbott Park, IL*

BACKGROUND: Appropriate interpretation of laboratory test results requires carefully established reference intervals based on a healthy population. Growth and development can markedly influence circulating concentrations of biomarkers, thus accurate reference intervals established from a healthy pediatric population are essential for test result interpretation. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program, a national research initiative aimed at closing the gaps in pediatric reference intervals, sought to develop a database of covariate-stratified reference intervals for a number of endocrine and special chemistry markers.

METHODS: Healthy children and adolescents were recruited as part of the CALIPER study. After informed parental consent was obtained, participants filled out a questionnaire including demographic information and provided a blood sample. We measured a number of specialty and endocrine and biochemical markers (Alpha-1 antitrypsin, AGP, Amylase P, Anti-CCP Anti-TPO, Beta 2 microglobulin, C Peptide, Ceruloplasmin, Cholinestarase E, hs-CRP, Cystatin C, DHEA-Sulfate, Glucose, IgE, Insulin, SHBG, Testosterone (2nd GEN, and Bioavailable/Free Testosterone indexes) using the Abbott ARCHITECT ci4100 system and reference intervals were established utilizing 367 - 763 samples per assay. The variance, age- and sex-specific concentrations of analytes were visually inspected from scatterplots of analyte concentration as a function of age for both genders. Pediatric reference intervals were calculated according to Clinical Laboratory Standards Institute (CSLI) C28-A3 guidelines. Partitions based on age and/or sex were determined and statistically evaluated using the Harris and Boyd method. After removal of outliers, reference intervals were calculated using the non-parametric rank method if the sample size was larger than 120, with values ranked and the 2.5th and 97.5th percentiles calculated. If the sample size was between 40 and 120, the robust method was used in reference interval calculation.

RESULTS: We observed a complex pattern of change in most analyte concentrations examined from the neonatal period to adolescence. The changes in concentration observed for each of the examined proteins were classified into 1 of 4 categories: (a) high variance and high concentration within the neonatal period that decreases abruptly shortly after birth: Beta-2-microglobulin, high-sensitivity CRP and Cystatin C; (b) gradual concentration increase with age: albumin BCG, albumin BCP and pancreatic amylase; (c) high variance and high concentration within the neonatal period that decrease gradually with age: Sex hormone-binding globulin (SHBG) and cholinesterase (less pronounced); and (d) high variance at birth that decreases abruptly around 1 year of age and increases again in adolescence: C-peptide, DHEA-S and 2nd GEN testosterone (males). Ceruloplasmin showed a unique expression pattern with a sharp increase in concentration during the neonatal period followed by a gradual decrease over time.

CONCLUSIONS: This study shows the complex expression profiles of several endocrine and chemistry biomarkers as a function of age and gender. This allowed for establishment of age- and sex-specific reference intervals for these biomarkers, which will aid in accurate diagnosis of pediatric patients monitored by immunoassays on the Abbott ARCHITECT ci4100 in healthcare institutions worldwide. It is however important that these reference intervals be validated by each laboratory for the local pediatric population as recommended by CLSI.

Wednesday, July 30, 2014

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

Point-of-Care Testing

B-276

Assessment of Harmonization among Siemens Point-of-care and Central Laboratory Blood Gas Platforms

S. Fennell, C. Bethoney, K. LaRock, D. Poirier. *Siemens Healthcare DX, Norwood, MA*

Background: Determine correlations between Siemens point-of-care (POC) and central lab blood gas platforms in order to demonstrate harmonization across the product portfolio.

Relevance: AACC's International Consortium for Harmonization of Clinical Laboratory Results has been working with a variety of stakeholders regarding harmonization among results from different methods and labs for the same measurand. [1] Malone states, "Harmonization means achieving comparable results among different measurement procedures". Further, "When lab measurement procedures give different results for the same specimen, patients may get the wrong treatment, because decision criteria are not appropriate for the procedure in use. In order to do this effectively, results need to be harmonized."

Methods: Method comparison studies were performed with whole blood among POC (RAPIDPoint®) and central lab (RAPIDLab®) blood-gas platforms in accordance with the CLSI EP-09 guideline. Correlation statistics including Deming slopes, intercepts, and coefficients of determination (r^2) were generated for the following comparisons:

- RAPIDLab 1265 Blood Gas System vs. RAPIDPoint 500 Blood Gas System
- RAPIDLab 348EX Blood Gas System vs. RAPIDPoint 500 Blood Gas System
- RAPIDPoint 405 Blood Gas System vs. RAPIDPoint 500 Blood Gas System

Results: Deming regression statistics for each comparison across intervals for each measurand are shown in Table 1. The slopes for each measurand fell between 0.96 and 1.25, with $r^2 \geq 0.9829$.

Conclusion: Harmonization at medical decision levels and average concentrations was demonstrated among Siemens POC and central lab blood gas platforms for the measurands evaluated.

[1] Malone B. AACC's Thought Leadership Series: Why Harmonization Matters.

Table 1. Method comparisons among POC and central lab platforms

Comparison	Measurand	n	Average Bias	Deming Slope	Intercept	r^2	Interval
RAPIDLab1265 Analyzer vs. RAPIDPoint500 Analyzer	pH (Units)	92	0.017	1.01	-0.024	0.9987	6.820 to 7.781
	pCO_2 (mmHg)	81	3.0	1.07	-1.0	0.9914	6.9 to 165.3
	pO_2 (mmHg)	92	7.1	1.02	4.0	0.9978	27.6 to 653.3
	Na^+ (mmol/L)	124	0.5	1.03	-3.9	0.9576	100.4 to 158.3
	K^+ (mmol/L)	105	0.10	1.10	-0.35	0.9982	0.62 to 10.00
	Ca^{2+} (mmol/L)	133	0.07	1.06	-0.07	0.9577	0.31 to 4.86
	Cl^- (mmol/L)	124	3	1.08	-5	0.9944	66 to 140
	Glu (mg/dL)	100	4	0.99	6	0.9981	26 to 603
	Lac (mmol/L)	100	-0.02	0.98	0.20	0.9870	0.31 to 27.13
	tHb (g/dL)	112	-0.1	1.01	-0.1	0.9997	2.5 to 23.8
	tBil (mg/dL)	110	1.1	1.03	0.7	0.9732	2.3 to 27.4

B-277

Investigation of Maltose Interference on the Roche ACCU-CHEK Inform II Blood Glucose Meter

L. Song, D. Stene, L. Boyd. *David Geffen School of Medicine at UCLA, Los Angeles, CA*

Background: Maltose can be present in the blood of patients who were treated with peritoneal dialysis using icodextrin or maltose-containing immune globulin up to 2 weeks after the treatment. Maltose can interfere with glucose measurement using glucose dehydrogenase pyrroloquinolinequinone (GDH-PQQ) and cause falsely elevated glucose results. Patients can develop severe hypoglycemia if treated with insulin in response to these falsely elevated glucose results. The Roche (Roche Diagnostics, Indianapolis, IN) ACCU-CHEK Inform I (Inform-I) blood glucose meter uses GDH-PQQ methodology. A mutant quinone GDH is used in ACCU-CHEK Inform II (Inform-II) which can distinguish glucose from maltose. In this study, we want to determine if maltose interference has been eliminated with Inform-II using samples containing maltose and samples from patients treated with icodextrin peritoneal dialysis.

Methods: Samples containing 240 mg/dL, 360 mg/dL, and 720 mg/dL of maltose were prepared by spiking a whole blood sample with maltose. At each level of maltose, a control sample was prepared by adding equal amount of water to an aliquot of the blood sample. Glucose results in these samples were measured in triplicates with both Inform-I and Inform-II. Previously frozen plasma samples from three patients who underwent icodextrin peritoneal dialysis were also tested with Inform-I, Inform-II, and results compared with that obtained with Beckman Olympus AU5400 (AU5400) which is free from maltose interference.

Results: Glucose results in samples containing different amounts of maltose and samples from the three patients obtained with Inform-I, Inform-II, and AU5400 are shown in the table below:

Sample Containing Maltose	**Glucose with AU5400 (mg/dL)**	**Glucose with Inform-I (mg/dL)**	Glucose with Inform-II (mg/dL)	**Falsely Increased Glucose with Inform-I (mg/dL)**	**Falsely Increased Glucose with Inform-II(mg/dL)**
240 (mg/dL)		241	**114**	151	10
360 (mg/dL)		313	121	225	16
720 (mg/dL)		563	128	491	34
Patient 1	217	327	238	110	21
Patient 2	196	245	218	49	22
Patient 3	276	286	289	10	13

Conclusion: Both the Inform-I and Inform-II exhibited maltose interference which increases with maltose concentration. However, significant reduction of maltose interference was observed with Inform-II. The increases in the glucose results obtained with Inform-II in samples of patients who underwent icodextrin peritoneal dialysis were minimal, therefore, may not change the clinical decisions to manage blood glucose levels in these patients.

B-279

Magnetic Immunoassay for Quantitative Point-of-Care Tests and Rapid Measuring of Protein Concentration

P. I. Nikitin[1], A. V. Orlov[1], V. A. Bragina[1], M. P. Nikitin[2], T. I. Ksenevich[1], B. G. Gorshkov[1]. *[1]General Physics Institute, Russian Academy of Sciences, Moscow, Russian Federation, [2]General Physics Institute and Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation*

Background: Currently, protein markers of diseases are widely detected by rapid Lateral Flow (LF) strips based on color or fluorescent labels. The measurements are carried out by recording such labels from the thin surface layer of the LF membranes only. The advantages of employment of magnetic beads (MB) as labels in bioassays are well established as the beads are not affected by color of the samples, reagent chemistry or photobleaching, are highly stable and could be counted over the whole volume of solid phase. MB can be used in quantitative immunochromatographic assays to facilitate rapid diagnostics of diseases and monitor therapy efficiency. In the present work, a rapid quantitative MB-based assay has been developed and demonstrated by measuring in human blood of concentration of the tumor marker of prostate specific antigen (PSA) used as a model. Such highly sensitive registration in wide dynamic range of concentration of PSA and other tumor markers is attractive for disease diagnostics and relapse monitoring after surgical removal of tumors.

Methods: The magnetic nanolabels were recorded over the whole volume of test zone on LF strips using original method of non-linear MB remagnetization and frequency mixing (P.Nikitin & P.Vetoshko, EP 1262766, 2001). Recently, this method was successfully used for toxin detection in complex biological media by MB counting at 3D-filter solid phase (A.Orlov et al. Anal. Chem. 2013, 85, 1154-1163). Direct comparison showed that the sensitivity of the electronic detection method is on the level of the gamma-radioactive technique for counting of MP based on the 59-Fe isotope (M.Nikitin et. al. J. Appl. Phys. 2008, 103, 07A30). Thus, MBs combined with highly sensitive detectors allow realizing many advantages of old radioimmunoassays, but in much more safe and affordable ways. In present work such advantages have been demonstrated by magnetic LF strips based on dry chemistry.

Results: It has been shown with blood samples of 25 patients that the limit of quantitative PSA detection computed in compliance with IFCC/CLSI guidelines for quantitative methods is 25 pg/mL over the wide dynamic range exceeding 3

orders of concentration magnitude. CV was less than 10% at low concentrations. Importantly, the developed dry chemistry assay features simplicity and 4 times better LOD at duration of 20 min as compared with several-hour-long commercially available ELISA kits. It has been shown that because of high sensitivity, quantitative registration, simplicity and short duration, the developed POC method combines the advantages of laboratory methods and rapid tests based on dry chemistry.

Conclusion: The replacement of traditional optical (gold, colored latex, etc.) labels in immunochromatographic assay formats by the magnetic beads combined with highly sensitive MB detection over the whole volume of the LF strips has allowed to develop a quantitative and highly sensitive in wide dynamic range of more than 3 orders of concentration magnitude immunoassay. The LOD of 25 pg/mL demonstrated by the magnetic immunochromatographic assay while detection of tumor marker PSA in human blood allows us to consider it as an attractive diagnostic POC platform for highly sensitive quantitative detection of proteins in biological fluids.

B-280

Development of a new rapid point-of-care assay for quantitative measurement of D-Dimer in whole blood and plasma

S. Yokokawa, M. Morita, K. Kohno, M. Yamamoto, H. Yago. *SEKISUI MEDICAL CO., LTD., Ryugasaki, Ibaraki Pref., Japan*

D-dimer is a fibrin degradation product (FDP) composed of cross-linked fibrin degraded by plasmin and well known as one of the markers for thrombotic disorders such as deep vein thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation (DIC) and for coronary artery diseases. We have developed a new rapid and quantitative assay for D-dimer in whole blood and plasma. This assay is based on lateral flow immunochromatography with colloidal gold and employs two different anti-human D-dimer mouse monoclonal antibodies. The test cartridge is inserted into the immunochromato-reader "RAPID PIA ™ (Sekisui Medical Co., Ltd.)", and a sample (120µL) of whole blood or plasma is added to the well of the cartridge. After 10 minutes, the reader automatically measures the density of colloidal gold captured at the test line by the antigen-antibody sandwich reaction. The lower detection limit for D-dimer was 0.2µg/mL, and the upper quantitation limit was 15 µg/mL. No prozone effect was observed in D-dimer samples of concentrations from 15 through 244 µg/mL. The within-run C.V. (n=5) at 0.55 µg/mL, 3.5 µg/mL, and 7.1 µg/mL was 1.8%, 5.1% and 4.9%, respectively. The between-run C.V. (n=5) at 0.55 µg/mL, 3.5 µg/mL and 7.1 µg/mL was 6.3%, 4.5% and 4.7%, respectively. The method comparison with the approved IVD reagent, the principle of which is latex-enhanced immunoturbidimetry, yielded a correlation coefficient of 0.992 and an equation of Y (present method) = 1.01X - 0.17 (n = 50 citrated plasma specimens). Furthermore, a high level of correlation was observed between citrated plasma and whole blood (R:0.997 ; slope:1.03;intercept:±0.00). We concluded that this newly developed assay is accurate, precise and simple for the measurement of D-dimer in whole blood or plasma at the bedside. We believe that this assay will be a useful tool for rapid screening patients with thrombotic disorders and coronary artery diseases.

B-281

Performance of the Nova StatStrip Glucometer in a Pediatric Hypoglycemic Population

C. Tan, K. Walker, B. L. Woolsey, P. S. Thornton, N. Farrell, V. Leung-Pineda. *Cook Children's Medical Center, Fort Worth, TX*

Background: In May of 2013, our hospital replaced the legacy glucometer with the Nova StatStrip. Our Department of Endocrinology has a fasting study protocol where patients are fasted until their blood glucose is below 50 mg/dL. At this point a variety of critical samples are drawn. However, due to the accuracy limitations of our legacy glucometer, the current protocol needs a confirmation of blood glucose concentrations from the core laboratory. Therefore, we sought to determine the performance of the Nova StatStrip in this pediatric hypoglycemic population. If the accuracy of the glucometer proved acceptable it could lead to a modification in the fasting study protocols that could lead to a decrease in the length of the study.

Objective: Our goal was to determine the accuracy of the Nova StatStrip glucometer in measuring samples close to the 50 mg/dL range in real clinical setting conditions.

Methodology: Precision of the Nova StatStrip glucometer at low glucose values was evaluated at four different values. Furthermore, quality control material and patient samples were evaluated in the Nova StatStrip, and compared to readings from our core laboratory analyzer without sample delay.

Results & Conclusions: Coefficient of Variations of the Nova StatStrip at low glucose levels was less than 4%. Comparing low glucose QC material showed no statistical difference between POC and core lab methods (n=23). Patient samples (n=30) co-relations indicated an average negative bias of 2 mg/dL when the Nova glucometer was compared to our central lab method. In addition, we saw a decrease in the rate of patient ID errors due to the use of a 2D barscan system present in the Nova StatStrip but absent from our legacy glucometer. The Nova StatStrip proved to have acceptable accuracy in measuring hypoglycemic samples when compared to our central laboratory method. These results support the use of POC glucose, in lieu of waiting for glucose results from the core laboratory, for activating our fasting study draws, shortening the length of the procedure for our patients**.**

B-282

Analytical and technical aspects of POCT-Troponin in the Emergency Department: Comparison with central laboratory hsTnT

J. von Recum[1], D. Meyer zum Büschenfelde[2], A. Slagman[1], J. Searle[1], F. Holert[1], C. Müller[1], M. Möckel[1]. *[1]Charité - University Medicine Berlin, Berlin, Germany, [2]Labor Berlin – Charité Vivantes GmbH, Berlin, Germany*

Introduction The use of point-of-care-testing (POCT) for cardiac troponin involves discrepancies in comparison to central laboratory based measurements. In this analysis, troponin-discrepancies are documented and analyzed to evaluate possible reasons and generate data that is likely to help eliminating factors of insecurity in association with technical and handling issues of different platforms and assays used.

Methods Cardiac troponin-T on AQT-90 (EDTA whole blood, Radiometer) POCT is the standard troponin test in our Emergency Department. We set up parallel measurements of hsTnT (heparin-plasma, Cobas602, Roche) in the central laboratory from the same blood draw and Troponin-I (EDTA whole blood, AQT90, Radiometer) in two separate timeframes of 3 and 4 months within one year (winter and summer period). Troponin-discrepancies were defined as outlined below (Table 1). In the first timeframe immediate re-measurement of the POCT-sample was performed. Measurements of admission-samples resulting from 4946 patients were analyzed regarding discrepancies between hsTnT/TnT and TnT/TnI.

Results 183 discrepancies were detected resulting from 164 patients. 37 between TnT and hsTnT and 146 between TnT and TnI (Table 1). 19 patients showed two discrepancies (hsTnT/TnT and TnT/TnI). Characterizing the discrepancies of timeframe 1 (n=20) we found 10 discrepancies in more than one measurement and 10 non-reproducible analytical errors. In three patients AQT TnT was constantly elevated while hsTnT and AQT TnI were negative. In two patients hsTnT was elevated while AQT TnT was negative. The latter were a 94years old female with a fall and a 81years old male with acute kidney failure.

Conclusion In our population the percentage of discrepancies between hsTnT and AQT TnT did not exceed 0.75% of nearly 5000 analyzed individuals. The use of AQT TnT at the POC is technically reliable under real life conditions. Further studies need to clarify the diagnostic accuracy, specifically when lower cutoffs are used for hsTnT.

Definition of discrepancies					
TnT (AQT) vs. hsTnT (Cobas)			TnT (AQT) vs. TnI (AQT)		
	hsTnT < 45 ng/L	hsTnT > 55 ng/L		TnI < 23 ng/L	TnI > 46 ng/L
TnT < 27 ng/L	ok	discrepancy	TnT < 17 ng/L	ok	discrepancy
TnT > 33 ng/L	discrepancy	ok	TnT > 33 ng/L	discrepancy	ok
Discrepancy Details in % (n=4946)					
	hsTnT < TnT	hsTnT > TnT		TnT > TnI	TnT < TnI
Timeframe 1 (n=2344)	0.73% (n=17)	0.13% (n=3)		3.5% (n=82)	0.17% (n=4)
Timeframe 2 (n=2602)	0.38% (n=10)	0.27% (n=7)		1.88% (n=49)	0.42% (n=11)
total (n=4946)	0.55% (n=27)	0.2% (n=10)		2.64% (n=131)	0.3% (n=15)
Characterization of the TnT discrepancies of timeframe 1 (n=2344)					
	hsTnT < TnT	hsTnT > TnT			hsTnT < TnT
persistent	0.34% (n=8)	0.09% (n=2)	persistent analytical discrepancies, to be investigated for possible interferences		0.13% (n=3)
non-persistent	0.38% (n=9)	0.04% (n=1)			

Table Definitions and details of Troponin discrepancies.

B-283

The modified method for microbilirubin determinations at low source setting-hospitals

K. Thongsukkaeng[1], P. Pheunsongkram[1], U. Doungwao[1], W. B. Treebuphachatsakul[2]. *[1]Phukhieo Hospital, Chaiyaphum Province, Thailand, [2]Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*

Background: Bilirubin determinations are often required in the routine management of newborns. Bilirubinometer requires a micro volume of blood sample and is convenient used at pediatric units as point of care testing. There is no bilirubinometer available at low source setting hospitals in rural area of Thailand. The objective of this study were to evaluated the performances of bilirubinometer and validate the performances of the modify method using a micro volume of plasma sample for bilirubin measurement in newborns.

Methods: Precision, accuracy, and linearity of bilirubinometer were evaluated. One hundred plasma samples of newborns at Phukhieo Hospital were determined for bilirubin. Sixty microliters of each blood sample was collected into heparin-containing capillary tube. All samples were immediately centrifuged, then measured for bilirubin by bilirubinometer and a modified method by the automated clinical chemistry analyzer. Paired data of bilirubin results were analyzed by using paired different T-test.

Results: Bilirubinometer has revealed good precision, accuracy, and linearity for bilirubin determination in newborns. Microbilirubin results obtained from bilirubinometer and a modified method were correlated ($r=0.978$) and paired difference data between two methods were not statistical significant differences ($p>0.05$).

Conclusion: Bilirubinometer was convenient used for microbilirubin in newborns on site and a modified method was the alternative way for low source setting in Thai Hospitals at clinical laboratory. However, laboratory should manage to provide shorten laboratory turnaround time for microbilirubin measurement.

B-284

Implementing a point of care (POC) laboratory in order to reduce patients' length of stay in the ED as well as meet ED critical care standards.

T. Nolen[1], H. Hasebe[2]. *[1]Bert Fish Medical Center, New Smyrna Beach, FL, [2]Mitsubishi Chemical USA Inc., Chesapeake, VA*

Background: Hospitals, providers and patients are all eager to reduce the length of emergency department (ED) visits. For hospitals, faster patient turnover means more patients can be seen, generating higher revenue. For physicians, faster turnover means releasing patients who don't need further care so they can spend more time with those who do. For patients, less time in the ED means they can return to daily life sooner and, potentially, reduce their medical costs. Speeding up the rate of turnover is dependent on reducing turnaround time (TAT) for lab results. We were interested to determine whether point of care (POC) laboratory testing, based in the ED itself, could lower TAT when compared to the performance of a satellite laboratory. To test this theory we set up a POC lab in the Bert Fish Medical Center's ED.

Methods: Although our POC lab operated within the ED, it was controlled by the main laboratory. Four emergency medical technicians, employed by the ED, managed all the testing, under the supervision of one medical technologist, a lab employee. A key difference about our approach to POC testing is that we did not use nurses to perform any testing procedures. Based on our research, nurses are already too busy to take on the additional burden of managing POC testing.

Results: In our POC, we used a more sensitive troponin assay with chemiluminecent POC testing helped improve TAT. With this test, the POC lab was 42% faster than the main lab when troponin test results were negative_54 minutes in the POC lab versus 97 minutes in the main lab. The impact was significant, since negative results made up 91% of the total. The remaining 9% positive results were retested in the main lab to exclude other cardiac conditions, so there was no time savings for this batch. In effect, testing at the POC level becomes a "lean" process because it cuts out the extra steps required for testing in the main lab.

Conclusion: Our study demonstrated that establishing a POC lab can yield a number of benefits. These include:

- Faster TAT for troponin testing, as well as testing for myoglobin and CK-MB
- Shorter ED stays, and as a result, shorter wait times for new patients in need
- Earlier detection of cardiac risk, due to the faster detection of troponin, leading to increased survivability
- Greater physician and patient satisfaction
- Improved relations between the lab and the ED, as they now work in unison

We also noted that the cost of running a POC lab is about $300,000 per year, versus $1-1.5 million for a satellite lab. In part, this is because the salaries of the medical assistants running the POC lab are lower than those of medical technologists in satellite labs. Even with the hiring of additional assistants, overall operating costs of the POC labs are lower.

Our conclusion is that given how the POC can perform more quickly and at lower cost than traditional satellite labs, hospitals should consider moving to the POC model.

B-286

Development of a fully-quantitative lateral flow assay system for the detection of a novel combination of sepsis markers

E. E. Oldridge[1], E. D. Carrol[2], T. Myers[3], N. Tort[4], J. Flint[1], C. Danks[1]. *[1]Forsite Diagnostics Ltd, York, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom, [3]Microlab Devices Ltd, Leeds, United Kingdom, [4]Biokit, Barcelona, Spain*

Sepsis, or severe bacterial infection (SBI), is the leading cause of death in intensive care units in high-income countries, and its incidence is on the rise. Sepsis is estimated to affect 18 million people worldwide and in 2003 $14.6 billion were spent on hospitalizations for the disease in the US. Sepsis diagnosis is often delayed due to inadequate diagnostic tools. Consequently, prompt diagnosis and treatment is of paramount importance to reduce morbidity and mortality associated with the disease.

We have developed an innovative point-of-care (POC) diagnostics system incorporating lateral flow assay technology to measure a novel combination of bio-markers: procalcitonin (PCT), neutrophil gelatinase-associated lipocalin (NGAL) and resistin. In combination, these markers provide a superior indication of SBI in febrile children presenting to the emergency department.

PCT, a precursor of calcitonin, is a 116 amino acid protein that has been proposed as a marker of disease severity in conditions such as septicemia, meningitis, pneumonia, urinary tract infection (UTI) and fungal and parasitic infection. NGAL, or lipocalin 2, is a 25 kD lipocalin that has a role in innate immunity and is highly up-regulated by inflammatory stimuli. Resistin has been shown to play an important regulatory role in adipogenesis, glucose homeostasis and insulin sensitivity. PCT is a well recognized marker of sepsis, and Resistin and NGAL have recently been shown to be significantly elevated in intensive care patients with sepsis.

The POC system incorporates 3 simplex lateral flow assays utilizing gold nano-particle technology to detect PCT, NGAL and Resistin. It requires no sample pre-treatment and gives fully-quantitative results within 10 minutes on a hand-held reader.

We show that the system is able to measure the bio-marker combination from human plasma, producing fully-quantitative values which are incorporated into a clinical diagnostic algorithm to assess response to therapy. The results show that the linear ranges of the individual assays span a large concentration range: PCT: 0.1-1000 ng/ml, Resistin: 5-1000 ng/ml and NGAL: 5-1000 ng/ml. We also discuss the ability of the assays to successfully differentiate between 5 clinical categories on which to base the clinical diagnosis and treatment: SBI rule out, SBI rule in, uncertain (further clinical assessment and investigations necessary), likely severe infection and likely life threatening.

This test has the potential to advance the effective use of bio-markers in the management of both child and adult sepsis and impact across the whole POC market by providing a better diagnosis, rapid treatment and reduced hospital admissions.

B-287

Impact of improved glucose monitoring in the neonatal intensive care unit: an evaluation of analytical and clinical performance of the point of care Nova Statstrip

J. Raizman[1], T. Henderson[1], J. Shea[2], S. Silverman[1], S. Redmond[1], A. Moore[1], J. Dubois[3], K. Adeli[1]. *[1]The Hospital for Sick Children, Toronto, ON, Canada, [2]Saint John Regional Hospital, Saint John, NB, Canada, [3]Nova Biomedical, Waltham, MA*

Objective: To evaluate the analytical and clinical performance of new glucose point of care testing (POCT) in the intensive care unit (NICU).

Methods: Within-run imprecision, correlation with a plasma hexokinase assay, and interferences with hematocrit were studied on the Nova-StatStrip and SureStep-Flexx meters. Meter and lab results were analyzed over 2 years in the NICU. Outcomes

measured were rate of hypoglycemia, frequency of critical results, average length of stay (LOS), clinical sensitivity/specificity for detecting critical results, and clinical accuracy.

Results: Imprecision (CV) using control material (2.8-15.7 mmol/L, n=20) and whole blood patient pool specimens (5.1-23.7 mmol/L, n=20) ranged from 3.19-4.97% and 1.39-7.21%, respectively, for SureStep, and 1.05-4.98% and 1.51-3.71%, respectively, for Nova. Method comparison to the Ortho Vitro950 hexokinase method (n=120) revealed correlations of y= 0.867x + 0.82 (R=0.990) with a mean bias of -0.64 mmol/L for SureStep, and y=1.016x+0.04 (R=0.997) with a mean bias of -0.21 mmol/L for Nova. Hematocrit interfered with SureStep by reducing glucose measurements at increasing hematocrit (23-67%) compared to Ortho, an effect absent on Nova. Studies to assess clinical performance of the meters revealed fewer readings per NICU visit (24%, p=0.001) in patients monitored with Nova compared to SureStep. This was associated with a reduction in frequency of hypoglycemia results (53%, p=0.053) as well as a trend towards critically low ≤3.0 mmol/L (35%, p=0.112) and high results ≥9.0 mmol/L (40%, p=0.009). The sensitivity/specificity for detecting critically low results was 70.2/98.7% and 80/99.5% for SureStep and Nova, respectively. Patients monitored with Nova trended towards increased LOS in the NICU (16%, p=0.181). Clinical comparisons demonstrated correlations of y=0.983x+0.24 (R=0.931) for Nova (n=607) and y=1.1x-0.036 (R=0.869) for SureStep (n=977) compared to lab values.

Conclusion: Nova demonstrated superior precision and accuracy compared to SureStep. Improved analytical performance translated into improved detection of critical glucose results, demonstrating the importance of implementation of accurate POCT in the NICU.

Clinical performance of the Nova StaStrip and SureStep Flexx in the NICU

	Nova StatStrip	SureStep Flexx	% Change	p-value
Total number of admissions	240	250	24	-
Total results per admission	10	13.1	24	0.001
Hypoglycemia results per admission	0.16	0.34	53	0.053
Critical low results per admission	0.68	1.04	35	0.112
Mean length of stay in NICU (days/patient)	25.1	21.1	16	0.181
Sensitivity for critical low results (%)	80	70.2	-	-
Specificity for critical low results (%)	99.5	98.7	-	-

B-288

Evaluation of new glucometers (Easy Touch GC) for bedside use

M. E. Adekiitan, G. E. Imana, O. O. Adedeji. *Lagos State University Teaching Hospital, Lagos, Nigeria*

BACKGROUND Glucometers have greatly improved the clinical care of diabetics. It shortens time for making critical decisions. It is portable, inexpensive and easy to use. Newly acquired glucometers (Easy Touch) in our hospital were evaluated by calibration and precision profile determination. The results were compared with values obtained using routine laboratory method.

METHOD Standard glucose solution 500mg/ dl was serially diluted in de-ionized water to 250mg /dl, 125mg/dl, and 100mg/dl. The solutions were analyzed with three glucometers to determine their linearity. .

Blood samples were taken in duplicate from 39 patients into fluoride oxalate containers for the determination of glucose concentrations. A set was analyzed in the Hospital Laboratory by spectrophotometric glucose oxidase method while the other was analyzed with the glucometers. Duplicate measurements of the blood samples by 3 individuals using the glucometers were performed to determine their precision profile.

RESULTS The glucometers showed good linearity using the standard glucose solutions. Their correlation coefficients were R1 = 0.883, R2 =0.983, R3 =0.949; R1, R2 and R3 represent the 3 glucometers respectively. However, the readings from the glucometers were much lower than the actual glucose concentration at ≥ 500mg/dl. This may be due to the use of de-ionized water as solvent for the standard solutions used in the calibration as against the matrix of blood. This could imply that the glucometer readings may not be accurate at critically high glucose levels.

Good correlations were obtained between the readings of the glucometers and laboratory results at blood glucose concentration <500mg/dl. Thus, the correlation coefficients were 0.824, 0.900 and 0.845 for glucometers R1, R2 and R3, respectively.

The coefficient of variations (CVs) obtained with the glucometers were 10.43%, 3.75% and 6.50% for R1, R2 and R3, respectively. With the routine laboratory method, the CVs were 10.94%, 9.84% and 11.69%. When the two methods were compared, the CVs obtained with the glucometers varied widely, whereas, the CVs of the laboratory method were fairly constant. This could imply that the performance of the glucometers was operator dependent because they were operated by 3 different individuals, which was not the case with the laboratory method that was performed by only one person.

The imprecision profile, representing, the mean differences between the readings of the glucometers and laboratory results, were 14.8 ± 4.67, 23.7 ± 6.08 and 15.5 ± 3.83 for glucometers R1, R2 and R3 respectively. These values were high and could have serious implications in the interpretation of glucose values for the management of diabetes mellitus.

CONCLUSION The readings from the glucometers showed inaccuracy at very high glucose concentrations and their performance could be operator dependent. In view of the good correlations with the laboratory method (at <500mg/dl), the glucometers should be standardized against the laboratory method regularly when used.

Keywords: Glucometer, calibration, precision.

B-289

Comparison of the UriScan® 2 ACR regent dipsticks for Albumin and Creatinine in Urine with Quantitative Methods

S. Lee, S. Park, M. Kang. *YD Diagnostice, YonginSi, KyungiDo, Korea, Republic of*

Background: Microalbuminuria is a predictive maker for renal disease and the identification of patients at high risk of developing complications of diabetes or hypertension. The UriScan® 2ACR(YD Diagnostics, Korea) is a urine chemistry point-of-care test for the semi-quantitative measurement of albumin and creatinine and calculation of albumin:creatinine ratio(ACR). The aim of this study is to comparing quantitative method and UriScan® 2ACR strip for measurement of albumin and creatinine ratio in urine.

Methods: The samples for this study used to random urine which were collected from a total 641 patients at three sites. The concentration of albumin and creatinine are measured to UriScan® 2ACR after quantifying by gold standard quantitative method.

Results: The clinical performance results of the UriScan® 2ACR strip s for detecting microalbuminuria (ACR) were showed to accuracy 86.9%. Also, the sensitivity, specificity, PPV (positive predictive value), and NPV (negative predictive value) is 89.2%, 85.6%, 77.4%, and 93.5%. The result of microalbumin measurements in the spot urine samples of UriScan® 2ACR strip were showed to accuracy 89.9%. Also, the sensitivity, specificity, PPV (positive predictive value), and NPV (negative predictive value) is 91.2%, 89.0%, 84.5%, and 93.9%, respectively. The results of creatinine were accuracy 70.0%.

Conclusion: UriScan® 2ACR strip tests had good agreement with gold standard quantitative methods. Therefore, measurement of UriScan® 2ACR strip in the spot urine sample is an efficient method for screening the general population for microalbuminuria. The dipsticks tests were easy to use, simple, and useful screening tool for point-of-care test.

B-290

Validation of the Abbott i-STAT total β-hCG cartridge for use in rural Alberta hospitals

A. K. Füzéry[1], L. Redman[2], S. M. H. Sadrzadeh[2], J. C. Wesenberg[3], A. A. Venner[3]. *[1]Alberta Health Services, Edmonton, AB, Canada, [2]Calgary Lab Services, Calgary, AB, Canada, [3]Alberta Health Services, Red Deer, AB, Canada*

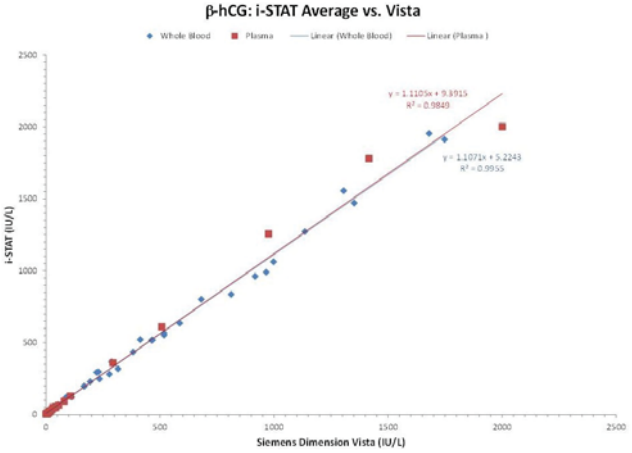
Background: Human chorionic gonadotropin (hCG) has significant clinical utility, yet many rural hospitals lack testing volume and/or instrumentation for quantitative measurement. However, many of these hospitals already have i-STAT analyzers, and Abbot's new β-hCG cartridge offers a feasible option for onsite quantitative testing. This study aimed to evaluate the i-STAT β-hCG cartridge.

Methods: Linearity, imprecision and accuracy were evaluated. For linearity, a plasma sample (hCG=1799 IU/L) was diluted from 1/2 to 1/256 and measured in duplicate. For imprecision, two levels of each of Clinica (24 IU/L and 1455 IU/L) and Bio-Rad Immunoassay Plus (6 IU/L and 21 IU/L) quality controls were measured daily over 20 days. Accuracy was assessed by measuring hCG in plasma samples on both the i-STAT and the Siemens Dimension Vista (n=37), Beckman Coulter DxI 800 (n=39), or Roche Cobas 6000 (n=42) analyzers. In addition, whole blood samples were

measured with the i-STAT; samples were then centrifuged and the plasma measured with central lab analyzers (n=43, n=34, n=52, respectively). The β-hCG ranged from <1 IU/L to 161,207 IU/L.

Results: Linearity was demonstrated from 9-1799 IU/L. Total imprecision was acceptable (Bio-Rad: mean=24 IU/L, CV=7.7%; mean=21 IU/L, CV=4.4%; Clinica: mean=24 IU/L, CV=5.1%; mean=1455 IU/L, CV=3.0%). Comparison of plasma on the i-STAT yielded acceptable correlations (Vista: regression line with slope=1.1105, y-intercept=9 IU/L, R^2=0.9849; DxI slope=0.9727, y-intercept=3 IU/L, R^2=0.9943; Cobas slope=1.0043, y-intercept=4 IU/L, R^2=0.9997). Comparison of whole blood on the i-STAT gave similar results (figure 1).

Conclusion: Linearity, imprecision and accuracy of the i-STAT β-hCG cartridge were acceptable for both whole blood and plasma samples. It can be utilized in clinical settings without access to a large chemistry analyzer with quantitative hCG. It is specifically useful for patients requiring a stat quantitative hCG result.



B-291

The Effect of Maltose on the Radiometer 837 Blood gas Analyzers

D. Semmel, G. Haddad, S. Fan. *Boston Medical Center, Boston, MA*

Background: Glucose measurement can be performed on commercially available blood gas analyzers (BGAs) for faster turnaround time, which are based traditionally on either the glucose dehydrogenase reaction or the glucose oxidase reaction. Other sugars, including maltose, may interfere with these reactions, causing the glucose measurement to be falsely elevated. Octagam 5% liquid is a commercially available intravenous immune globulin. Its preparation contains maltose, which has the potential to interfere with glucose measurement on the BGAs. Patients treated with this drug who also necessitate blood glucose levels may demonstrate falsely elevated measurements if their testing is performed on the BGAs. As it is not feasible to screen every patient for interfering drug preparations before performing glucose measurements, we felt compelled to demonstrate whether the maltose's effect on glucose measurement was clinically significant when using the BGAs.

Methods: To simulate patients being treated with Octagam, two levels of clinically relevant maltose concentrations were prepared using maltose solution admixed with whole blood from patients known not to be on any medication produced with maltose or other sugar based preparations. There were a total of eleven specimens, whose glucose levels ranged from 25 to 550 mg/dL. Simulated high and low drug levels were prepared by spiking concentrated maltose into whole blood specimens, resulting in a "high level" of 6.4 g/L to simulate maximum dosage and "low level" of 1.6 g/L to simulate low dosage. The spiked samples were tested for glucose in duplicates on the Radiometer 837 BGAs. Results were then corrected for the spiked volume, and averages of the duplicates were plotted against the original glucose concentrations.

Results: The correlation coefficient (R^2) of the best fitting line for both high and low maltose levels were good. Additionally, the predicted glucose measurements at various levels can be extrapolated from the best fitting line. As seen by this best fitting line, there is no clinically significant difference between the original and spiked measurements, either in the high level or low level specimens.

Conclusions: There is no clinically significant difference between the original and spiked results. Therefore, there is no need to eliminate the use of the Radiometer BGAs as a laboratory instrument for fast and accurate glucose measurements in urgent specimens for patients undergoing treatment with maltose-containing drug preparations or to screen patients for use of such drug preparations.

B-292

Novel POC analysis for CBC, using PixCell Medical device, HemoScreen

A. Bransky[1], Y. Ben-Yosef[1], B. Marom[1], H. Ben-Asher[1], C. D'Souza[2]. *[1]PixCell Medical Technologies Ltd., Yokneam Ilit, Israel, [2]University of Westminster, Faculty of Science & Technology, Department of Biomedical Sciences, London, United Kingdom*

Background: Point of care (POC) in the community and hospital out patients is increasing to enable rapid sample testing for anaemia, monitoring oncology treatment and reduce patient waiting times. Current devices provide single parameters within a Complete Blood Count (CBC). However, the HemoScreen developed by PixCell Medical will provide a CBC and five-part differential within minutes.

Objective: To present a novel device, HemoScreen, for performing CBC using viscoelastic-focusing and microfluidic technology linked with image-based analysis and to demonstrate its ease of use and reliability for use in POC settings.

Methodology: HemoScreen, uses a disposable cartridge with 20ul of blood, finger prick or venous sample, placed into an analytical device. Employing viscoelastic-focusing and flow-based optical imaging, the cells are focused into a single plane, analysed by image-processing and classification algorithms to calculate the cell counts and related red cell indicies. The HemoScreen will offer a safe, reliable maintenance free method for performing CBC including possible flagging for morphological abnormalities. Internal quality control includes a built-in self-test for electronics, mechanics and software, carried out before each test, and at regular intervals during device operation. EQA is undertaken using a commercial control set at three different levels, low, normal and high.

Currently, 3 samples with 10 replicates each have been processed for precision. Approximately 60 samples were tested for accuracy, following the CLSI standard criteria. The Sysmex XE-2100 served as a reference method.

Results and validation: HemoScreen precision and accuracy results are summarized in the table below:

	Precision N=30	Accuracy		
Parameter	**CV (%)**	**Correlation coefficient (r)**	**Slope**	**Intercept**
WBC (x10^9/L)	8.0	0.988	0.979	0.26
RBC (x10^{12}/L)	3.6	0.958	0.966	0.167
HGB (g/dL)	6.1	0.949	1.013	-0.125
MCH (pg)	7.2	0.809	0.916	1.91
HCT (%)	4.0	0.963	1.003	-0.048
MCV (fl)	1.0	0.934	0.976	1.691
RDW (%)	1.3	0.957	0.894	1.552
PLT (x10^9/L)	5.5	0.960	0.948	6.7

Additional studies are being performed as it has been shown that precision can be further improved by optimizing the current method.

Conclusion: The HemoScreen is an innovative device that has potential to deliver a CBC within minutes, for use in POC settings, pharmacies, physician offices, oncology, neonatal and adult ICU, or even in the home.

B-293

Novel POC analysis for Leukocytes and five-part differential, using PixCell Medical device, HemoScreen

C. D'Souza[1], A. Bransky[2], B. Marom[2], H. Ben-Asher[2], Y. Ben-Yosef[2]. *[1]University of Westminster, Faculty of Science & Technology, Department of Biomedical Sciences, London, United Kingdom, [2]PixCell Medical Technologies Ltd., Yokneam Ilit, Israel*

Background: Point of care (POC) is increasing to enable rapid testing while reducing patient waiting times. Current POC devices provide single parameters within a Complete Blood Count (CBC). However, the HemoScreen developed by PixCell Medical will provide a CBC and five-part differential within minutes. Haemoglobin and neutrophil counts in particular are essential for the monitoring of cytotoxic chemotherapy and sepsis whereas a 5-part differential is useful in all patients. The HemoScreen will offer all the parameters at the patient bedside or doctor's office that are currently only provided by the hospital laboratory.

Objective: To introduce the HemoScreen device for Leukocytes with five-part differential technology and demonstrate ease of use, safety and reliability of the device for use within POC settings.

Methodology: HemoScreen uses a disposable self-contained reagent cartridge, which employs micro-fluidic technology. The cartridge uses 20ul of capillary blood collected directly from the finger or venous blood. Once inserted into the analyser, digital imaging, and advanced algorithms are employed to calculate CBC results. Following

RBC lysis the differential is obtained by chemical staining of leukocytes. Leukocytes are classified based on multiple attributes such as cell size, nuclei size, nuclei lobes and cellular content. Contamination and device maintenance are eliminated as the flow of liquids occurs within the cartridge.

Intra-assay precision and accuracy were conducted using venous whole blood samples analysed in accordance with CLSI standards on both the Sysmex XE-2100 (reference method) and the HemoScreen. Three samples with 10 replicates were tested for precision. For accuracy 32 samples have been processed. Further samples will be used for evaluation of accuracy, including highly abnormal patient samples to validate this device.

Results and Validation: Results of comparability study are summarized below:

	Precision N =30		**Accuracy N=32**			
Parameter (Units)	**CV (%)**	**Acceptance Criteria**	**Correlation coefficient (r)**	**Slope**	**Intercept**	**Acceptance Criteria**
WBC ($x10^6$/L)	8.0	CV <10%	0.988	0.979	0.26	r>0.95
NEUT ($x10^6$/L)	8.0	CV <10%	0.989	1.081	-0.107	r>0.95
LYMP ($x10^6$/L)	10.9	CV <15%	0.965	1.041	0.135	r>0.9
MONO ($x10^6$/L)	15.5	CV <20%	0.931	0.976	0.135	r>0.8
EOS ($x10^6$/L)	22.8	CV<40%	0.987	1.032	0.012	r>0.9
BASO ($x10^6$/L)	8.8	CV<40%	Calculations were not done due to a low count			

Conclusion: A five-part differential is essential when monitoring oncology and septic patients in a POC setting and the HemoScreen has the potential to deliver this rapidly.

B-294

Extensive Evaluation of Sample Interferences on Point-of-Care Glucose Meters

A. N. Steele, Z. Godwin, M. Howes, N. K. Tran. *University of California Davis, Davis, CA*

Background: Intensive insulin therapy (IIT) guided by tight glycemic control (TGC) reduces morbidity and mortality in critically ill patients. Accurate glucose measurements are necessary for safe TGC. However, endogenous and exogenous interferences, such as ascorbic acid (AA), beta-hydroxybutyrate (BHB), galactose (GAL), glutathione (GLUT), lactose (LAC), and N-acetylcysteine (NAC) may impact glucose monitoring systems (GMS) accuracy. These compounds may be the result of medical interventions and/or critical illness. The objective of this study was to determine the effect of these five interferences on current generation POC GMS performance and the impact of autocorrecting biosensors in improving glucose measurement accuracy.

Methods: We investigated the effects of AA, BHB, GAL, GLUT, LAC, and NAC on the Nova Biomedical (Waltham, MA) StatStrip hospital (GMS 1-5) and Xpress meters (GMS 6-10), Roche Diagnostics (Indianapolis, IN) Inform II meter (GMS 11-13), and Abbott Laboratories (Abbott Park, IL) Precision Xceed Pro (GMS 14). All POC GMSs incorporated autocorrecting features within their biosensors. Whole blood from 12 healthy adult (age≥18 years) volunteers was used for testing. Specimens were adjusted to two clinically relevant levels (moderate, high) for each interferent and at different five glucose levels (range: 50-500 mg/dL). A negative control (without interfering compound) was also included for each sample series. Samples were tested on each GMS five times for each individual glucose and interference level. Results were compared to a plasma reference. Two-way ANOVA followed by pairwise analyses compared each GMS versus the reference method at each interference level.

Results: AA significantly affected GMS 11-13 at both levels (mean [SD] bias: -56.1 [38.9] mg/dL, P<0.001), while GMS 14 was only significantly affected at high AA levels (-18.0 [31.2] mg/dL, P<0.001) only. BHB significantly affected GMS 3 (-30.8 [20.8] mg/dL, P<0.001) and 7 (-28.6 [19.5] mg/dL, P<0.001) at high interference levels, GMS 4 (-28.6 [13.6] mg/dL, and P<0.001) and 8 (-30.0 [18.3] mg/dL, P<0.001) at moderate levels. For GMS 14, BHB affected the device at both interference levels (42.4 [37.7] mg/dL, P<0.001). GAL significantly affected GMS 1 (-29.0 [15.5] mg/dL, P<0.001), 3 (-38.6 [24.3] mg/dL, P<0.001), 9 (-23.4 [18.8] mg/dL, P<0.001), 11-13 (81.6 [13.1] mg/dL, P<0.001), and 14 (-47.1 [35.4] mg/dL, P<0.001) at both interference levels. GLUT significantly impacted GMS 11-13 (43.5 [27.2] mg/dL, P<0.001). LAC significantly affected GMS 11-14 at moderate and high interference levels. NAC significantly affected GMS 11 (20.0 [34.0] mg/dL, P<0.001) and 12 (19.0 [35.6] mg/dL, P<0.001) at high levels, and significantly affected GMS 14 at both interference levels (-114.5 [44.4] mg/dL, P<0.001).

Conclusions: Accurate glucose monitoring improves glycemic control and outcomes in critically ill patients. Sample interferences results in erroneous GMS measurements and increases the risk for dangerous hypoglycemic events during IIT. GMS 1-10 was observed to be the most robust against the evaluated interfering substances. We advise caution for facilities using GMS 11-14 due to significant AA, BHB, GAL, GLUT, LAC, and NAC inferences observed by our study.

B-295

Extensive Evaluation of Hematocrit Interference on Point-of-Care Glucose Meters

A. N. Steele, Z. Godwin, M. Howes, N. K. Tran. *University of California Davis, Davis, CA*

Background: Intensive insulin therapy (IIT) guided by tight glycemic control (TGC) reduces morbidity and mortality in critically ill patients. Accurate glucose measurements are necessary for safe TGC. However, confounding factors, such as abnormal hematocrit (HCT), results in inaccurate measurements on point-of-care (POC) glucose monitoring systems (GMS). Abnormal HCT is common in intensive care unit patients as a result of pathologic and iatrogenic mechanisms. Therefore, accurate POC glucose measurements are necessary for safe IIT for TGC. The objective of this study was to determine the effect of altered HCT on current generation POC GMSs and the impact of autocorrecting biosensors in improving glucose measurement accuracy.

Methods: We investigated the effects of abnormal HCT on the Nova Biomedical (Waltham, MA) StatStrip hospital (GMS 1-5) and Xpress meters (GMS 6-10), Roche (Indianapolis, IN) Inform II meter (GMS 11-13), and Abbott (Abbott Park, IL) Precision Xceed Pro (GMS 14). All POC GMSs incorporated autocorrecting features within their biosensors. Whole blood from 12 healthy adult (age≥18 years) volunteers was used for testing. Specimens were adjusted to five different HCT levels for five glucose levels (range: 50-500 mg/dL). Samples were tested on each GMS five times for each individual glucose and interference level. Results were compared to a plasma reference. Two-way ANOVA followed by pairwise analyses compared each GMS versus the reference method at each HCT level.

Results: Study results are summarized in **Table 1.** Asterisks indicate HCT significance GMS bias against the reference method.

Conclusions: Abnormal HCT is common in critically ill patients. Automatic hematocrit correction is instrumental for accurate glucose monitoring and TGC. GMS 1-10 exhibited acceptable performance at all HCT levels with the exception of GMS 3. Performance was acceptable for GMS 11-13. However, we advise caution for facilities using GMS 14 due to significant bias over a broad range of HCT levels.

Table 1. Comparison of Mean (SD) GMS Results

HCT	GMS 1	GMS 2	GMS 3	GMS 4	GMS 5	GMS 6	GMS 7	GMS 8	GMS 9	GMS 10	GMS 11	GMS 12	GMS 13	GMS 14
20	-13.8 (8.9)	-12.4 (12.3)	-13.8 (8.7)	-16.6 (12.5)	-8.0 (9.2)	-15.4 (12.9)	-14.0 (14.9)	-8.6 (10.0)	-24.4 (24.6)	-9.0 (9.9)	13.0 (9.1)	14.0 (9.2)	14.0 (7.4)	-8.8 (13.2)
30	-12.6 (5.1)	-12.6 (11.5)	-16.8 *** (12.3)	-14.0 (5.4)	-12.6 (6.4)	-6.0 (3.7)	-12.4 (9.2)	-11.2 (6.1)	-13.2 (8.4)	-9.0 (11.4)	12.2 (10.6)	13.8 (11.8)	8.8 (8.6)	-18.4 *** (12.7)
40	-15.8 (8.0)	-18.2 (16.9)	-22.2 *** (15.2)	-19.2 (11.1)	-12.2 (8.2)	-15.0 (5.8)	-13.0 (14.5)	-17.6 (12.0)	-13.2 (17.7)	-8.2 (5.3)	4.6 (12.5)	8.6 (9.2)	2.6 (10.2)	-38.8 *** (31.7)
50	-19.6 (6.7)	-22.0 (15.7)	-23.2 *** (8.2)	-18.2 (10.7)	-18.0 (12.8)	-18.8 (8.2)	-16.8 (9.4)	-20.0 (9.8)	-17.0 (9.6)	-11.6 (6.8)	0.4 (22.7)	3.2 (9.4)	-4.6 (16.5)	-52.6 *** (30.8)
65	-24.8 (12.3)	-27.2 (18.6)	-25.4 (20.6)	-25.4 (9.2)	-25.6 (16.1)	-24.6 (12.2)	-18.0 (16.8)	-25.8 (15.8)	-21.2 (12.4)	-14.6 (7.5)	-7.2 (17.6)	-7.8 (6.1)	-16.0 (18.7)	-72.8 *** (45.0)

Note: Glucose levels were measured in mg/dL
Abbreviations: Glu, Glucose; SD, standard deviation
***P<0.001 as determined by Two-Way ANOVA and Tukey's HSD

B-296

Optimization of the turn-around-time of CRP measurement in the emergency setting by using the Microsemi® analyzer

H. Baum, H. Sander, A. Leni, K. Mages. *Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany*

Introduction: C-reactive protein (CRP) is an established marker in the diagnosis and follow-up of patients with infectious diseases. In the acute setting it can help to assess and prioritize these patients and therefore improve diagnosis and further treatment. The Microsemi® CRP analyzer is a small analyzer, which determines with the use of only 18 µl EDTA-blood a complete blood cell count and CRP within 4 minutes. Furthermore, the Microsemi can be linked to a LAN for rapid result upload into a hospital information system (HIS). The aim of this study was to determine the turn-around-time (TAT) of CRP measured with the Microsemi analyzer and compared it with a fully automated method in a routine labor setting.

Material and Methods: Serum and EDTA samples from 66 patients with an urgent test-request were selected for analysis out of the daily routine. Serum-CRP was measured on a Vitros® 5600 analyser whereas the Microsemi was used to test CRP with EDTA samples . Time of blood collection was determined using the time of ordering in the order-entry system of the hospital information system (HIS). Using the laboratory information system (LIS) the time of arriving in the lab, uploading the result from the routine analyzer into the LIS, and reporting the result into the HIS

were collected. For the Microsemi the measuring time for CRP is 4 minutes. To these 4 minutes we added a mean time for other work of 5 minutes.

Results: The method comparison showed a good correlation between both assays with r = 0.9888 and CRP (Microsemi) = 1.057 x CRP (Vitros) - 0.235.

Mean time from blood collection to arrival in the lab was 32 (2-235) minutes. Mean time from arrival of the blood in the lab to reporting of the results into the HIS was 37 (21-103) minutes for the Vitros and 9 minutes of the Microsemi. Taking together it results in a mean total TAT of 69 (23 - 338) minutes for the Vitros and 41 (13-244) minutes for the Microsemi. Therefore, using the Microsemi the results could be reported in mean 28 (10-94) minutes earlier compared to the routine processing procedure.

Conclusion: Using the Microsemi for CRP measurement in the emergency situation there is the possibility to report much faster this critical parameter to the clinician without loss of analytical accuracy.

B-297

Evaluation of Clinitest® hCG device susceptibility to high-dose hook effect by intact human chorionic gonadotropin (hCG) and hCG beta-core fragment at concentrations observed in early natural pregnancy

D. M. Milhorn, N. L. Korpi-Steiner. *UNC, Chapel Hill, NC*

Background: Qualitative detection of urinary human chorionic gonadotropin (hCG) using point-of-care testing devices is common practice in the evaluation of suspected pregnancy. False-negative findings due to high-dose hook effect are known to occur at elevated urinary concentrations of intvact hCG and/or hCG beta-core fragment (hCGβcf) with select POC hCG devices. In early pregnancy, hCG and hCGβcf concentrations vary depending on days relative to ovulation/conception. The aims of this study were to evaluate hook effect of intact hCG alone, hCGβcf alone, and combinations of hCG and hCGβcf exhibited in early pregnancy using the Clinitest® hCG device with accompanying Clinitek Status (Siemens Healthcare Diagnostics) readout. The distribution of days relative to ovulation at the time of Clinitest® hCG testing for patients presenting at 1 institution was further evaluated.

Methods: Hook effect by intact hCG and hCGβcf using Clinitest® hCG devices with Clinitek Status readout was evaluated using hCG-negative urine matrix containing 7 levels of purified intact hCG or hCGβcf (0 - $2x10^6$ pmol/L) alone, and combinations of intact hCG and hCGβcf (n=20) corresponding to physiological concentrations (± 50 pmol/L and ± 2% for concentrations ≤ 10,000 pmol/L and > 10,000 pmol/L, respectively) detected every other day in early natural pregnancy between 13 and 49 days relative to ovulation (n=37, intact hCG range 10 – $5.9x10^4$ pmol/L; hCGβcf range 10 – $2.3x10^5$ pmol/L; data courtesy of McChesney et al. Human Reprod 2005. 20(4):928-35). Prepared samples were tested in duplicate. Estimated days relative to ovulation at the time of Clinitest® hCG testing for female patients ≥ 18 years presenting to UNC Hospitals with borderline or positive Clinitest® hCG results (n=182) were calculated using retrospective analyses of estimated date of delivery (EDD), date of Clinitest hCG testing, and a gestation slide chart (Perrygraf) with assumed 40 weeks gestational age at EDD and 14 day relationship between gestational age and days relative to ovulation.

Results: Clinitest® hCG results per Clinitek Status readout for urine samples containing intact hCG or hCGβcf were as follows: positive for intact hCG at all concentrations (500-$2x10^6$ pmol/L) tested, positive between 500 to $5x10^4$ pmol/L hCGβcf, borderline at $5x10^5$ pmol/L hCGβcf, and negative at 0, $1x10^6$ and $2x10^6$ pmol/L hCGβcf. Positive Clinitest® hCG results were detected for combinations of intact hCG and hCGβcf in urine corresponding to concentrations detected in early pregnancy between 16 to 49 days relative to ovulation. The median estimated days relative to ovulation at the time of Clinitest® hCG testing among 182 pregnant females presenting at 1 institution was 38 days, with an inter-quartile range of 27.5 to 58.5 days.

Conclusion: The Clinitest® hCG device with Clinitek Status readout demonstrated no detectable hook effect by intact hCG. Hook effect at high concentrations of hCGβcf was observed though did not interfere with Clinitest® hCG detection of combined intact hCG and hCGβcf concentrations known to occur in early natural pregnancy. The majority of pregnant females presenting at 1 institution have Clinitest® hCG testing performed during early pregnancy and are unlikely to exhibit false-negative Clinitest® hCG results due to hook effect by hCGβcf.

B-298

Comparison of Six Point-of-Care Glucose Monitoring (POCGM) Devices in Diabetic and Hemodialysis Patients.

W. Tamimi[1], N. Alotaibi[2], A. Alsadhan[2], S. Aljasser[1], R. Dafterdar[3], A. Khan[2], A. Alajlan[1]. *[1]King Fahad National Guard Hospital, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia, [2]King Fahad National Guard Hospital, Riyadh, Saudi Arabia, [3]Medical Services Department, Ministry of Defense, Riyadh, Saudi Arabia*

Background: Many patients with diabetes control their blood sugar level on a daily basis with Point-of-Care Glucose Monitoring (POCGM) devices. Accurate readings are of high importance to successfully self-manage their diabetes. There is variety of POCGM home-use devices available to measure glucose for diabetic patients. Each has a different degree of accuracy and imprecision. Therefore, in this study, we have evaluated six glucose meters from different manufacturers for patient's home-use. Patients & Methods: A total of 80 blood samples were collected from venous blood obtained from 20 healthy adults, 40 diabetic and 20 hemodialysis patients during July 2012 in our hospital. For each hemodialysis patient two blood samples were collected before and after dialysis. Blood glucose level was measured in these samples by different six POCGM devices from different manufacturers (denoted as A, B, C, D, E & F) and was compared to the reference glucose- hexokinase method Architect 16000 (Abbott). Two manufacturers (A & B) utilize strip that used the enzyme PQQ-GDH (pyrroloquinolinequinone dependent glucose dehydrogenase). The test strips utilize flavin adenine dinucleotide (FAD) with glucose-dehydrogenase (GDH) enzyme by manufacturers C, D and E. The strip from manufacturer F utilizes glucose oxygenase enzyme. Results: When the ISO 15197 standards were applied, most of POCGM devices have shown a good agreement and accuracy with the reference glucose-hexokinase method in the range of 82-98% with the exception of one device (F) which has shown only 39% agreement. All of the six devices have shown an average negative bias with the reference glucose-hexokinase method in the range of -6.2% up to -24% (p-value <0.0001). Conclusion: The home-use POCGM devices produced comparable results in relation to the glucose-hexokinase reference method. Devices that use FAD-glucose-dehydrogenase method have shown better accuracy in these studied populations.

B-299

Evaluation of Glucose Meter Accuracy Using Locally-Smoothed Median Absolute Difference (LSMAD) Analysis

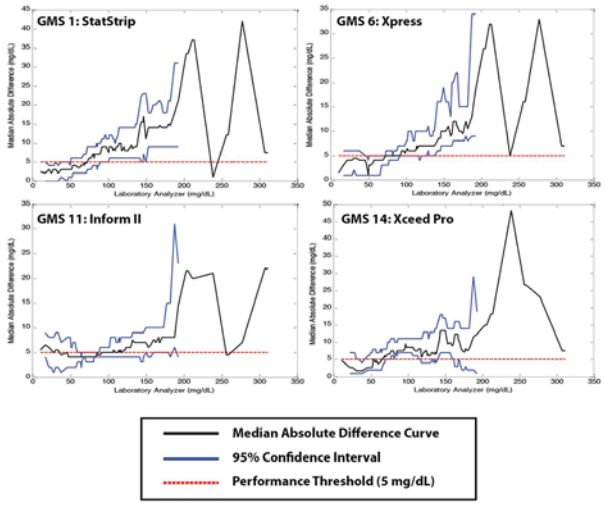
M. Howes, A. N. Steele, Z. Godwin, N. K. Tran. *UC Davis, Davis, CA*

Background: Glucose monitoring system (GMS) accuracy is crucial for the management of critically ill patients. Intensive insulin therapy (IIT) is used for tight glycemic control (TGC) and relies on accurate GMS measurements. Poor GMS performance results in inappropriate insulin dosing and increases risk for dangerous glycemic excursions. Performance assessment of GMSs is instrumental for determining appropriate devices for critical care TGC. We hypothesize that traditional measures of performance may be inadequate for evaluating current generation GMSs when compared to the new locally smoothed median absolute difference (LSMAD) method.

Methods: We evaluated the performance of the Nova Biomedical (Waltham, MA) StatStrip hospital (GMS 1-5) and Xpress meters (GMS 6-10), Roche Diagnostics (Indianapolis, IN) Inform II meter (GMS 11-13), and Abbott Laboratories (Abbott Park, IL) Precision Xceed Pro (GMS 14) against a plasma reference. We collected 202 unique remnant arterial blood gas samples for paired testing. Traditional parametric statistics including ANOVA, Bland-Altman, and least square linear regression (LSLR) analyses were compared to the non-parametric LSMAD method. A 5 mg/dL tolerance threshold served as a LSMAD performance benchmark.

Results: We found no statistically significant differences via ANOVA, Bland-Altman, or LSLR analyses. With LSMAD analysis (**Figure 1**), GMS 1-10 exceeded the 5 mg/dL tolerance threshold for mean (SD) values greater than 86.9 (17.67) mg/dL. GMS 11-13 exhibited breakout points at two mean points: 11.0 and 17.0 mg/dL. For GMS 14, we observed a breakout point of 54 mg/dL.

Conclusions: Accurate glucose monitoring is instrumental for appropriate IIT in critically ill patients. Current generation GMSs have improved performance. Traditional methods used to analyze device performance proved inadequate in identifying significant differences between GMSs. In contrast, LSMAD illustrated the median performance of GMS 11-14 to be inadequate at clinically significantly hypoglycemic ranges_suggesting that these devices may be inappropriate for critical care.



B-300

Evaluation of point-of-care (POC) glucose test performance of Patient Care Technicians (PCT) and Registered Nurses (RN)

S. N. Narla, J. Epps, K. Hermayer, P. Arnold, Y. Zhu. *Medical University of South Carolina, Charleston, SC*

Background: The glucose results attained by POC glucose meters are highly dependent on critical thinking and performance of the personnel performing the test. In the majority of clinical settings, either PCTs or RNs perform the POC glucose tests. The objective of this study is to evaluate the POC glucose test performance of PCTs and RNs by comparing the accuracy of their POC glucose results with the clinical chemistry (CC) lab analyzer results.

Method: Abbott's Precision XceedPro glucose meter based on the glucose dehydrogenase method was used for POC test. In the CC lab, glucose is analyzed on Beckman Coulter UniCel®DxC800 analyzers by an oxygen rate method employing a Beckman Coulter Oxygen electrode. Glucose results acquired with both POC glucose and CC lab analyzer with a difference in blood collection time of ≤5 mins were chosen and the data was collected retrospectively for 15 days, i.e., from 01.16.2014 to 01.30.2014. The average % variance between POC and CC lab tests was analyzed for the tests performed by PCTs and RNs. The POC and the CC lab test results were analyzed based on the personnel performing the POC tests by paired two-tailed t test.

Results: 402 tests were analyzed with glucose ranges of 35 mg/dL to 463 mg/dL in which 155 were performed by PCTs, 221 by RNs and 26 by other personnel. The average %variance of tests performed by PCTs compared to the CC lab was 8.40, and the results of POC and the CC lab were statistically significant (p=0.0121). Also, 51(33%), 47(30 %) and 57(37%) of these tests showed >10%, 5%-9.9% and <5 % variance respectively compared to the CC lab. For tests performed by RNs, the average % variance was 7.31, and the results by POC and the CC lab were not statistically significant (p=0.7910). Also, 52(23%), 66(30%) and 103(47%) of these tests showed >10%, 5%-9.9% and <5% variance respectively. According to CLSI, for glucose <100 mg/dL and >100 mg/dL, the difference from the POCT to CC lab test should not exceed 12 mg/dL and 12.5% respectively. 24% of tests performed by PCTs and 17% tests performed by RNs did not meet these criteria. Also in both the groups, there were 15 individuals with at least 2 tests showing >10% variance, out of which 12 were throughout the 15-day period and 5 with at least 2 tests >10% variance on the same day, there were 2 individuals who fell in both categories.

Conclusion: Overall performance by both RNs and PCTs is within the acceptable (±20%) allowable error. However, there is no statistical significance between POC and CC lab results for tests performed by RNs, whereas the tests performed by PCTs were statistically significant. Also, there are some individuals whose performance is not up to the required standards, therefore, it is important to identify these people and educate them about the potential sources of errors caused by the operators and critical thinking. It is also important to perform timely education and competency assessment on the personnel performing the POC testing.

B-301

An analytical evaluation of the Abbott i-STAT hCG test cartridge

A. M. Sowder[1], M. L. Yarbrough[2], R. Nerenz[2], R. Mortensen[3], A. M. Gronowski[2], D. G. Grenache[1]. *[1]University of Utah School of Medicine, Salt Lake City, UT, [2]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, [3]ARUP Laboratories, Salt Lake City, UT*

Background: The qualitative detection of human chorionic gonadotropin (hCG) in urine is commonly used as a rapid test to determine pregnancy status. Urine hCG tests are less analytically sensitive than quantitative serum hCG tests and are prone to false-negative results. Despite these drawbacks, urine hCG tests are often favored over serum hCG tests because they can be performed at the point-of-care. The ability to perform quantitative hCG testing in whole blood at the point-of-care would be advantageous. The i-STAT total hCG cartridge (Abbott Diagnostics, Abbott Park, IL) is a quantitative hCG test to be used with whole blood or plasma with an intended use for the detection of early pregnancy. The purpose of this study was to perform an analytical validation of the i-STAT hCG test.

Methods: hCG-free whole blood was obtained from volunteers. Residual serum and/or plasma samples sent to the laboratory for physician-ordered hCG tests were used as a source of hCG. Aliquots of the hCG-free whole blood were used to prepare samples with specific target hCG concentrations. Whole blood and plasma were used to evaluate the precision, linearity, analytical sensitivity, and accuracy of the i-STAT hCG test. Institutional Review Board approval was obtained for this study.

Results: Precision was determined from two samples analyzed in two replicates, twice per day for 10 days. Whole blood repeatability and within-laboratory CVs were 14.4 and 15.6% at 10.1 IU/L and 6.5 and 6.5% at 1176.6 IU/L, respectively. Plasma repeatability and within-laboratory CVs were 5.6 and 9.9% at 11.2 IU/L and 4.2 and 4.8% at 1273.7 IU/L, respectively. Linearity was evaluated from six samples prepared to span the claimed analytical measuring range of 5-2,000 IU/L. For whole blood, linear regression produced a slope of 0.99, a y-intercept of 6.1, and an r2 of 0.999. For plasma, linear regression produced a slope of 1.0, a y-intercept of 1.6, and an r2 of 0.999. Analytical sensitivity was determined from a set of five samples prepared to contain 0, 5, 10, 15, and 20 IU/L of hCG and each analyzed in 10 replicates. The limit-of-blank, defined as the mean+3 SD of the 0 IU/L sample, was 0 IU/L for whole blood and plasma. The limit-of-detection, defined as LOB+3 SD of the 5 IU/L sample, was 2.9 and 1.7 IU/L for whole blood and plasma, respectively. The limit-of-quantitation, defined as the hCG concentration that yielded a CV of ≤20% was 8.0 and <5 IU/L for whole blood and plasma, respectively. Accuracy was evaluated using 20 samples tested in one replicate on the i-STAT and compared to corresponding plasma hCG concentrations measured on the Architect Total β-hCG (Abbott Diagnostics) assay. For whole blood (i-STAT) vs. plasma (Architect), Deming regression produced a slope of 0.96, a y-intercept of -33, and r of 0.997. For plasma (i-STAT) vs. plasma (Architect), Deming regression produced a slope of 1.09, a y-intercept of -22.6, and r of 0.994.

Conclusions: The Abbott i-STAT total hCG cartridge demonstrates acceptable performance for quantifying hCG in whole blood or plasma.

B-303

Evaluation of Accu-Chek Inform II® performance with cobas c501® and Modular P800® Using Specimens from Patients in Emergency Department, Medical-Surgical and Intensive Care Units.

V. M. Genta[1], L. Wyer[2], M. Ferguson[1], S. S. Church[2], D. Graubner[3], Y. Shen[1]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Healthcare, Norfolk, VA, [3]Sentara Helthcare, Norfolk, VA*

Background: Glucose meters, offering a rapid evaluation of patient blood glucose values at the point-of-care, promote prompt medical intervention. The interchangeability of the glucose meter results with those obtained with the laboratory method is essential for their seamless interpretation. The performance of the glucose meter method may be affected by the blood matrix of patients treated in different hospital units. We report the results of a limited study comparing Accu-Chek Inform II with the laboratory method using blood specimens from patients treated in Medical-Surgical Unit (MSU), Intensive Care unit (ICU) and Emergency Department (ED).Methods: Patient specimens: 1230 (MSU 520, ICU 290, ED 420). Glucose meters: Accu-Chek Inform II (Roche Diagnostics), Strip lot #471353; Laboratory Methods: cobas c501 and Modular P800 (Roche Diagnostics).The patient specimens were collected by venipuncture in green-top tubes and assayed in parallel and within 30 minutes with both methods. For 324 patients the HCT value was available.The observations

were collected in Minitab® (Version 16, Minitab) statistical software and were analyzed with multivariable weighted least squares regression analysis (MWLSR), locally weighted scatterplot smoother (lowess), regression diagnostics and graphic representations.Results: The scatterplot of the glucose meters values (y axis) by the laboratory methods values (x axis) by the hospital unit, showed a linear relationship between methods. This was confirmed by the lowess. This plot showed increased variability for increasing glucose values; this prompted the use of a weighted least squares regression model. The absolute (for values 30-100 mg/dL) and relative (for values 101-600 mg/dL) difference plots showed that for the grand majority (99%) of specimens the differences were within the total error (CLIA's criterion target value +/- 6mg/dl, or +/-10%, greater).The MWLSR model (y=3+1.0x+2Unit+0.7HCT) showed that while there were no statistically significant differences between regression lines for HCT (P=0.42), there were statistically significant differences for Units (P<0.0001). The slope for ED (beta1=1.01) was statistically significantly different from those for MSU (beta1=0.95) and ICU (beta1=0.95). However, these differences were not clinically significant. The pure error test by data subsetting showed possible lack of linearity for high values.The lowess for the plot of the standardized deleted residuals by the fitted value showed a slight curvature for values >400mg/dL and 9 potential outliers (3<|value|<4).The leverage (Hi<0.1), Cook's distance (<0.05) and DIFTs(<|0.06|) did not show any influential observations. Conclusion: This study showed that there was a linear relationship between Accu-Chek Inform II method and the laboratory methods (cobas c501 and Modular P800). For the grand majority of the specimens, the absolute and relative differences were within the CLIA's criterion for total error. Consequently, these results suggest that the two methods can be used interchangeably with the laboratory methods for evaluating patient glucose blood levels. However, due to the limited number of specimens some matrix effects secondary to either disease or treatment may not have been manifest. Further studies should be performed to corroborate these findings.

B-304

Analytical validation of a blood glucose meter device in the emergency department of a university hospital

M. S. Feitosa, N. C. C. S. Nogueira, R. L. Furlan, L. S. Vasconcellos. *Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: The university hospital in Belo Horizonte is a 500 bed tertiary hospital. Despite federal regulation that demands the use of hospital equipments, the glucose meters (and its strips) in the emergency department are appropriate for domestic use only, and they are not monitored by laboratory staff. Furthermore, their analytical performance has not been validated. As part of a project of implementation of glucose meters suitable for hospital use, we conducted analytical validation of the Precision XCEED PRO (PXP®) – Abbott, quality control and calibration in the emergency department, which is reference for several clinical conditions in the public health system.

Methods: Imprecision, accuracy and linearity were evaluated as part of the validation plan. Within-run imprecision was first evaluated using two level QC solutions tested 10 times each. Between-run imprecision was conducted running each control four times for five days. Accuracy was performed comparing 20 results of PXP® with Vitros® 5600 in the core laboratory. A capillary sample was collected to perform glucose testing in PXP®. Immediately after, one fluoride tube and one arterial heparin tube were collected and sent to the core lab to be tested in Vitros® 5600. The experiment was conducted for five days, so at least five patients were tested each day. Linearity was performed using a five level linearity kit from *RNA Medical*. Each level was tested in quadruplicate.

Results: Within-run imprecision was 3.45% for control 1 (mean = 88.7mg/dL) and 3.26% for control 2 (mean=273.0 mg/dL). Between-run imprecision within 5 days were 6.47% (mean = 88.9mg/dL) and 4.35% (mean = 287.2mg/dL). All values were considered acceptable. Comparison between capillary samples and fluoride plasma were performed with correlation coefficient (r) = 0.99, slope = 1.087, intercept = -4.53 and 95% of samples with results in the acceptable range < 20% of total error, according to ISO 15197 specifications. Comparison between heparin samples and plasma fluoride were performed also with correlation coefficient (r) = 0.992, slope = 0.969, intercept = -4.53mg/dL and all samples with results in the acceptable range < 20% of total error. Linearity was evaluated ranging from 24mg/dL to 427 mg/dL with software QCM®3.0.

Conclusions: Precision EXCEED PRO (PXP®) is suitable for hospital environment. Validation experiment performed with this particular device showed that its performance meets the quality requirements established by the laboratory and the literature.

B-306

Evaluation of the Performance of a Commonly-Used Glucometer in a Tertiary Hospital in Nigeria.

C. P. Onyenekwu, E. U. Egbuagha, E. C. Azinge. *Department of Clinical Pathology, Lagos University Teaching Hospital, Surulere, Lagos, Nigeria*

Background:Point-of-care-testing (POCT) for glucose is the most common type of near patient testing performed at various sites within the Lagos University Teaching Hospital (LUTH). A previous study at our hospital among POCT operators found among others, a lack of awareness concerning evaluation of POCT devices and the use of quality control materials for POCT; absence of liaison with the central laboratory for the verification of suscpicious results, and absence of external quality assurance programs for POCT. This finding prompted us to evaluate the accuracy of the Accu-Chek® Active (Roche) glucometer, which is the glucometer in use at 90% of the sites performing POCT for glucose, including the emergency units and the diabetic clinic.

Methods:The study was approved by the Health Research and Ethics Committee of LUTH and was conducted over a four-week period. Glucose levels in capillary blood samples from 31 diabetics and 18 non-diabetics attending the Lagos University Teaching Hospital (LUTH) were measured with an Accu-Chek Active glucometer calibrated to plasma samples. All glucometer readings were conducted by the same individual, in order to reduce operator variability. Venous plasma was collected from the patients into fluoride oxalate vacutainers within five minutes of finger-prick tests. The central laboratory measured the plasma glucose on Siemens Dimension® Xpand® Plus analyser. The laboratory analysis was performed within one hour after sample collection. Precision studies were not conducted as control solutions for Accu-Chek Active are apparently not easily available in Nigeria. Accuracy was assessed with Pearson's correlation, % bias and Bland-Altman plot of the results from the two methods. The ISO 15197:2013 and the American Diabetes Association (ADA) requirements for glucose testing were employed in assessing quality. Data was extracted on Microsoft Excel® and analysed using Analyse-It® for Excel software version 2.30, Leeds, United Kingdom.

Results:The glucometer results were highly correlated with those of the central laboratory (r=0.99), bias ranged from 0.0 to 20.0%. Higher bias levels were observed with high glucose results. A Bland-Altman plot of the difference between each pair of results (glucometer and central laboratory), against the central laboratory result, showed increasing variability of glucometer results at high glucose levels. The glucometer met the ISO recommendation with 95.9% of the results having a bias <15%, however, it did not meet the ADA requirements of <5% bias for glucose testing.

Conclusion:The Accu-Chek Active glucometer had a high correlation with the central laboratory method but showed increased variability at high glucose levels. This may have implications for patient care particularly at the diabetic outpatient and emergency care units where insulin-dose adjustments are made according to glucometer readings, without verification of results from the central laboratory.

B-307

Utilization of a Superior Monoclonal Antibody Pair against Procalcitonin in Development of Fluorescence-based Lateral Flow Immunoassay

L. Q. Wang, S. He, Y. Z. Guo, D. F. Li, Y. Liu, J. Ge. *Abzymo Bioscience Ltd., Beijing, China*

Background: After the first report in 1993 on Procalcitonin (PCT) in patients with bacterial infection elevated significantly, the use of PCT in identifying the bacterial or non-bacterial origin of systemic inflammation has been gaining widespread support. Because the level of PCT in the blood stream of healthy individuals is below 0.5 ng/ml, we aimed to generate a set of specific monoclonal antibodies with higher affinity and develop a rapid and sensitive POCT test for use in the emergency rooms and clinical laboratories.

Methods: Balb/c mice (6-8 weeks) were immunized with recombinant PCT emulsfied with Freund's or Titermax adjuvant. Four times of intraveneous injections were given at 50μg per injection in 3 week intervals. ELISA were conducted for monitoring PCT-specific sera titer, four mice with the highest titer were selected for the cell fusion. Three days before the fusion, the last shot was done intraveneously. The splenocytes of the four mice were fused with the mouse myeloma cell line SP2/0 using PEG-1500. The microplate wells exhibiting hybridoma growth were screened for the production of anti-PCT antibodies with both direct and indirect methods. Positive hybridoma cultures with higher titer and specificity were selected and subcloned by three round of limited dilutions. All the 98 MAbs were purified by protein A-sepharose affinity chromatography and were evaluated by colloidal gold-based LFT. The LFT test containing 4763 kinds of combination in which each MAb was coated as the

capture antibody and the others were detected as the detecting antibody. After that, 18 matched MAb pairs were selected for further development of fluorescence-based lateral flow immunoassay (FLFIA) and the antibody pair (PCT79/PCT83) was shown to have the best sensitivity, specificity, stability and coincidence rate. Furthermore, the results from clinical samples tested by FLFIA with this antibody pair (PCT79/PCT83) were compared and evaluated with those of electrochemiluminescence immunoassay (ECLIA) method.

Results: Ninety-eight hybrid clones were screened eventually using the above-mentioned methods. After the evaluation in colloidal gold tests,18 matched MAb pairs were screened from 4763 combination. In FLFIA, the best antibody pair (PCT79/PCT83) was selected with the sensitivity of 0.1 ng/ml, detection range of 0.1~100 ng/ml; inter-assay CVs <15%. The results from clinical samples revealed that our strip using the antibody pair (PCT79/PCT83) correlates well with the ECLIA method (R=0.988).

Conclusion: We described here that we have generated a superior MAbs pair against PCT which is proved to have promising potential applications in development of FLFIA. They seem to be the ideal candidate antibodies suitable for development of a quantitative POCT.

B-308

Bioelectronic Platform for Sensitive and Versatile Diagnostic Applications*

D. Georganopoulou[1], A. Gaustad[1], E. Van Groll[1], R. Hoo[1], T. J. Meade[2], P. Bao[1]. *[1]Ohmx Corporation, Evanston, IL, [2]Northwestern University, Evanston, IL*

Background A new bioelectronic platform is described that is designed for multiple Point-of-Care (POC) diagnostic applications, including protein, DNA, and small molecule diagnostics. A self-assembled monolayer (SAM) technology is presented that demonstrates quantitative, ultra-sensitive, precise, and accurate measurement of a number of clinical analytes in biological samples (e.g. whole blood, urine, semen, prostatic fluid, saliva, etc). Cyclic voltammetry techniques measuring a ratiometric signal allow for a rapid, self-calibrating, fully quantitative dose response with broad dynamic range (over 4 logs of analyte concentration). This capability allows any applicable clinical assays to be executed on the Ohmx platform, using a minimal sample volume (1-50 uL), with performance levels similar to reference lab tests. The analytical performance of the sensor, and the clinical validation for multiple analytes (Hemoglobin A1c, hs Troponin I, TSH, hs CRP, DNA, and lactate) are discussed.

Methods Following standard solution bioassays (immunoassays, hybridization, or enzymatic reactions) an electrophore substrate specifically reacts with the self-assembled monolayers on the gold micro-electrodes. For all assays, a dose response spanning the analytes' relevant clinical range was obtained using commercially available calibrators. Further clinical validation is presented with 50 clinical samples tested for A1c, 72 samples tested for Troponin I and 50 samples for TSH. The clinical samples were provided by hospital collaborators and pre-tested with clinical immunoanalyzers. A correlation statistical study is shown between the Ohmx test and reference methods.

Results The Ohmx HbA1c is a single measurement test in 2 uL of whole blood, a TAT of 3 minutes with a linear response ranging from 2.7% (6.0 mmol/mol) to 19.8% (192.9 mmol/mol), an intraday precision of CV less than 3% and with R^2= 0.93 linear correlation with BioRad clinical immunoanalyzer. The Ohmx troponin I assay is a high sensitivity assay that has an LOD of 1 pg/mL with a TAT of 15 min, with CVs from 3-10% and a linear correlation with R^2= 0.95 with Siemens immunoanalyzers. TheTSH Ohmx test has an LOD of 0.0024 uIU/mL, a TAT of 15 min and R^2= 0.9 correlation with the Beckman Coulter DXI. SNP differentiation is shown for DNA factors II, IV, and MHTFR, and a lactate test is shown with a linear range from 0.2 to 28 mM.

Conclusions There is significant correlation between the Ohmx tests and clinical immunonalyzers. Analytical and clinical performance for all POC tests offered rivals performance of reference labs. The platform versatility is shown with tests that include proteins, DNA, and small molecules with a cartridge that has a COGS of $0.40.

*Assays currently under development and not for clinical use

B-309

Analytical and Diagnostic Characteristics of High-Sensitivity Troponin Assays: Examination of PATHFAST cTnI

E. Spanuth[1], B. Ivandic[2], R. Thomae[3], E. Giannitsis[4]. *[1]DIAneering GmbH, Heidelberg, Germany, [2]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [3]Mitsubishi Chemical Europe, Düsseldorf, Germany, [4]Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany*

Background The analytical characteristics of high-sensitivity cardiac troponin (cTn) assays comprise an imprecision (CV) at the 99th percentile value ≤ 10% and measurable concentrations above the limit of detection (LoD) and below the 99th percentile in at least 50% of healthy individuals. The PATHFAST cTnI assay (Mitsubishi Medience Corporation, Japan) has already shown promising analytical validity and is usable for point-of-care testing. We thought to evaluate it`s analytical and diagnostic characteristics and to examine whether the assay could be classified as "high-sensitive".

Methods To establish the analytical criteria cTnI was determined using PATHFAST in 120 healthy individuals (60 men and 59 women, 21-69 years old, median 42 years) in whom cardiac disorders were excluded by extensive evaluation including cardiac magnetic resonance imaging and NT-proBNP < 125 ng/L. The diagnostic characteristics were investigated by comparison of cTnI and cTnT (Roche`s high-sensitivity assay cobas® hs-cTnT) in 181 patients admitted to the chest pain unit at presentation, 3 and 6 hours later. The results were related to the discharge diagnoses.

Results The cTnI concentrations measured in the healthy individuals ranged from 0.4 to 17.2, mean 2.1 (95% CI:1.6-2.6) ng/L, without age dependency. Men revealed higher levels than women, means (IQR) were 2.8 (1.2-2.6) and 1.1 (0.7-1.3) ng/L. The CLSI nonparametric method revealed a 99th percentile value of 16 ng/L below the manufacturer recommended value of 20 ng/L. The quantification of cTnI above the LoD (1.0 ng/L) and below the 99th percentile was possible in 79 of 120 individuals. The imprecision profile according to NCCLS revealed 20%, 10% and 5% CVs at cTnI concentrations of 2, 3 and at 20 ng/L, respectively. In the patient group the discharge diagnosis was non-ST-segment elevated myocardial infarction (NSTEMI) in 72 patients. The cTnI median values at 0, 3 and 6 hours were 46, 166 and 399 ng/L, respectively. To evaluate the diagnostic validity for detection of NSTEMI the results of PATHFAST cTnI and cobas hs-cTnT were compared by ROC analysis. AUC values at 0, 3 and 6 hours were 0.919, 0.962 and 0.958 for cTnI and 0.923, 0.964 and 0.969 for hs-cTnT, respectively. cTnI revealed AUC values for absolute changes from admission to 3 hours and from admission to 6 hours of 0.920 and 0.931 in NSTEMI patients.

Conclusions PATHFAST cTnI demonstrated complete fulfillment of the analytical criteria for high-sensitive cTn assays: The imprecision (CV) at the manufacturer recommended 99th percentile value was 5%. Quantification of cTnI was possible in 65.8% of healthy individuals. The examination of the diagnostic characteristics revealed complete concordance with cobas hs-cTnT detection of NSTEMI as well as for assessment of absolute changes of cTn values (rise and/or fall) during the course over time in NSTEMI patients. PATHFAST cTnI showed highly sensitive detection of NSTEMI with increasing sensitivity at admission and after 3 to 6 hours, not going along with decreased specificity. The PATHFAST cTnI assay allows high-sensitivity determination of cTnI within 16 min from whole blood samples and might be useful at the point-of-care setting for early rule- in and rule-out diagnosis of NSTEMI.

B-311

Evaluation of a point-of-care HbA1C method in an underserved community clinic and measurement of the impact of implementing a high-quality assay with immediate results.

A. V. Jean-Noel[1], B. Williams[1], M. M. Perez[2], M. Spinola[2], F. Azzara[2], H. Bidwell[2], G. L. Horowitz[1], N. V. Tolan[1]. *[1]Beth Israel Deaconess Medical Center and Harvard Medical School, Department of Pathology and Laboratory Medicine, Boston, MA, [2]Bowdoin Street Health Center, Beth Israel Deaconess Medical Center, Boston, MA*

Background: Measurement of hemoglobin A1c (HbA1C) assesses glycemic control and provides an estimate of the average glucose concentration (eAG) over the previous 2-3 months. The American Diabetes Association recommends that %HbA1C be measured twice annually for diabetic patients meeting glycemic goals, in an effort to reduce the risk of microvascular disease. In this study, we evaluated a NGSP-certified CLIA-waived point of care (POC) method for HbA1C by finger-stick and measured the impact on workflow and compliance with pay-for-performance goals in an underserved community clinic.

Methods: The boronate affinity Afinion AS100 HbA1C POC assay (Alere Analytics) was evaluated for performance by the clinic nursing staff, in addition to the laboratory technologists. Precision studies, across various cartridge and QC lots, included the calculation of intra- (n=10) and inter-assay (n=30, 150 days) coefficients of variation (CV) for two levels of manufacturer QC (6% and 8% HbA1C). Method correlation against the Cobas Integra 800 Tina-quant HbA1C immunoassay method (Roche Diagnostics) was assessed through linear regression, mean bias, Bland-Altman plots and evaluation of clinical concordance. This included EDTA samples (n=27) and concurrent venipuncture/capillary samples (n=20). EDTA samples collected from patients with HbC and HbS were also included. Compliance with our institution's pay-for-performance goal of measuring HbA1C twice annually (6±3mo) for diabetic patients, was compared before and after implementing POCT.

Results: The intra- and inter-assay precision was <2% and ≤3% CV, respectively. For HbA1C ranging from 3.9-14.9%, the method correlation for EDTA samples (y=0.94x+0.37, r2=0.99) and paired venipuncture/capillary samples (y=0.93x+0.28, r2=0.97) showed excellent agreement. The mean (±SD) of bias of %HbA1C was -0.1%(±0.3) and -0.2%(±0.2) for EDTA and paired venipuncture/capillary samples, respectively. There was no significant bias observed for samples with the hemoglobin variants included. Overall, clinical concordance was 91%(43/47) for %HbA1C within the ranges of 10%(5/6), where the absolute bias was less than ±0.5%. Representing 32% of all HbA1Cs ordered at this clinic, 189 tests were performed by POCT in the first two months, allowing for real-time therapy assessment and feedback to diabetic patients. Of these samples, 146(77%) were collected without any additional laboratory testing ordered for the patient that would have required venipuncture. Perhaps more important, this means 43(23%) patients, who had venipuncture for other laboratory testing, also had POCT performed for the sole benefit of the real-time assessment. Introduction of POCT was associated with an initial 6% increase in the clinic's compliance with a pay-for-performance goal of serial testing performed within nine months. In addition, POCT was provided for 57(32%) patients whose prior HbA1C determination exceeded these performance goals.

Conclusion: Accurate representation of long-term method performance is possible when the evaluation is conducted by the end-user. Compared to the immunoassay method, the Afinion HbA1C POC method demonstrated excellent precision, accuracy, and clinical concordance. With on-going cross-checks, this study demonstrates the potential for these methods to be used interchangeably; including eAG calculation, screening for, and diagnosis of diabetes. Replacing venipuncture collection with POCT was shown to initially, increase compliance with pay-for-performance goals.

B-312

A Whole Blood POC Enzymatic Creatinine Assay that Meets the eGFR Reporting Requirements by NKDEP

C. Xu, M. Curley, L. Boone, H. Chuang, O. Beeks, H. Visnick. *Instrumentation Laboratory, Bedford, MA*

BACKGROUND: Estimated Glomerular Filtration Rate (eGFR) from serum creatinine is considered a better assessment of renal function than serum creatinine alone. To assure the reported eGFR is within clinically acceptable error limits, the NKDEP Laboratory Working Group has recommended the total error (TE) limits for creatinine measurement in the critical creatinine range (1-1.5 mg/dL). A rapid whole blood creatinine assay with eGFR in the point of care test (POCT) environment is gaining attention. This publication assessed the TE of our biosensor based whole blood creatinine assay per NKDEP recommendations for eGFR capability.

METHODS: Performance of the creatinine assay was assessed based on the NKDEP Whole Blood Protocol, and was tested on six GEM Premier analyzers after appropriate modifications to accommodate the creatinine sensors. Heparinized whole blood samples with creatinine concentrations from 1.0-1.5 mg/dL were collected. The hematocrit levels were adjusted to form four sample test groups: plasma, low Hct (20-30%), normal Hct (no adjustment), and high Hct (50-60%). Whole blood samples were assayed in random order with 10 replicates per sample type per cartridge, followed by plasma samples (N=10). Three level IDMS traceable reference serum pools were assayed before and after the test samples. The samples were tested in parallel on an ABL 800 Flex analyzer (Radiometer) as reference.

RESULTS: The sample results were normalized to the IDMS traceable reference serum. The mean creatinine was 0.993 mg/dL, the 3.5% bias (95% CI: 3.03%, 4.01%) vs. ABL, and the 3.5% imprecision (95% CI: 2.8%, 4.2%) were both within the 5% and 8% limits of NKDEP recommendations. The bias and within-run precision per cartridge were plotted against the acceptable TE budget for reporting eGFR (Fig.1).

CONCLUSIONS: The biosensor based creatinine assay under development demonstrated analytical performance in whole blood with a TE meeting the NKDEP recommendations for reporting eGFR.

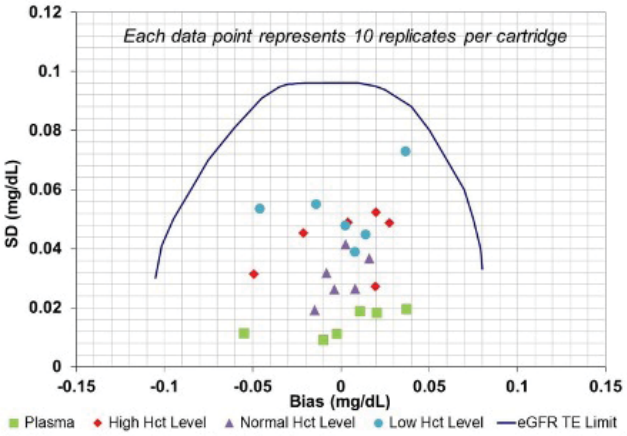


Fig. 1 Bias and imprecision of whole blood Creatinine assay recovery at normal Cratinine level

Wednesday, July 30, 2014

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

Cardiac Markers

B-314

One year retrospective cTroponin T observations : Women present themselves at a higher age with ACS.

J. P. van Straalen, J. C. Fischer, M. M. Vis, A. Sturk, R. J. de Winter. *Academic Medical Center, Amsterdam, Netherlands*

Background: Generally presumed women present themselves with Acute Coronary Syndrome (ACS) at higher age than men.

Method: We studied the use of cardiac Troponin T (cTnT) in our patient population with respect to results, age and gender. cTnT was measured with hs cTnT assay (Roche Diagnostics) using a limit of detection of 0.005 µg/l and an upper limit of reference value of >0.015 ug/L. Data on cTnt results from a serially sampled patient population (results, gender and age) were extracted from the laboratory information system over a one year period for those patients with cardiologists involved in their medical treatment. In this patient group only the result of the first blood drawing was incorporated in the dataset investigated.

Results: 1665 patients (703 females (42%), 962 males (58%)) were included. 696 of these patients had a cTnT< 0.015 µg/l and 969 patients a cTnT >0.015 ug/L.

Table1

Conclusion: Our data indicate that women do present themselves at higher age with respect to men with increased cTnT level as indicator of ACS.

cTroponin T >0.015 µg/l				Age (years)	
	N-patients	Mean	25 th percentile	50 th percentile	75 th percentile
Female	364	74	67	76	83
Male	605	67	57	67	79

B-315

Association of Lipid, Inflammatory, Cardiac, and Renal Biomarkers with C-Reactive Protein in Cardiovascular Risk Categorization - A Factor Analysis Approach

S. Jovicic[1], S. Ignjatovic[1], R. Kangrga[2], M. Dajak[2], N. Majkic-Singh[1]. *[1]Center for Medical Biochemistry, Clinical Center of Serbia, and Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, [2]Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia*

Background. C-reactive protein (CRP) strongly and independently predicts cardiovascular complications, and its use is recommended for risk assessment in primary prevention by several institutions. Also, there is evidence of other factors, contributing to and maintaining the intensity of atherosclerotic processes, which might identify cardiovascular risk contribution not originated from traditional risk factors. The aim of this study was to examine, using factor analysis, the nature of influence of biomarkers of inflammation, lipid metabolism, renal, and cardiac function on cardiovascular risk, and their possible connection and relations to CRP values.

Methods. Principal component analysis was used to investigate clustering of inflammatory markers [serum amyloid A (SAA), fibrinogen, α1-acid glycoprotein (A1AGP), haptoglobin, C3 and C4 complement components], lipid metabolism [total, HDL, non-HDL and LDL cholesterol, triglycerides, apolipoprotein A-I (apo A-I), apolipoprotein B (apo B), lipoprotein (a) (Lp(a))], renal [creatinine, cystatin C (Cys-C), estimated glomerular filtration rate (eGFR)], cardiac function [N-terminal pro-natriuretic peptide type B (NT-proBNP), high sensitivity cardiac troponin T (hs-cTnT)], and traditional cardiovascular risk factors [age, gender, body mass index (BMI), systolic blood pressure (SBP)], obtained from 242 apparently healthy individuals.

Results. Factor analysis identified five clusters, i.e. principal components (factors), which explained 65.3% of the total variance (29.0% factor 1, 13.2% factor 2, 9.0% factor 3, 8.5% factor 4, and 5.6% factor 5). Based on factor loading of ≥ 0.5 clasters were interpreted as 1) „systemic inflammation“ (fibrinogen, SAA, A1AGP, haptoglobin, C3 and C4 complement components); 2) „cardiorenal factor“ (creatinine, uric acid, Cys-C, hs-cTnT and gender); 3) „atherogenic cholesterol“ (LDL and non-HDL cholesterol); 4) „hemodynamic factor“ (age and NT-proBNP); and 5) „metabolic factor“ (triglycerides and HDL cholesterol). The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.75. In multiple regression analysis, five factor model had the best predictive value for CRP concentrations >1 mg/L (OR 6.53, 95% CI 4.06-10.50, P<0.0001 for “systemic inflammation”; OR 1.44, 95% CI 1.04-2.00, P=0.028 for „cardiorenal factor“; OR 1.76, 95% CI 1.23-2.5, P=0.002 for „atherogenic cholesterol”; OR 1.91, 95% CI 1.33 - 2.73, P<0.0001 for „hemodynamic factor“;OR 1.90, 95% CI 1.33 - 2.73, P<0.0001 for „metabolic factor“), while „cardiorenal factor“ and „atherogenic cholesterol“ completely lost their significance (P>0.05) for predicting CRP concentrations >2 mg/L and >3 mg/L. The ability of the factor-based logistic regression model was compared with multivariable logistic model containing all 25 variables in predicting the presence of CRP concentrations >1 mg/L, >2 mg/L, and >3 mg/L. The area under the receiver operator characteristics curve (AUC) of the five factor model was 0.889 and was not statistically significantly different from the 25 variable model (AUC=0.922) (P=0.2113). However, the differences between the two models examined were statistically significant in predicting the values of CRP>2 mg/L and CRP>3 mg/L.

Conclusion. Systemic inflammation, cardiorenal function, atherogenic lipid profile, hemodynamic and metabolic status might independently contribute to the pathophysiology of chronic, subclinical inflammation in atherosclerosis. They might represent underlying dimensions accompanying the elevation of CRP concentration and increased cardiovascular risk.

B-316

Verifying A Cut-Off Value for the Beckman TnI+3 Assay on the DxI 800 and Access-2 Analyzers.

V. PATEL, J. Qiu, J. Wang, A. Khan, J. Ekpe, M. Sydlowska, B. Goldsmith. *TEMPLE UNIVERSITY HOSPITAL, PHILADELPHIA, PA*

Background: Cardiac troponin (cTn) assays have been available in clinical laboratories for nearly two decades and considered a highly sensitive marker for myocardial damage. An elevation of cTn is used, together with other diagnostic criteria, to rule in/out a myocardial infarction (MI). Laboratories measure either cTnI or cTnT isoforms of troponin. Following a recall of cTnI reagents from the DxI Immunoassay analyzer in October 2010, Beckman Coulter recently re-introduced a Troponin-I (AccuTnI +3) assay for the DxI 800 and Access-2 analyzers. We evaluated whether the stated cutoff determined by the manufacturer was appropriate for our patient population.

Design: We measured plasma cTnI concentrations in 94 patients presenting to our Emergency Department (ED) in whom no history or evidence of cardiac disease was found following a review of each patient’s chart. Our population consisted of 30 Males (ages 16-63 years) and 64 females (ages 5-54 years). Specimens were spun, aliquoted, frozen within 24 hours at -20o C, and analyzed within 30 days of collection. Where a history could not be determined results were not included. cTnI was measured using the AccuTnI+3 assay (Beckman Coulter, Brea, California) on both the DxI 800 and Access-2 Immunoassay Systems. EP Evaluator (Data Innovations, Burlington, VT) and Excel spreadsheet calculations were used to evaluate the data.

Results: cTnI results for the DxI 800 showed (ng/mL): Males range=0.00-0.76, mean 0.03; Females range=0.00-0.36, mean 0.02. For the Access-2: Males range=0.00-0.86, mean 0.04; Females 0.00-0.50, mean 0.02. We determined the cutoff for plasma specimens assayed on the DxI 800 of 0.03 ng/mL and for the Access-2 of 0.04 ng/mL. The ranges (males and females) demonstrate that there were results on non-cardiac patients above the cutoff.

Conclusions: The 99th percentile at a coefficient of variation (CV) of <10% is used to define the cutoff concentration between a non-MI and a MI event. Though the manufacturer determined a cutoff of 0.2 ng/mL, 0.04 ng/mL was selected to provide one consistent cutoff for our hospital. We suggest using non-cardiac specimens that reflect the population to determine an appropriate cutoff. Caution must be used to ensure, as best as possible, exclusion of patients with a history or presenting symptoms of cardiac disease.

B-317

Short Term Variation Important In Evaluating Goodness of Troponin Assays For Diagnosing Myocardial Damage

G. S. Cembrowski[1], A. R. Cembrowski[2], A. N. Kunst[1], D. T. Holmes[3], F. S. Apple[4]. *[1]Alberta Health Services, Edmonton, AB, Canada, [2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada, [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [4]Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, Minneapolis, MN*

Background: While total imprecision is usually used to evaluate the quality of troponin assays, most diagnoses of myocardial necrosis are based on serial troponin changes within 8 to 12 hours. We have used a novel method to isolate the short term variation in sequential, intra-patient results, a mixture of intra-individual biologic variation (sb) and co-existing analytic variation (sa). We have used this method to derive the short term variation of two troponin assays, the Ortho VITROS Troponin I ES and the Beckman Coulter AccuTnI.

Methods: Two different data sets were extracted: all of the patient troponins that were measured by the main Beckman DxI analyzer at University of Alberta Hospital over a 20 month period and serial VITROS troponins of 1271 patients in whom the diagnosis of myocardial infarction (MI) was being considered (measured on one of two VITROS systems over 6 months at Hennepin County Medical Center). For both clinical environments, patient bloods were collected into lithium heparin. Two subsets of patient results were studied and compared, those patients whose serial troponins were all less than 41 ng/L and patients with serial troponins all less than 61 ng/L. We tabulated the pairs of intra-patient troponins that were separated by 2 to 3h, 3 to 4h, 4 to 5h, 5 to 6h, 6 to 7h, 8 to 9h, and 9 to 10h. The standard deviations of duplicates (SDD) of the paired troponins were calculated for each time interval. The graphs of SDD vs. time interval were approximately linear; the y intercept (y0) provided by the weighted linear regression equation represents the sum of sa and sb with y0 = (sa2 +sb2)1/2.

Results: For patients with series of troponins under 41 ng/L (average of 14 and 13 ng/L for Beckman and Ortho, respectively), the average short term variation (including biologic variation) was 3.0 ng/L (SEM=0.8 ng/L) for the Beckman and 1.1 ng/L (SEM=0.3 ng/L) for the Ortho. For patients with series of troponins under 61 ng/L (average of 18 and 15 ng/L for Beckman and Ortho), the short term variation was 3.0 ng/L (SEM=1.3 ng/L) for the Beckman and 2.2 ng/L (SEM=0.6 ng/L) for the Ortho.

Conclusion: Perhaps not surprisingly, the total short term variation (including biologic variation) is approximately equal to the short term variation derived from running low level quality control materials. The biologic variation of troponins for patients with healthy myocardiums is thus very low. For the Beckman DxI, the short term variation (3 ng/L) at a level of 25 or 35 ng/L is considerable. If +/-3SD limits are used (99.7% confidence limits), then variation around these levels is +/- 9 ng/L, variation that can cause both false positive and false negative interpretations. For the Ortho VITROS, there will be fewer misinterpretations. We recommend that serial patient troponin values be collected and "mined" for total variation. The analytical systems that minimize the short term total variation are most useful diagnostically.

B-318

Paraoxonase-1 enzyme activity assay for clinical samples: Validation and correlation studies

M. Garelnabi[1], A. Younis[2]. *[1]University of Massachusetts Lowell, Lowell, MA, [2]Department of Obstetrics and Gynecology, Mercer University School of Medicine, & Central Georgia Fertility Institute, Macon, GA*

Introduction: Paraoxonase-1 (PON1) enzyme is reported in various types of tissues and linked to numerous pathophysiological disorders; representing a potential biomarker in many pathological conditions such as cardiovascular diseases. Methods: We conducted several small studies to evaluate PON1 performances affected by sample types, storage, and interferences. In addition; we carried out some limited studies to compare the performance of the clinical research widely used PON1 assay to similar commercially available PON1 kit assay method. Results: Type of anticoagulant studies have shown that samples collected in NaF, citrate, ETDA clot activator, and sodium heparin have increased PON1 levels 49%, 24.5% and 19.8%; 11.4% and 8% respectively compared to the serum. Whereas samples in lithium heparin have 10.4% decreases in its PON1 levels compared to the serum. Biological interference such as hemolysis has little effect on PON1 levels; however samples spiked with lipids have shown 13% reduction on PON 1 levels. The method comparison studies between the PON1 method commonly available for PON1 assay and a similar non-ELISA commercially available PON1 kit method have resulted in a weak Spearman correlation displaying a coefficient $R^2 = 0.40$ for the range 104.9- 245.6 U/L. Conclusion: The current study will bridge some of the gaps on our understanding to the enzyme performances. The outcome should encourage additional studies in clinical setting to investigate other missing aspects of the factors known to affect PON1 enzyme function and performance.

B-319

Assessing the incremental value of additional biomarkers versus a high-sensitivity cardiac troponin I assay for predicting a short-term serious cardiac outcome in an early chest pain population

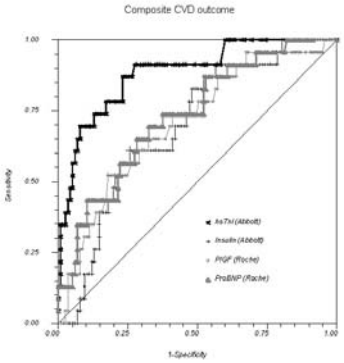
C. Shortt[1], G. Pond[1], K. Phan[2], K. Sohn[3], A. Worster[1], S. Hill[1], P. A. Kavsak[1]. *[1]McMaster University, Hamilton, ON, Canada, [2]McGill University, Montreal, QC, Canada, [3]University of Toronto, Toronto, ON, Canada*

BACKGROUND: Patients presenting with chest-pain to the emergency department (ED) who are at risk for a cardiovascular event in the short-term, must be identified. With the advent of high-sensitivity cardiac troponin (hsTn) assays, most ED patients will have measurable cardiac troponin concentrations. It is unclear whether additional biomarkers of myocardial stress, inflammation, vascular or other endocrine functions can improve prognostic ability when compared to hsTnI alone.

METHODS: After ethics approval, the presentation EDTA plasma sample (stored -80^{0}C) from patients (onset of chest-pain within 6h) in the RING study (Clin Chem Lab Med 2011;49:1915–8) were measured for these analytes (platforms):hsTnI, insulin, myeloperoxidase, 25-OH vitaminD (Abbott:ARCHITECT); IL-6 (Beckman:Access); PlGF, sFlt-1, proBNP (Roche:Elecsys); EGF, NTproBNP, E-selectin, P-selectin, sICAM-3, Thrombomodulin, IL-6, IL-10, MCP-1, bFGF, PlGF, sFlt-1, VEGF (MSD: Multi-Array:); IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNgamma, TNFalpha, IL-1a, IL-1b, MCP-1, EGF (Randox:Evidence). Using MedCalc, Statsdirect, and R program, we performed ROC curve analyses to select the top three biomarkers with the highest AUC for the composite outcome (percutaneous intervention, coronary artery bypass surgery, significant arrhythmia, refractory ischemic pain, heart failure, myocardial infarction, stroke, cardiac arrest, or death) at 72h and combined these three biomarkers with hsTnI to assess if inclusion of these biomarkers increased the AUC.

RESULTS: Of 140 patients (median age (interquartile range)=60y (49-68); 65% male), 23 had a composite outcome. Abbott hs-cTnI by itself had an AUC of 0.88 (95%CI:0.82-0.93) for prediction of the composite outcome. The top three biomarkers were Roche proBNP (AUC=0.73; 95%CI:0.65-0.81); Roche PlGF (AUC= 0.71; 95%CI:0.63-0.78); and Abbott insulin assay (AUC=0.70; 95%CI:0.61-0.77). The addition of insulin, PlGF and proBNP were not significant (p-values of 0.94, 0.36 and 0.12 respectively) and lowered the AUC (0.86) for the Abbott hsTnI assay.

CONCLUSIONS: The Abbott hsTnI assay alone, provides superior short-term prognostic utility in patients presenting early after chest-pain.



B-320

A high sensitive Homocysteine (hsHCY) assay improves this predictive biomarker's clinical value

G. Liang[1], A. Jaffe[1], J. Larkin[2], R. Jaffe[1]. *[1]Health Studies Collegium, Ashburn, VA, [2]ELISA/ACT™ Biotechnologies, Sterling, VA*

Plasma Homocysteine (HCY) levels predict accelerated development of heart and blood vessel disease and HCY is one of the few tests that predict all cause morbidity

and mortality. A significant practical limitation to HCY testing has been the leakage into plasma of red cell HCY starting within minutes after phlebotomy severely limiting community clinical use of HCY assays. A more stable specimen with reduced variability is needed, especially at lower, more predictive levels. Strategic, clinical, and lab results for an hsHCY assay that addresses this need is reported here.

Specimens (N=30) were collected in tubes containing EDTA (current preferred specimen) and a novel cell preservative EAB. Specimens were stored at 4±1°C until analysis. Plasma was isolated and analyzed at 0.5, 4, 8, 24, 48, 72, and 96 hours after phlebotomy using a Diazyme™ Analyzer 700. The data of HCY values were statistically analyzed by one-way ANOVA.

HCY levels started to increase within 30 minutes in EDTA specimens while the EAB cell preservative sample values were stable for at least 48 hours. Indeed initial HCY levels in EDTA samples averaged 32% higher than ones in the EAB preservative ($p<0.0001$). The initial HCY variance in EDTA is 10.23 ± 2.23, while the variance in EAB is 7.63 ± 1.60. This suggests that HCY is released into plasma from red blood cells in EDTA samples starting minutes after phlebotomy and does not occur in EAB cell preserved specimens over 48 hours.

Initial and more precise HCY can be measured and maintained for at least 48 hours when the EAB cell preservative is used. HCY increase appears to starts within minutes after blood draw in EDTA but not EAB preservative. The EAB cell preservative allows a more stable and accurate HCY assay for this important predictive biomarker. More studies of high sensitivity HCY (hsHomocysteine) are needed to further validate this predictive biomarker clinically and globally.

B-321

Development of a New Latex-Enhanced Immunoturbidimetric Assay for the Determination of Type IIA Secretory Phospholipase A2 (sPLA2), a Biomarker of Increased Cardiovascular Risk

G. Shanbhag, P. Lowry, M. Benchikh, A. Chacko, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Secretory phospholipase A2 (sPLA2) enzymes are biomarkers of increased cardiovascular risk and are targets of emerging therapeutic agents. They are associated with incident coronary artherosclerosis in healthy men and women and with recurrent adverse cardiovascular events in patients with acute coronary syndromes. sPLA2-IIA is a member of the phospholipase A2 family and is a potent biomarker for cardiovascular risk assessment. It is widely expressed in hepatocytes, macrophages, platelets and vascular smooth muscle cells and is up regulated in response to pro-inflammatory compounds such as interleukin-1β, interleukin-6, tumor necrosis factor-α, interferon-γ and oxidized low-density lipoprotein (LDL). The objective of this study was to develop a new quantitative, latex-enhanced immunoturbidimetric immunoassay to determine levels of sPLA2-IIA in human serum / plasma which have prognostic value in patients with Coronary Heart Disease (CHD) and can be employed to assess risk of future cardiovascular events. The assay is applicable to a variety of automated, clinical analyser systems, which ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

Methods: The assay is a latex-enhanced immunoturbidimetric assay based on the principle of measuring changes in scattered light. The latex particles are coated with anti-sPLA2-IIA antibodies, which in the presence of sPLA2-IIA rapidly agglutinate. When a sample containing sPLA2-IIA is introduced, the agglutination reaction is initiated and the change in scattered light is measured as a change in absorbance that is directly proportional to the concentration of sPLA2-IIA in the sample. The assay is applicable to a variety of automated systems. A correlation study was conducted using a commercially available enzyme immunoassay. Results:The assay presented a limit of detection of ≤15 ng/mL sPLA2-IIA (analytical range: 0 to 500 ng/mL sPLA2-IIA). The within-run precision and the total precision expressed as %CV, were typically <5% and <8% respectively. In the correlation study, citrate plasma samples, spanning the analytical range, were tested with this immunoturbidimetric assay and a commercially available enzyme immunoassay. Linear regression on the resulting data generated an r-value >0.9.

Conclusion: The data generated indicates that this latex-enhanced immunoturbidimetric assay is applicable to the detection of sPLA2-IIA in human plasma and serum. The assay is of value as a new analytical tool for assessment of cardiovascular risk in clinical settings. Its applicability to a variety of automated clinical analysers ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

B-324

Comparison of hs-cTnT and a conventional cTnI assay for the detection of ischemia induced myocardial injury and type 4 myocardial infarction post PCI.

V. Van Hoof, A. Vorlat, D. Coenen, R. Hammami, S. van Kerckhoven, J. Bosmans, S. Haine, T. Vandendriessche, C. Vrints, M. Claeys. *University Hospital Antwerp, Edegem, Belgium*

Background: comparison of a high sensitive troponin T (hs-cTnT) assay with a conventional troponin I (cTnI) assay, for the follow-up of patients post-percutaneous coronary intervention (PCI), more particular for the detection of ischemia induced myocardial injury (IIMI) and type 4 myocardial infarction (type 4 MI).

Methods: PCI and stenting was performed in 103 stable cardiac patients with significant coronary artery stenosis. hs-cTnT (Modular, Roche) and conventional cTnI (Dimension Vista, Siemens) were measured at 4 time points (0, 90, 180 and 360 min post-PCI). IIMI during stenting was defined as at least one cTn value > upper reference limit (URL = 99th percentile of the reference population) and an absolute rise of >50% of the 99th pctl URL in the 360 min blood sample. Type 4 PCI MI was defined in the 360 min blood sample according to the Third Universal Definition of Myocardial Infarction: elevation of cTn values (>5 x 99th pctl URL) in patients with normal baseline values (=<99th pctl URL) or a rise of cTn values >20% if the baseline values were elevated and were stable or falling (Thygesen et al Circulation 2012;126:2020-2035).

Results: both assays correlated fairly well (r= 0,9081, 95%CI 0,8898 to 0,9235). IIMI during stenting was detected in 68 patients (66%) with the hsTnT assay and in 69 patients (67%) with the TnI assay. The number of patients who answered the criteria for IIMI at 90 min and at 180 min was significantly less (18.2% for TnI and 20.9% for hsTnT at 90 min; 27.3% for TnI and 38.2% for hsTnT at 180 min). A frequency table for IIMI showed agreement between both methods for 31 patients without IIMI and for 65 patients with IIMI (93% concordance). Discrepancies were found for 7 patients (3 positive for hsTnT but negative for TnI and 4 positive for TnI but negative for hsTnT). Chi square test and Fisher's exact test were very significant ($p<0.0001$). The contingency coefficient between both methods was 0.637. Type 4 MI was detected in 33 patients (32%) with the hsTnT assay and in 31 patients (30.1%) with the TnI assay. A frequency table for type 4 MI showed agreement between both methods for 61 patients without type 4 MI and for 22 patients with type 4 MI (81% concordance). Discrepancies were found for 20 patients (11 positive for hsTnT but negative for TnI and 9 positive for TnI but negative for hsTnT). Chi square test and Fisher's exact test were very significant ($p<0.0001$). The contingency coefficient between both methods was 0.465.

Conclusion: myocardial injury caused by a short period of myocardial ischemia during PCI was detected with comparable sensitivity by the hsTnT assay and by the conventional TnI assay. For both assays, values at 90 min and at 180 min underestimated the presence of IIMI as established at the 360 min time point. There were more discrepancies between both assays for the detection of type 4 MI post PCI.

B-325

Quality Assessment and Reagent Lot-to-Lot Consistency in High-Sensitivity and Contemporary Troponin T Assays

A. K. Saenger[1], U. Klause[1], A. Ziegler[2], S. Menassanch-Volker[2], K. Hallermayer[3]. *[1]Roche Diagnostics, Indianapolis, IN, [2]Roche Diagnostics, Rotkreuz, Switzerland, [3]Roche Diagnostics, Penzberg, Germany*

Background: Introduction of high-sensitivity cardiac troponin T (hs-cTnT, Roche Diagnostics) in 2009 outside the US has facilitated and expedited earlier diagnosis of NSTEMI and demonstrated prognostic value in a variety of patient populations. Accompanying this trend are new analytical challenges with hs-cTn assays due to the significant impact small analytical confounds may have in the diagnosis of AMI and interpretation of serial troponin results. Lot-to-lot reagent stability for hs-cTn, particularly at low concentrations, is of utmost importance.

Objective: Assess lot-to-lot performance of the hs-cTnT and contemporary cTnT assays across multiple reagent lots, instruments and targeted at various cTnT concentrations.

Methods: For the period between 2008-2014 (cTnT Gen. 4) and 2013-2014 (hs-cTnT/hs-cTnT-STAT), we reviewed all cTnT reagent lot data (Roche Diagnostics, Penzberg). Acceptability of reagent performance utilized protocols designed to assess variability and bias between reagent lots and instruments. Residual pooled patient specimens were used in all assessments of reagent lot-to-lot performance. Data were analyzed collectively and validated between multiple platforms (Cobas e411/e601/e602; Elecsys 2010; Modular E170).

Results: Unique reagent lot data were available for the hs-cTnT, hs-cTnT-STAT and cTnT Gen.4 assays (n = 3, 5 and 42, respectively). Lot-to-lot reagent performance demonstrated minimal bias across the reportable range for hs-cTnT (0-1%), hs-cTnT-STAT (3-17%) and cTnT (5-7%). Very low bias at the 99th percentile of the hs-cTnT assay (14 ng/L) with the STAT (range: 13.00-13.09 ng/L) and routine assays (13.91-13.98 ng/L) was observed. The largest bias for hs-cTnT was noted at the LoD (13-17% at 5 ng/L).

Conclusion: Excellent lot-to-lot comparability was achieved with the hs-cTnT and cTnT assays, allowing laboratories to confidently integrate the assays into their clinical AMI decision making protocols. Assurance of reagent processes and transparency regarding performance criteria is critical for implementation of troponin and interpretation in the context of serial sampling and biological variability.

Assay (Concentration)	Lots Tested (n)	Target Concentration	Actual Conc. Range (Min – Max)	% Bias at Target Concentration (Low/High)
hs-cTnT (ng/L); 2013-2014	3	5	4.97 – 4.97	-1/-1
		10	9.94 – 9.98	-1/0
		14	13.91 – 13.98	-1/0
		30	29.81 – 29.99	-1/0
		50	49.68 - 50.01	-1/0
		100	99.36 - 100.05	-1/0
hs-cTnT STAT (ng/L); 2013-2014	5	5	4.17 - 4.36	-17/-13
		10	9.08 - 9.21	-9/-8
		14	13.00 - 13.09	-7/-7
		30	28.59 - 28.65	-5/-4
		50	47.97 - 48.31	-4/-3
		100	96.43 - 97.35	-4/-3
cTnT Gen. 4, (ng/mL); 2008-2014	42	0.03	0.0286 - 0.0322	-5/-7
		0.05	0.0503 - 0.0510	-5/-7
		0.1	0.0952 - 0.1073	-5/-7
		0.5	0.4759 - 0.5366	-5/-7
		1.0	0.9518 - 1.0732	-5/-7
		5.0	4.7590 - 5.3660	-5/-7

B-326

Cystatin c as biomarker for Coronary Artery Disease patients in southern India.

P. Srilakshmi[1], R. Kondreddy[1], V. Madrol[1], A. R. Saeid[2], S. J. Dhoipode[2], S. Shakila[2], H. A. Beloch[2], A. M. Jarari[3], L. T. Peela[4], J. R. Peela[2]. *[1]Department of Biochemistry, Mamata Medical College, Khammam, India, [2]Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [3]Department of Biochemistry, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [4]Great Eastern Medical School, Srikakulam, India*

BACKGROUND: Coronary Artery Disease is the leading cause of mortality and morbidity across the globe. Prevalence of Coronary Artery Disease is alarmingly increasing in developing countries. India is also experiencing the same with the increasing urbanization, changing lifestyles and obesity. Present study is focused on estimation of Cystatin-C in Coronary Artery Disease patients in correlation with lipid profile, obesity and other risk factors.

Materials and methods: The study was conducted in the department of biochemistry, Mamata Medical College and General Hospital, Khammam, Andhra Pradesh, India. The patients attending outpatient and wards of cardiology and general medicine departments of hospital and local cardiac centers were included in this study. Study group comprised of 145 patients diagnosed as Coronary Artery Disease with an age group of 30-50 patients with associated risk factors i.e., Diabetes, hypertension, smoking. And alcoholism. 66 sex and age matched subjects were recruited as control group (non Coronary Artery Disease cases) using the same criteria.

All patients and controls were measured cystatin-C and lipid profile by using authentic methods available. These patients were also divided as per their Body Mass Index with associated risk factors.

Results: In present study, there is significant increase in the values of serum cholesterol ($p<0.0001$), Low density lipoprotein cholesterol (0.0001) and significant decrease in High density lipoprotein cholesterol ($p<0.0001$) level was observed in Coronary Artery Disease cases when compared with controls. Cystatin-C ($p<0.0001$) was significantly elevated in people with Coronary Artery Disease when compared with controls. The levels of cystatin-C is correlating positively with total cholesterol, low density lipoprotein cholesterol, Body Mass Index and waist circumference.

Conclusion: Cystatin-C is one of the promising early risk marker for the Coronary Artery Disease patients. Importance of Cystatin-C as one of the biomarker for Coronary Artery Disease patients, positive correlation with cholesterol and body mass index will be discussed.

Mean and standard deviation values of Cystatin-C and Lipid Profile

Parameter	Mean ± (Controls)	Mean ± SD Cases)	t-value	p-value
Cholesterol	162.5 ± 25.9830	201.9 ± 56.8112	5.37	< 0.0001
HDL-C	43.1818 ± .7746	39.0138 ± .6248	-5.22	< 0.0001
LDL Cholesterol	119.3 ± 24.9667	161.9 ± 54.0915	6.10	< 0.0001
Cystatin-C	0.9738 ± 0.2067	1.3883 ± 0.3822	8.27	< 0.0001
BMI	23.3668 ± 3.0605	24.3015 ± 3.1302	2.00	0.0467
WC	87.8939 ± 7.7819	93.1778 ± 8.8080	4.15	< 0.0001

B-327

Diagnostic Accuracy of the Trinity Biotech Meritas® Cardiac Troponin I Point of Care Assay

M. M. Murakami, Y. Sandoval, S. W. Smith, K. M. Schulz, K. M. Smith, P. R. Stack, B. J. Kilburn, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

Background: The diagnosis of acute myocardial infarction (MI) is based on clinical factors and an increased cardiac troponin (cTn), with a rising and /or falling cTn pattern required. In addition, utilizing point-of-care (POC) technology to measure cTn may assist in a more rapid management of patients presenting to rule in or rule out MI. The goal of this study was to validate the diagnostic accuracy of the Trinity Biotech Meritas POC cTnI assay based on the 99th percentile value (19 ng/L).

Methods: 293 Patients presenting with symptoms suggestive of myocardial ischemia presenting to Hennepin County Medical Center's emergency department with cTnI orders based on clinical indications were evaluated in this study. Plasma (EDTA) was obtained at 0h (baseline), and 6h. cTnI was measured by the Meritas cTnI assay (LoD, 12 ng/L; 10% CV at 24 ng/L). All charts were adjudicated for MI, predicated on the Third Universal Definition of Myocardial Infarction guidelines based on the 99th percentile of the Abbott ARCHITECT cTnI assay used routinely in the hospital.
Results: MI was diagnosed in 8.6% (n=25) patients. MIs were comprised of 15 type 1 and 10 type 2. Clinical sensitivity improved over the time of serial testing, from 64.0% at 0h to 88.0% over 6h. Specificity was 88.8% at 0h and 86.8% over 6h; with ROC AUC values of 0.84 and 0.94 at 0h and 6h, respectively. In a subset of 92 patients in which EDTA whole blood was measured in parallel to plasma, similar ROC AUCs were found, 0.96 vs. 0.97, for maximum cTnI over 6h (p=0.43). Conclusion: Our findings demonstrate that the Trinity Biotech Meritas POC cTnI assay is a diagnostically useful aid in ruling in and ruling out acute MI in an emergency room setting.

B-328

Improved Diagnostic Accuracy for Myocardial Infarction of the Abbott ARCHITECT High Sensitivity Assay Compared to the Contemporary cTnI assay in an unselected US Population

Y. Sandoval, M. M. Murakami, S. A. Love, K. M. Schulz, R. Ler, J. Nicholson, S. W. Smith, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

Background: High-sensitivity (hs)-cTn assays have not yet been cleared for clinical use in the US. This study compared the diagnostic accuracy for myocardial infraction (MI) of the Abbott ARCHITECT hs-cTnI assay to the Abbott ARCHITECT contemporary cTnI assay.

Methods: Retrospective analysis of 310 unselected patients with symptoms suggestive of acute coronary syndrome (ACS), in which serial cTnI measurements were obtained on clinical indication. Fresh EDTA plasma specimens were simultaneously measured with both the contemporary and hs-cTnI assays. Unique to this study was adjudication of MI [using the 3rd Universal Definition of MI subtype (type1 and type 2) classification] independently predicated on the 99th percentiles of a normal population for a) the contemporary cTnI assay (99th percentile, 30 ng/L), b) the hs-cTnI assay (overall 99th percentile, 26 ng/L), and c) the gender-specific hs-cTnI (99th percentiles: male 34 ng/L and female 16 ng/L). Sensitivity, specificity, and positive and negative predictive values were calculated using baseline and maximum values.
Results: 24% Fewer MIs were identified based on the hs-cTnI assay: 33 (10.7%) using the overall hs-cTnI assay cutoff and 32 (10.3%) using the gender-specific cutoffs

compared to 43 (13.9%) using the contemporary cTnI assay. Using the hs-cTnI assay, type 1 MIs decreased from 14 to 11 and type 2 MIs decreased from 29 to 22. The table demonstrates our diagnostic accuracy findings. ROC areas under the curve showed that baseline diagnostic accuracy was improved using the hs-cTnI assay: overall cutoff 0.734; female cutoff 0.763, male cutoff 0.705; compared to the contemporary cTnI assay: 0.691.

Conclusions: Our data demonstrate that the hs-cTnI assay would not result in an over diagnosis of MI, and with careful adjudication, appears to result in fewer misclassifications. The hs-cTnI assay demonstrated superior specificity, with the best diagnostic accuracy in females. Future outcomes studies will be important.

hs-TnI and cTnI Sensitivity & Specificity				
Troponin Assay (99th percentile)	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV %(95%CI)
Baseline cTnI (>30 ng/L)	55.8 (39.9, 70.9)	65.5 (59.5, 71.2)	20.7 (13.7, 29.2)	90.2 (85.1, 94)
Baseline hs-TnI (>26 ng/L)	54.6 (36.4, 71.9)	73.2 (67.6, 78.3)	19.6 (12.0, 29.2)	93.1 (88.9, 96.1)
Baseline Gender-Specific hs-TnI (M>34 and F>16 ng/L)	56.3 (37.7, 73.6)	71.2 (65.5, 76.5)	18.4 (11.2, 27.5)	93.4 (89.2, 96.3)
Maximum cTnI (>30 ng/L)	100.0 (91.8, 100.0)	58.1 (51.9, 64.0)	27.7 (20.9, 35.5)	100.0 (97.7, 100.0)
Maximum hs-TnI (>26 ng/L)	100.0 (89.4, 100.0)	68.8 (63.0, 74.3)	27.7 (19.9, 36.7)	100.0 (98.1, 100.0)
Maximum Gender-Specific hs-TnI (M>34 and F>16 ng/L)	100.0 (89.1, 100.0)	65.8 (59.9, 71.4)	25.2 (17.9, 33.7)	100.0 (98.0, 100.0)

B-329

Zonulin as a potential biomarker of metabolic inflammation and pulmonary endothelial permeability

T. B. Dschietzig[1], R. Kluesener[2], F. P. Armbruster[1], C. Melzer[2]. *[1]Immundiagnostik AG, Bensheim, Germany, [2]Dept. of Cardiology, Charité Berlin (Campus Mitte), Berlin, Germany*

Background: In obesity and metabolic syndrome, disturbed intestinal permeability and low-grade chronic systemic inflammation appear to act in a vicious circle called metabolic inflammation. Zonulin, a tight junctions modulator and key regulator of intestinal permeability, has been shown to be up-regulated in individuals with type-1 diabetes and to play a role in gut-related dysfunctional auto-immunity. In addition, there are some preliminary reports indicating a possible association between zonulin and metabolic inflammation or type-2 diabetes as well. Moreover, zonulin is implicated in the regulation of general endothelial/epithelial permeability, and its association with increased pulmonary permeability has been demonstrated in animal experiments.

Methods: This study aimed at investigating plasma zonulin and its dependence on various clinical and biochemical factors in 225 patients carrying automatic implantable cardioverters/defibrillators (AICD), with 75% of them suffering from systolic heart failure, 69% from coronary artery disease (CAD), and 27% from type-2 diabetes (T2D).

Results: Univariate linear regression analysis showed that zonulin levels were associated with plasma creatinine, plasma nitrotyrosine, severity of CAD, left ventricular ejection fraction, and NYHA functional class, but not with high-sensitivity C-reactive protein (hsCRP), body mass index, weight, height, sex, or age. After multiple linear regression analysis, the negative association with creatinine ($p = 0.006$) and the positive one with NYHA class ($p = 0,013$) remained significant. In the subgroup of individuals with T2D, multiple regression revealed a significant positive affection of zonulin by hsCRP only ($p = 0.025$).

Conclusion: These findings may support reports on zonulin's involvement in the phenomenon of metabolic inflammation in T2D patients. The association of zonulin with NYHA may reflect its newly established role in altering endothelial/pulmonary permeability in heart failure. The robust
negative correlation with creatinine is unexpected and needs further clarification in experimental and clinical studies.

B-330

A Multi-Center Analytical Evaluation of the ARCHITECT *STAT* High Sensitive Troponin-I Assay

M. Krintus[1], P. Boudry[2], N. Capell[3], U. Koller[4], G. Lefevre[5], L. Lennartz[6], J. Lotz[7], M. Nybo[7], M. Plebani[8], J. Shih[9], O. Skadberg[10]. *[1]Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland, [2]CHR Mons-Hainaut, Mons, Belgium, [3]Dr. Peset University Hospital, Valencia, Spain, [4]Vienna Hospital Association, Hospital Hietzing, Vienna, Austria, [5]AP-HP, Hopital Tenon, Paris, France, [6]Abbott Laboratories, Wiesbaden, Germany, [7]Odense University Hospital, Odense, Denmark, [8]University Hospital, Padova, Italy, [9]Abbott Laboratories, Abbott Park, IL, [10]Stavanger University Hospital, Stavanger, Norway*

Introduction: Troponin is the preferred biomarker for the diagnosis of acute myocardial infarction in the presence of symptoms of ischemia, with the recommended cutoff at the upper reference limit (URL) or 99th percentile value with precision of <10%. Troponin assays have improved in sensitivity to meet these guidelines.

Objective: This study evaluated the analytical performance of a new high sensitivity troponin-I assay on the ARCHITECT instrument to confirm the data provided by the manufacturer.

Methods: The ARCHITECT *STAT* high sensitive Troponin-I (hsTnI) assay is a double monoclonal, sandwich assay with chemiluminescent detection. Nine laboratories in Europe participated in this study using either ARCHITECT $i2000_{SR}$ or $i1000_{SR}$ instruments in their routine laboratories. Total precision, limit of blank (LoB), Limit of detection (LoD), limit of quantitation (LoQ), linearity and interference were determined following guidance from CLSI documents EP5-A2, EP17-A, EP6-A, and EP7-A,respectively. Method comparison was performed using the ARCHITECT *STAT* Troponin-I assay(contemporary TnI) as the referent method and 2598 samples that spanned the dynamic range of the assays. The 99th percentile URL was determined using 1769 samples from healthy populations (screened with a questionnaire) or blood donors from seven countries. Ethics approval or waiver was received for specimens collected for the reference interval and method comparison studies.

Results: Precision ranged from 1.5 to 8.9% using the manufacturer's controls. The LoB, LoD and LoQ ranged between 0.1-1.4, 0.5-2.1 and 4.6-8.5 ng/L, respectively, confirming the information in the package insert. The lowest concentrations corresponding to a total CV of 10% was 5.6 ng/L. Common interferences did not affect the hsTnI results. The overall 99th percentile URL was determined to be 19.3 ng/L and was higher in men (27.0 ng/L) than in women (11.4 ng/L). Troponin was detectable in between 52.1 and 87.8% of the apparently healthy population depending on the LoD value used. Concordance between the investigated hsTnI assay and the contemporary TnI assay at the 99th percentile cutoff was found to be 95%.

Conclusions: These results demonstrate that the ARCHITECT *STAT* high sensitive troponin-I assay is a precise and highly sensitive method for measuring troponin I on a high throughput analyzer. This new assay meets the criteria of a high sensitivity troponin test with the 10% CV concentration below the 99th percentile URL.:

B-331

Comparison of sCD14-ST (Presepsin) with Eight Biomarkers for Mortality Prediction in Patients Admitted with Acute Heart Failure

E. Spanuth[1], G. Hess[2], E. Giannitsis[3]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]Prof Hess Medical Consulting GmbH, Mainz, Germany, [3]Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany*

Background sCD14-ST represents a 13 kDa fragment of sCD14 which is released after conversion of CD14++ monocytes into CD14+/16+ monocytes after M-CSF activation. sCD14-ST may play a role in acute heart failure (AHF) as monocyte TLR4 expression has been shown to be increased in this condition and as it was related to disease severity.

Objective To evaluate the diagnostic and prognostic value of sCD14-ST in AHF in comparison with cardiovascular markers (NT-proBNP, hscTnT, GDF-15, sFlt-1,), inflammatory markers (C-reactive protein (CRP) and procalcitonin (PCT)), kidney markers (neutrophil gelatinase-associated lipocalin (NGAL)), and soluble ST2.

Methods The marker concentrations were measured in base-line plasma samples obtained from 60 patients (50 to 90 years old, median 77 years; 26 females, 34 males) with AHF attending the emergency room (ER). Patients with myocardial infarction or sepsis were excluded. Outcome measure was mortality at 2 years. sCD14-ST was determined by using the PATHFAST Presepsin assay (Mitsubishi Chemical), NT-proBNP, hscTnT, GDF-15, sFlt-1, PCT, and CRP by using the ELECSYS tests (Roche

Diagnostics), and ST2 and NGAL using the Presage assay (Critical Diagnostics, San Diego, CA, USA) and the NGAL Rapid ELISA Kit (BioPorto Diagnostics, Denmark), respectively.

Results Baseline NT-proBNP ranged from 361 - 27287 ng/L, median (IQR) = 5773 (2207 - 8488) ng/L confirming the diagnosis of AHF. During the 2years follow up 25 patients (41.7%) died. The results of the biomarker determination are summarized in the table.

Tab. 1: Prognostic validity criteria of 9 markers for mortality prediction in emergency patients with acute heart failure

	Survivors, n=35 Median (IQR)	Non-survivors, n=25 Median (IQR)	p-value	ROC analysis AUC (95% CI)
sCD14-ST, ng/L	763 (601-1144)	1414 (1069-1712)	0.0001	0.789 (0.662-0.885)
GDF-15, ng/L	2885 (1766-4582)	3979 (2725-7717)	0.0392	0.681 (0.546-0.798)
ST2, µg/L	57 (34-83)	79 (50-120)	0.0453	0.675 (0.540-0.792)
PCT, µg/L	0.022 (0.02-0.037)	0.044 (0.02-0.13)	0.0314	0.667 (0.531-0.785)
hscTnT, µg/L	21 (12-33)	31 (20-47)	0.0280	0.646 (0.509-0.767)
CRP, g/L	13.1 (3.8-25.3)	25.1 (7.28-62.0)	0.1227	0.640 (0.504-0.762)
NT-proBNP, ng/L	5453 (1901-6919)	6161 (3518-9664)	0.1609	0.607 (0.470-0.732)
sFlt-1, ng/L	113 (90-179)	145 (106-210)	0.3410	0.605 (0.468-0.731)
NGAL, ng/L	1.60 (0.85-3.0)	1.69 (0.74-2.69)	0.7472	0.505 (0.370-0.639)

Conclusion sCD14-ST, hscTnT, PCT, GDF-15 and ST2 differed significantly between survivors and non-survivors.

Surprisingly, sCD14-ST was found to be the best prognostic marker for mortality prediction in patients admitted with AHF to the ER. The data may provide new information on the pathogenesis of heart failure and may improve therapeutic approaches in the future.

B-332

Validation of a Novel Equation for Estimating Low-Density Lipoprotein Cholesterol

J. W. Meeusen, A. Lueke, A. S. Jaffe, A. K. Saenger. *Mayo Clinic, Rochester, MN*

Background: Aggressive low density lipoprotein cholesterol (LDL-C) lowering strategies are recommended for primary and secondary prevention of cardiovascular events. A newly derived equation for LDL-C estimation was recently reported which addressed limitations in the commonly used Friedewald calculation method (LDL-C_F). The novel method (LDL-C_N) adjusts for the large inter-individual variability in triglyceride (TG) to very-low-density lipoprotein (VLDL-C) ratio using an adjustable factor empirically determined based on patient TG and non-high-density lipoprotein cholesterol (non-HDL-C). LCL-C_N reportedly classified patients with superior concordance to measured LDL-C compared to the Friedewald method, particularly in patients with LDL-C <70mg/dL. We evaluated the performance of the novel method in a data set from patients with LDL-C directly measured by ß-quantification (BQ-LDL-C), the gold-standard reference method.

Methods: Retrospective analysis identified 23,055 subjects, excluding those with TG >400mg/dL. Serum total cholesterol (TC) and TG were measured on a Roche Cobas c501. High-density lipoprotein cholesterol (HDL-C), LDL-C (BQ-LDL-C) and VLDL-C concentrations were determined by ß-quantification. Our analytical performance of these lipid measurements is directly certified by the Center for Disease Control and Prevention Lipid Standardization Program. The Friedewald LDL-C was calculated as (LDL-C_F =[TC] - [HDL-C] - [TG/5]); and the novel method was calculated as (LDL-C_N =[TC] - [HDL-C] - [TG/X]), where X is an adjustable factor based on the 180-cell method described by Martin, *et al (JAMA* 2013;310(19):2061-2068).

Results: Overall, LDL-C_F underestimated BQ-LDL-C, while LDL-C_N tended to overestimate BQ-LDL-C. The median LDL-C_N was 112mg/dL (IQR 88-141), significantly higher than the BQ-LDL-C median of 108mg/dL (IQR 84-136; P<0.0001). The median difference for [LDL-C_F] - [BQ-LDL-C] was -1mg/dL (95%CI -1-1) and the median difference for LDL-C_N was 3mg/dL (95%CI 1-4).

Both estimation methods significantly deviated from the reference LDL-C method when BQ-LDL-C was <70mg/dL (P<0.0001). The median difference for LDL-C_F was -2mg/dL (95% CI -3 - -1) compared to +2mg/dL (95%CI 1-3) for LDL-C_N. Consequently, the LDL-C_N method calculated fewer <0mg/dL compared to LDL-C_F (1 vs. 9, respectively).

Overall, LDL-C_F correctly classified 16,593 (72%) patients and LDL-C_N correctly classified 16,749 (73%) patients according to guideline cutoffs. LDL-C_F was more sensitive at identifying patients with BQ-LDL-C <70 mg/dL at 85% compared to 76% for LDL-C_N. However, LDL-C_F was less specific at 86% compared to 91% for LDL-C_N. The largest discrepancy in classification was observed in subjects with a BQ-LDL-C <70 mg/dL and triglycerides between 200-399 mg/dL, where sensitivity and specificity for LDL-C_F were 88% and 86%, compared to 53% and 99% for LDL-C_N.

Conclusions: We compared both novel and Friedewald estimated LDL-C against the gold standard LDL-C reference method, in contrast to the prior study which relied on validation of a subset of samples by ß-quantification to allow the use of the vertical auto profile method for direct LDL-C measurement. In our patient cohort, the novel method significantly overestimated LDL-C. Conversely, the Friedewald method tended to underestimate LDL-C; however the bias was not statistically significant. We conclude that the novel method has some benefits but whether the improvements are significant enough over the Friedewald calculation to justify making the change in routine clinical practice is unclear.

B-333

Elevated Serum Levels of Von Willebrand Factor Antigen (vWF:ag) Predict for Early Death and Shorter Survival in Patients with Primary Systemic Light Chain (AL) Amyloidosis Independently of Cardiac Biomarkers

I. Papassotiriou[1], E. Terpos[2], N. Simos[1], A. Gougoutsi[2], N. Kanellias[2], E. Eleutherakis-Papaiakovou[2], D. Chiotis[2], G. Amentas[2], M. Kavasi[2], C. Pamboucas[2], E. Psimenou[2], S. Toumanidis[2], M. Roussou[2], M. A. Dimopoulos[2], E. Kastritis[2]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece*

Background: Cardiac involvement is the main determinant of prognosis in AL amyloidosis, but the role of endothelium in this disease has not been extensively studied and no marker of endothelial dysfunction has been evaluated, as yet. We aimed to study the prognostic role of vWF:Ag in patients with AL amyloidosis who were treated with novel agents.

Patients and Methods: The analysis included 81 consecutive patients with newly diagnosed AL amyloidosis, with median number of involved organs was 2; heart was involved in 62%, kidneys in 74%, peripheral nerve in 24%, liver in 9% and soft tissue in 21% of patients. Median NTproBNP level was 2,318pg/mL (range 33-75,000pg/mL); 36% had NTproBNP levels ≥4,000pg/mL (Roche Diagnostics, CH) and 28%, 38% and 34% of patients had Mayo stage -1, -2 and -3, respectively. vWF antigen (vWF:ag) levels were measured using a latex particle-enhanced immunoturbidimetric assay (ACL Top 3G, Instrumentation Laboratory, USA).

Results: The median serum level of vWF:Ag in patients with AL amyloidosis was 181U/dL (range 20-557U/dL), significantly higher compared to healthy controls (median: 84U/dL, range 48-124U/dL; p <0.001). There was no significant association of vWF:Ag levels with renal, cardiac, nerve or liver involvement, as well as with the levels of NTproBNP or Mayo stage and there was no significant correlation with the degree of renal dysfunction, serum albumin levels or proteinuria. Levels of involved free light chains did not have any correlation with vWF:Ag levels either. The prognostic significance of vWF:Ag revealed that vWF:Ag levels within the top quartile (≥230U/dL) were associated with a very poor outcome (median survival 4 months vs. 47 months, p=0.001). Because the most important predictor of early death in patients with AL amyloidosis is cardiac involvement, we performed a multivariate analysis, which included NTproBNP levels: vWF:Ag levels ≥230U/dL were independently associated with survival (HR:2.64, 95%CI1.2-5.8, p=0.01), along with NTproBNP levels ≥4,000pg/ml (HR:4.17, 95%CI1.98- 8.8, p<0.001). As survival curves indicated that patients with vWF:Ag ≥230U/dL had a significant probability of early death, we performed an analysis to identify whether vWF:Ag is a significant predictor of early death, adjusting for the levels of NTproBNP ≥4,000pg/mL. Thus, in multivariate analysis, vWF:Ag ≥230 U/dL was independent predictor of death within 6 months from initiation of therapy (HR:15, 95%CI2.6-84, p=0.002) together with NTproBNP ≥4,000pg/mL (HR:16.8, 95%CI3-94, p=0.001). A combination of the two risk factors was able to identify patients at a very high risk of early death: 75% of patients with both risk factors present died within 3 months from initiation of therapy.

Conclusion: vWF:Ag levels are elevated in patients with AL amyloidosis but are not correlated with other features of the disease, such as pattern of organ involvement and cardiac biomarkers. For first time, we found that high levels of vWF:Ag are associated with a high risk of early death and shorter survival in patients with AL amyloidosis, independently of cardiac biomarkers. In addition, vWF:ag levels improve the prognostic ability of cardiac biomarkers in patients with AL amyloidosis. Our data also justify the investigation of the role of endothelial dysfunction in AL amyloidosis.

B-335

Cardiac troponin T and cardiac troponin I in patients with Chronic Renal Disease: A Meta-Analysis.

D. C. Gaze, P. O. Collinson. *St George's Hospital & Medical School, London, United Kingdom*

BACKGROUND: There are considerable methodological differences amongst studies evaluating the prognostic significance of an elevated cardiac Troponin T and I (cTnT, cTnI) in patients with Chronic Kidney Disease (CKD). This makes it difficult to study the factors involved or the mechanisms responsible for cTn elevations in CKD. The heterogeneity of patients included and the small sample size in many of the studies as well as the use of multiple assays and cut off values has resulted in imprecise estimation of prognostic significance. This systematic meta-analysis reviews the clinical value of measuring cardiac troponin in CKD.

METHODS: Suitable papers were identified from Medline/EMBASE searches. Each paper was abstracted for demographics, clinical outcome, assay used and cut off value. Primary end-point was all-cause mortality. Meta-analysis was performed reporting diagnostic odds ratio (dOR) by the DerSimonian-Laird random effects model. Publication bias was assessed by Funnel plots and Egger's regression asymmetry test; heterogeneity by Q-test. Summary areas under the receiver operator characteristic curve(sROC) were calculated. Meta-regression was used to assess the independent effects of sample size and increasing renal dysfunction on the dOR.

RESULTS: 183 datasets were identified. 107(58%) reported using cTnT and 96(52%) using cTnI. 33% of studies did not include follow up. In total, 22,820 patients were included. Median age=61(38-73)years. 58% were male. Median length of follow up was 24mts. The overall dOR for all-cause mortality was 2.88 (95%CI=2.23-3.71), (Sensitivity=53%,Specificity=65%,sROC=0.749) irrespective of assay used and data were heterogeneic without evidence of publication bias. For cTnT, 18,058(79%) subjects were studied. The dOR was 2.79 (95%CI=2.12-3.70, (Sensitivity=64%,Specificity=60%,sROC=0.943). The 2ndgen and 3rdgen assays had similar dOR. For cTnI, 16,747(73%) subjects were studied. The dOR was 2.38 (95%CI=1.78-4.51, (Sensitivity=22%,Specificity=88%,sROC=0.905). Sample size had a positive influence on the overall dOR(p=<0.0001) which was negated for cTnT(p=0.829) but significant for cTnI(p=<0.0001). 30(15%) reported pre-dialysis creatinine concentrations (median=682.31,95%CI=639-763µmol/L) which was negatively correlated to the dOR irrespective of assay used but of no significance(p=0.09), which was negated for cTnT(p=0.687)but significant for cTnI(p=0.039).

DISCUSSION: cTnT and cTnI are predictors of all-cause mortality in patents with CKD but no evidence of acute coronary syndrome. The evidence base is larger for CKD patients who have measurable cTnT compared to cTnI. The ability to predict all-cause mortality is higher with cTnI compared to cTnT, but the values are similar. cTnI exhibits greater specificity in CKD compared to cTnT for the determination of all-cause mortality, suggesting the overall diagnostic performance of cTnT and cTnI in CKD patients may reflect different associated risk of death possibly due to differences in pathophysiology. These data suggest that the presence of renal dysfunction alone is not related to the ability of cTn to predict all-cause mortality.

B-336

A simple fluorescence total homocysteine microassay for very low sample volume

Y. Tan, S. Li, L. Tang-Hall, N. Zhang, Q. Han, R. M. Hoffman. *AntiCancer, Inc, San Diego, CA*

Background: Total plasma/serum homocysteine (tHCY) is an independent risk factor for cardiovascular and other diseases. However, the tHCY assays on the current market are limited due to the sample volume which is not suitable for routine screening for newborn or for small animal studies. Therefore, we developed a low sample-size novel portable HCY fluorescence assay to meet those needs.

Methods: The novel portable tHCY fluorescence assay is a single-enzyme two-step assay. Reagent I (RI) is a combination of a reducing reagent, homocysteine α γ-lyase, and a compound consisting of a Schiff-based of N,N-dibutyl-p-phenylenediamine (DBPDA) and pyridoxal 5′-phosphate (PLP). A sample (5 µl) of either plasma or serum is added to RI where reduction of tHCY takes place and enzymatic reaction occurs, producing H2S, which binds to the DBPDA-PLP, producing a pre-chromophore. The first-step takes 5 min. In the second-step, Reagent II (RII), an oxidant [Ferric Chloride (FeCl3) in acid] is added. The oxidation reaction is complete in 10 minutes, and produces a chromophore, which can be measured by fluorescence at Ex 660/Em 710 nm. The total assay time is 15 min. The assay is carried at room temperature. A fluorescence reader, including a small portable device, is the only equipment needed.

Results: This novel portable tHCY fluorescence assay was compared to the A/C Portable tHCY Assay [FDA 510(k) 080851] for 40 plasma samples. The correlation coefficient is 0.95 and the slope is 0.96. The precisions of within and between assay were below10%. The linearity of tHCY in the assay is 4.2-44.8 µmol/L, and the detection limit is 4.2 µmol/L. The interferences of L-CYS, L-MET, lipid and protein were all below 10%.

Conclusion: This new portable tHCY fluorescence assay is a highly-sensitive and simple single enzyme two-step assay that can be used with a very small sample volume. A simple portable fluorescence reader is the only equipment required. This assay only needs a 5 µl sample, which meets the needs for newborn routine tHCY screening and for small animal studies.

B-337

Development of a New Specific and Highly Sensitive Enzyme-Linked Immunosorbent Assay to Detect Heart Type Fatty Acid Binding Protein in Human Serum

M. Summers[1], C. Richardson[1], T. McFadden[1], R. McConnell[1], K. Makris[2], J. Lamont[1], S. FitzGerald[1]. *[1]Randox Laboratories Limited, Crumlin, United Kingdom,* ***[2]Laboratory of Metabolic Bone Diseases, KAT General Hospital, Athens, Greece***

Background: The human genome comprises nine putative fatty acid-binding proteins (FABPs) with an approximate molecular weight of 15 kilodaltons. FABPs are structurally well conserved but interestingly exhibit only moderate sequence homology. FABPs bind and transport intracellular hydrophobic ligands through cellular compartments. Heart-type FABP or FABP 3 (H-FABP) is a member of the FABP family that exhibits high expression in both cardiac and skeletal muscle. Due to its low molecular weight and cytoplasmic location, H-FABP is released quickly into the circulation following myocardial injury. Consequently, this protein has been demonstrated to be an early indicator of acute myocardial infarction. Furthermore, elevated levels of H-FABP have also been reported following acute stroke. The availability of analytical methods allowing the determination of this protein in serum is relevant in clinical settings. This study reports the development of a new specific and highly sensitive enzyme-linked immunosorbent assay (ELISA) for the detection of H-FABP, which represents a useful analytical tool to facilitate clinical research applications.

Methods: A colorimetric sandwich immunoassay was employed. The in-house made monoclonal capture antibody was immobilised and stabilised on the 96-well microtitre plate surface. The analyte, if present in the sample, is bound to the capture antibody and then a second in-house made monoclonal antibody labelled with horseradish peroxidase is bound to the analyte. Absorbances were read at 450nm. The signal is proportional to the concentration of the analyte in the sample. Recognition of native H-FABP was confirmed with analysis of 15 selected stroke samples (6 ischemic stroke and 9 haemorrhagic stroke) compared with 15 healthy controls. Difference was assessed with Kruskal Wallis Test(Medcalc version 12.7.8.0).

Results:The ELISA was specific for H-FABP showing %cross-reactivity values ≤0.05% with the other FABP family members. The assay presented a functional sensitivity of 0.2 ng/ml (assay range of 0 to 50ng/ml). Native recognition of H-FABP in serum samples was demonstrated by comparing H-FABP concentrations in 15 selected stroke samples (Median 5.016ng/ml) versus 15 controls (Median 1.041ng/ml).The stroke samples were significantly higher than the healthy controls (P<0.0001).

Conclusion: The results show that the developed ELISA for measurement of H-FABP in human serum is both highly specific and sensitive. Furthermore, the median concentration values were significantly higher in the selected stroke patient samples when compared with controls. This indicates clinical utility of the developed ELISA in the context of stroke. This represents a new analytical tool for clinical research applications related to this important biomarker.

B-338

Troponin concentrations in young women measured by high-sensitivity troponins T and I

T. C. Aw, S. K. Phua. *Changi General Hospital, Singapore, Singapore*

Introduction: The high-sensitivity troponins (hs-cTn) have a reported limit of detection (LOD) of 5 ng/L (Apple. Clin Chem 2012;58:59) for hs-TnT (Roche) and 1.5 ng/L for hs-TnI (Abbott) (Aw. Clin Chim Acta 2013;422:26-8). Both assays have lower 99th percentile upper reference limits (P99URL) in women than men. The hs-TnI URL in females aged 40-65 is 17.9 ng/L with 11.1% of the hs-TnI results undetectable (below the LOD) (Aw. Clin Chim Acta 2013;422:26-8). Such low hs-cTn levels are more noticeable in younger subjects. Disproportionate representation of

low troponin levels will confound the determination of the P99URL for women. We decided to investigate hs-cTn levels in a large cohort of young women below 40 years.

Methods: We measured serum troponins (hs-TnT and hs-TnI) in 260 apparently healthy (via questionnaire) ambulant female subjects aged 20-39 years. An additional 94 women (aged 30-39) were recruited to determine a robust P99URL for hs-TnI in the entire cohort of 354 women. Pregnant subjects and those with a personal or family history of hypertension, diabetes, renal, heart, or muscle disease were excluded. Statistical analyses were performed using MedCalc 12.0 (Mariakerke, Belgium).

Results: Overall 98.5% (256/260) of the subjects studied had hs-TnT values below the assay LOD while only 27.7% (72/260) of the hs-TnI values were undetectable as shown in the Table below. Undetectable hs-cTn values are most pronounced in the 20-29 age group. The female (age 20-39) P99URL for hs-TnI (n=354) is 7.7 ng/L (90% CI 5.1-12.6), median hs-TnI and interquartile range were1.7 and 0.7-2.4 ng/L respectively.

Table. Proportion of hs-cTn values < LOD

Age Group (n)	hs-TnT < LOD (%)	hs-TnI < LOD (%)
20-29 (72)	100	72.2
30-34 (91)	97.8	7.7
35-39 (97)	97.9	11.3
Overall (260)	98.5	27.7

Conclusion: hs-cTn values are much lower in healthy younger females (age 20-39) than older subjects. These women may not be suitable subjects for inclusion in the reference population for the determination of hs-TnT P99URL while women 30-39 can be included for hs-TnI reference range studies.

B-339

Turnaround time for a sensitive cardiac troponin I assay at Point of Care

R. H. Christenson[1], S. Duh[1], S. Carden[2]. *[1]Univ of Maryland Sch of Med, Baltimore, MD, [2]Univ of Maryland Medical Center, Baltimore, MD*

Objective: We compared turnaround times for cardiac troponin I (cTnI) measurement in the adult Emergency Department (ED) from order to analytical system (pre-analytical), system to result (analytical/post-analytical) and Brain-to-Brain (Order to Result) for the Pathfast point-of-care system (Misubushi) and for a Vitros 5600 central lab analyzer and automated track system (Ortho Clinical Diagnostics).

Relevance: Point-of-Care availability has been shown to improve the disposition time for patients with suspected myocardial ischemia in the ED. Both cTnI assays have ≤10% total CV at the 99th percentile of normal subjects. We compared the difference in turnaround times in a busy ED associated with a 750-bed tertiary care medical center. This study helps define turnaround time expectations for in an ED population presenting to a large metropolitan hospital.

Methodology: ED orders for both the Pathfast point-of-care and the Vitros automated systems are placed by clinicians in the hospital information system and barcode labels print; this is '*Order'* time. When the specimen is presented to the point-of-care system the label is scanned; after the sample is tubed to the lab, the specimen is placed on the automated track system and scanned; the time scanned is *'Instrument'* time. Both the point-of-care and automated lab systems are interfaced and auto-verify results if no issues impacting analytic testing quality are detected; this is *'Report'* time. The total turnaround time, is termed *'Brain-to-Brain'.* Results for 73 patients were audited to determine if there were differences in diagnostic results between the Pathfast point-of-care and automated Vitros system. The study was conducted for 100 consecutive days.

Results: Data are displayed in the table. There was no diagnostic difference between the Pathfast and Vitros systems ($p<0.001$).

Conclusions: Use of the Pathfast system decreased Brain-to-Brain turnaround time by 30 minutes or more (24% to 60% faster) compared to the automated OCD system.

Testing Phase	Pre-analytical		Analytical/Postanalytical		Brain-to-Brain	
Description	Order to Instrument (time, h:min)		Instrument to Report (time, h:min)		Total Time: Order to Report (time, h:min)	
Instrument System	Pathfast POC TnI	Ortho Vitros cTnI	Pathfast POC cTnI	Ortho Vitros cTnI	Pathfast POC cTnI	Ortho Vitros cTnI
Sample Size	262	829	262	829	262	829
10th Percentile	0:03	0:14	0:21	0:42	0:25	1:02
25th Percentile	0:11	0:21	0:21	0:45	0:33	1:10
Median	0:23	0:31	0:21	0:48	0:45	1:21
75th Percentile	0:42	0:46	0:21	0:52	1:04	1:38
90th Percentile	1:05	1:03	0:21	0:57	1:27	1:57

B-341

Reducing CK-MB Utilization: The Calgary Laboratory Services (CLS) Experience

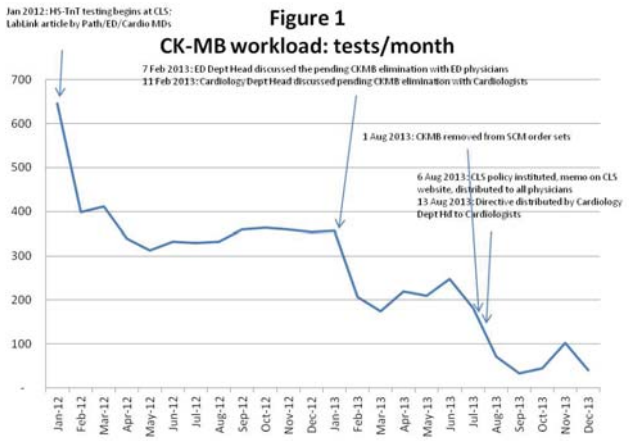
E. Flynn, I. Seiden-Long, L. deKoning. *Calgary Laboratory Services, Calgary, AB, Canada*

Background: Creatine kinase-MB isoenzyme (CK-MB) was the main cardiac biomarker in Calgary until Troponin T (TnT) testing was implemented in 2001,. However, CK-MB continued to be used not only for acute myocardial infarction (AMI), acute coronary syndrome (ACS) evaluation but also for other indications. High-sensitivity cardiac Troponin T (hs-TnT) testing became available in early 2012. Hs-TnT could be used for virtually every clinical condition where CK-MB had previously been the test of choice. At the time of hs-TnT implementation, CLS was performing >4000 CK-MB tests annually. A laboratory utilization initiative was directed towards significantly reducing CK-MB testing.

Methods: (1) Stakeholder consultation with Calgary Zone Departments of Cardiology, Cardiovascular Surgery, and Emergency Medicine; (2) Stakeholder and lab agreement to restrict CK-MB ordering to Cardiologists and Cardiovascular Surgeons only; (3) Educational article in the CLS newsletter (LabLink) to all medical providers; (4) On an agreed-upon date, all computerized physician order entry (CPOE) order sets containing CK-MB used by non-Cardiology specialists were removed from the CPOE database (Sunrise™ Clinical Manager [SCM]). Monthly CK-MB test workloads were obtained from the laboratory information system (Millennium®, Cerner) pre- and post-intervention; (5) Monthly lists were compiled via SCM of non-Cardiology/non-Cardiovascular Surgery providers who continued to order CK-MB; e-mails were sent to the individual providers as to the superiority of hs-TnT over CK-MB.

Results: Monthly CK-MB workload decreased from a pre-intervention baseline of 646 tests/month (at the time of hs-TnT implementation in January 2012) to a low of 34 tests/month (September 2013) post-intervention (see Figure 1).

Conclusion: The above interventions resulted in a 95% reduction in CK-MB testing in the Calgary Zone. Multiple utilization strategies including engagement of clinical stakeholders, education and limiting CPOE resulted in sustained workload reduction for this test.



B-342

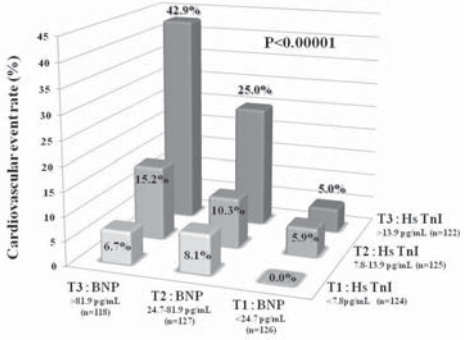
Prognostic Values of Combination of High-Sensitivity Cardiac Troponin I and B-Type Natriuretic Peptide in Outpatients with Chronic Kidney Disease

F. Kitagawa[1], M. Hasegawa[2], A. Kuno[1], J. Takeda[1], T. Ishikawa[1], H. Naruse[1], Y. Yuzawa[2], J. Ishii[1]. *[1]Department of Joint Research Laboratory of Clinical Medicine, Fujita Health University Hospital, Toyoake, Japan, [2]Department of Nephrology, Fujita Health University Hospital, Toyoake, Japan*

Background: The risk stratification for cardiovascular events is clinically important in patients with chronic kidney disease (CKD: estimated GFR<60mL/min/1.73m^2). We prospectively investigated whether combining cardiac troponin I (hsTnI), measured with a new high-sensitivity assay, and B-type natriuretic peptide (BNP) would be effective for the risk stratification in 371 outpatients (median age of 69 years) with CKD not on dialysis. They were also divided into tertiles of serum hsTnI levels; T1: <7.8 pg/mL, T2: 7.8-13.9 pg/mL and T3: >13.9 pg/mL. They were divided into tertiles according to plasma BNP levels; tertile 1 (T1): <24.7 pg/mL, T2: 24.7-81.9 pg/mL

and T3: >81.9 pg/mL. Results: Median estimated GFR was 24mL/min/1.73m^2. A medical history of cardiovascular disease was present in 32.9%. Log hsTnI levels were positively correlated with log BNP levels (r = 0.43, p < 0.0001). Cardiovascular event rate were 3.2%, 10.4% and 32.0% in T1, T2 and T3 of hsTnI, and were 2.4%, 13.4% and 30.5% in T1, T2 and T3 of BNP, respectively (p < 0.0001 in both). On a multivariate Cox regression analysis, both hsTnI (p = 0.002) and BNP (p = 0.0002) were independently associated with cardiovascular events. Cardiovascular event rates according to combined setting of hsTnI and BNP tertiles were shown in Figure. Conclusions: The combination of hsTnI and BNP may be useful for predicting adverse outcome in this population.

Fig. Cardiovascular event rates according to combined setting of hsTnI and BNP tertiles.



B-343

Presepsin (sCD14-ST) in Acute Coronary Syndromes and Heart Failure

E. Spanuth[1], R. Thomae[2], E. Giannitsis[3]. *[1]DIAneering - Diagnostics Engineering & Research, Heidelberg, Germany, [2]Mitsubishi Chemical Europe, Düsseldorf, Germany, [3]Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany*

Background Recently, research activities revealed that TLR4 expression on circulating peripheral CD14+ monocytes may play a pivotal role in the pathogenesis of cardiac diseases. Monocyte activation is triggered by different factors like LPS, ischemia, tissue hypoxia, left ventricular distension, or increasing filling pressures of the heart. It was shown that CD14++ monocytes could be induced by M-CSF (macrophage colony stimulating factor) and changed into CD14+CD16+ monocytes and shed off CD14 into blood circulation as soluble sCD14. Simultaneously, a 13 kDa fragment of sCD14 is formed, named sCD14-ST or presepsin.

Objectives To evaluate the diagnostic value of presepsin in cardiovascular conditions.
Methods 112 healthy volunteers, 40 patients with low grade chronic heart failure (CHF) and 106 patients admitted with chest pain and/or dyspnea were included. Discharge diagnosis was unstable angina pectoris (UAP) in 17, non-ST-elevation myocardial infarction (NSTEMI) in 29, and acute heart failure (AHF) in 60 patients, respectively. Presepsin and cTnI were measured by using the PATHFAST system (Mitsubishi).
Results The control group revealed 95% and 99% upper reference limits of 258 (90%CI: 237-276) and 304 ng/L (90% CI: 288-320) ng/L, respectively.Presepsin differed significantly between controls and patients (Fig. 1). The difference between healthy controls and patients with UAP was more significant with presepsin than with cTnI (p<0.0001 and p<0.0071). This finding could be confirmed by ROC analysis yielding the AUC values 0.992 for presepsin and 0.681 for cTnI, p=0.0001. Detection of NSTEMI revealed AUC values of 0.982 for presepsin and 0.966 for cTnI. Using a threshold of 304 ng/L for the diagnosis of NSTEMI presepsin reached sensitivity/specificity values of 100/98,2% compared to 74,1/100% for cTnI.

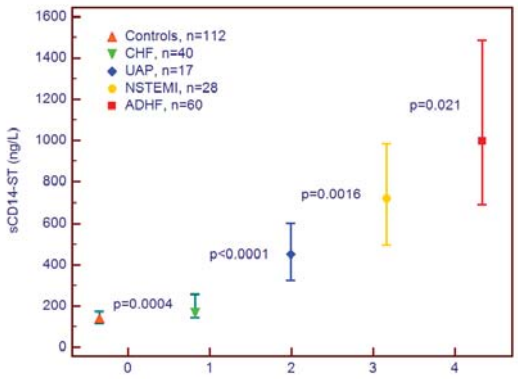


Fig. 1: Presepsin plasma concentrations (Medians, IQR) in healthy controls and patients with cardiac diseases

Conclusion Presepsin concentration showed a strong relationship with the severity of cardiac diseases reflecting inflammatory processes in the pathogenesis of cardiovascular conditions. Our data show that presepsin could provide incremental diagnostic information to distinguish cardiac and non-cardiac chest pain. Further investigation is needed to confirm the possible rule-out diagnosis of myocardial infarction as well as discrimination between cardiac and non-cardiac chest pain at admission in the ER.

B-344

Troponine T results in a patient population: Should women have their own upper reference limit for cardiac Troponin T?

J. P. van Straalen, J. C. Fischer, M. Vis, A. Sturk, R. J. de Winter. *Academic Medical Center, Amsterdam, Netherlands*

Background: For a reference population, differences in 99th percentile values for cardiac Troponin T (cTnT) are reported between men and women (1). This study investigates these characteristics in the relevant patient population itself.

Method: Data with respect to cTnT results and gender were extracted from the laboratory information system over a one year period for all patients with cardiologists involved in their medical treatment. cTnT was measured with the hs cTnT assay (Roche Diagnostics, limit of detection 0.005 µg/l). If cTnT was serially sampled in a patient, only the cTnT result of the first drawing was incorporated in the dataset.

Results: The dataset comprised 3515 individual patients (1491 female, 2024 male). The results profile of women and men was as follows:

cTnT<0.005 µg/L: 334 female (22%) and 214 male (10%).

0.005<cTnT< 0.015 µg/L: 519 female (35%) and 733 male (36%).

Elevated levels of cTnT (>0.014 µg/L): 638 female (43%) and 1077 male (53%). So, using 0.014 µg/L as a decision threshold for women as well as for men, elevated levels of cTnT relative to one reference value, are predominantly found in males.

Assuming that the risk for ACS in this patient population is the same for women as for men, in our dataset we established the cTnT decision level for which 53% of the women, i.e. identical to the percentage men, should have an elevated level of cTnT. To reach this target, we found that the cTnT threshold for women should be adjusted to 0.010 µg/L (793 female patients positive instead of 638 female patients).

Conclusion: The results support the use of a lower reference value for women: At cTnT<0.005 µg/L the percentage of females of the whole female patient population is twice that of men. The cTnT decision value for women to reach the same percentage of positive patients as for men was found to be 0.010 µg/L. These decision thresholds coincide with 99th reference limits in a reference population (reference value females <0.010 µg/L; males <0.0145 µg/L) (1).

1. Mitiwala SR, Sarma A., Januzzi JL, O'Donoghue ML. Biomarkers in ACS and heart failure: should men and women be interpreted differently? Clin Chem 2014; 60: 35-43.

B-345

Plasma cotinine is associated with social deprivation and subclinical atherosclerosis

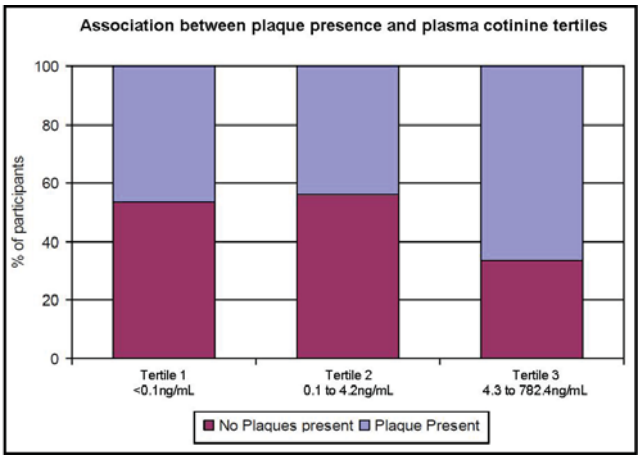
A. J. Dunlop, H. E. Turner, J. J. Allison, I. Clunie, B. L. Croal, D. W. S. Stephen, K. A. Deans. *Aberdeen Royal Infirmary, Aberdeen, United Kingdom*

Background: Cotinine is the primary metabolite of nicotine and is the preferred biomarker for verification of self-reported history of cigarette smoking. Cigarette smoking is a well-recognised classical risk factor for cardiovascular disease. The aims of this work were: to use plasma cotinine to verify self-reported smoking history; to study the association between plasma cotinine and social deprivation, and to examine the association between plasma cotinine and subclinical atherosclerosis (ultrasound evidence of carotid plaque).

Methods: Plasma cotinine was measured by an in-house liquid chromatography-tandem mass spectrometry method. Intra-assay Coefficient of Variation (CV) was 4.7% at a cotinine concentration of 1ng/mL; 0.9% at a concentration of 10ng/mL and 1.4% at 100ng/mL. Inter-assay CV was 6.7% at a mean cotinine concentration of 1.8ng/mL and 5.2% at 372.3ng/mL. EDTA plasma samples (n=572) from the Psychological, Social and Biological Determinants of ill-health (pSoBid) study were analysed for cotinine.

Results: Current smokers had higher cotinine concentrations (250 (95% CI 171.1 to 385.8)ng/mL) than ex-smokers (0.1 (0.0 to 1.7)ng/mL), p<0.0001 and non-smokers (0.0 (0.0 to 0.6)ng/mL), p<0.0001. Cotinine was higher in the most deprived group (4.4 (0.2 to 283.2)ng/mL) compared to the least deprived (0.0 (0.0 to 0.5)ng/mL), p<0.001. Cotinine was associated with carotid plaque presence (OR of 1.46 (1.19 to 1.79) per tertile increase in cotinine), p<0.001 (see Figure). After adjusting for self-reported smoking history and area-level social deprivation, this association persisted (adjusted OR 1.40 (1.04 to 1.87)), p=0.025.

Conclusion: Cotinine is independently associated with subclinical atherosclerosis, even after adjustment for self-reported smoking history and social deprivation.



B-346

The value of delta troponin for differential diagnosis of troponin elevation in non AMI patients in the unselected emergency room population.

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

Objective: To determine if delta troponin allows reliable exclusion of AMI in the non AMI population when more sensitive troponin assays are used for the diagnosis the universal definition of myocardial infarction.

Methods: The study was a sub study of 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 for measurement of a panel of cardiac markers. An additional blood sample was taken at admission and 90 minutes from admission, separated and the serum stored frozen until subsequent analysis. Samples were analysed for cardiac troponin I (cTnI) by

the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30-50,000 ng/L 10% CV 60ng/L 99th percentile 70 ng/L; the Beckman AccuTnI enhanced (B) (Access 2, Beckman-Coulter) range 1 - 100,000 ng/L, 10% CV 30 ng/L, 99th percentile 40 ng/L, the Siemens Ultra (S) (ADVIA Centaur, Siemens Healthcare Diagnostics), range.6 - 50,000 ng/L, 10% CV 30 ng/L 99th percentile 50 ng/L. and cardiac troponin T (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 utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. All patients were followed up for 3 months for major adverse cardiac events death, myocardial infarction, readmission with unstable angina or need for urgent revascularisation (MACE). Delta troponin was calculated as the difference between the second and first samples. A significant delta was considered as 50% of the reference interval. Only those with a troponin with an initially uncertain diagnosis who had sequential sampling were studied further.

Results: Samples were available from 608/1132 patients enrolled in the study. MACE occurred in 7 patients. The number of patients with at least one elevated troponin for each method was as follows, cTnI CS (>70 ng/L) 8 (1.3%) no MACE, cTnI S (>40 ng/L) 12 (2.0%) 1 MACE, cTnI B (>40 ng/L) 6 (1.0%) no MACE and for cTnT (>14 ng/L) 18 (3.0%) no MACE. Troponin elevation did not predict MACE. Addition of the delta reduced the number of misclassifications as follows cTnI CS to 3/8, cTnI S to 11/12, cTnI B to 5/6 and for cTnT to 9/18.

Conclusion: In this group troponin elevation occurred in 1.0-3.0% of patients. Additional of delta troponin provides only modest further exclusion of alternative elevations of cTnI. Clinicians need to interpret small elevations of cardiac troponin in the appropriate clinical context but they carry a good short term prognosis.

B-347

Absolute or relative deltas for diagnosis of myocardial infarction and how should they be calculated.

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

Objective: To compare delta values with peak troponin for the diagnosis of myocardial infarction when more sensitive troponin assays are used for the diagnosis using the universal definition of myocardial infarction.

Methods: The study was a sub study of 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 for measurement of a panel of cardiac markers. An additional blood sample was taken at admission and 90 minutes from admission, separated and the serum stored frozen until subsequent analysis. Samples were analysed for cardiac troponin I (cTnI) by

the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30-50,000 ng/L 10% CV 60ng/L 99th percentile 70 ng/L; the Beckman AccuTnI enhanced (B) (Access 2 , Beckman-Coulter) range 1 - 100,000 ng/L, 10% CV 30 ng/L, 99th percentile 40 ng/L, the Siemens Ultra (S) (ADVIA Centaur, Siemens Healthcare Diagnostics), range.6 - 50,000 ng/L, 10% CV 30 ng/L 99th percentile 50 ng/L. and cardiac troponin T (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. Delta troponin was calculated for all permutations as follows (where a = 0 minute and b = 90 minute sample). Absolute delta (b - a), absolute positive delta (b - a for b>a and a - b for a>b), relative delta (b - a)/a, relative positive delta (b-a)/a for b>a and (a-b)/b for a>b. Diagnostic accuracy was compared for different diagnostic strategies by receiver operator characteristic (ROC) curve analysis and comparison of area under the curve (AUC) for diagnosis by each delta calculation and for peak troponin value.

Results: Samples were available from 617/1132 patients enrolled in the study, 357 male age 23.7-92.8 years median 53.8 years. Delta troponin was diagnostically equivalent to peak troponin for all four troponin methods. Absolute delta was superior to relative delta for cTnI B (p 0.0007) and cTnT (p 0.0491) and just failed to reach significance for cTnI CS (p 0.064). Absolute and absolute positive delta had equivalent diagnostic performance for all methods. For all methods, expressing relative delta as a positive percentage made diagnostic performance worse when compared to absolute delta or peak troponin or both. Absolute positive delta was superior to relative positive delta for cTnI B and cTnT.

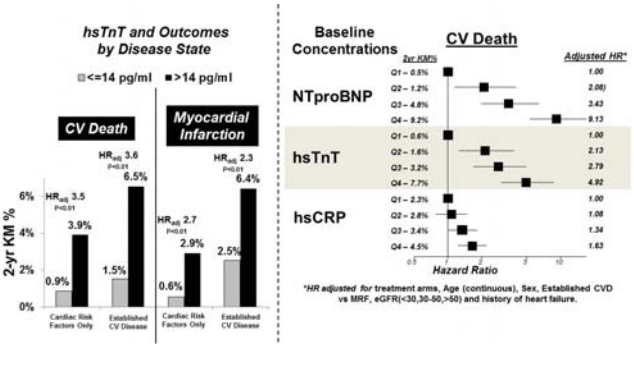
Conclusion: Absolute delta is superior to relative delta for the diagnosis of MI. The calculation should be the subtraction of the two values in temporal sequence.

B-348

Prognostic implications of simultaneous biomarker assessments in patients with type 2 diabetes mellitus - observations from the SAVOR-TIMI 53 Trial

P. Jarolim, B. M. Scirica, D. L. Bhatt, M. A. Cavender, M. J. Conrad, A. A. Umez-Eronini, K. A. Im, D. A. Morrow. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

Background: Cardiac biomarkers improve risk stratification and therefore offer an attractive strategy for cardiovascular (CV) screening. We evaluated the incremental prognostic value of multiple biomarkers reflecting different pathophysiologic processes in stable outpatients with type 2 diabetes mellitus (T2DM) and established CV disease (CVD) or cardiac risk factors only to ascertain the benefit of biomarker screening. Methods: Baseline specimens from 12,182 patients enrolled in the SAVOR–TIMI 53 (Does Saxagliptin Reduce the Risk of Cardiovascular Events When Used Alone or Added to Other Diabetes Medications) trial and followed for a median of 2 years were tested using the high sensitivity troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and high sensitivity c-reactive protein (hsCRP) assays (all Roche Diagnostics). hsTnT was categorized according to the 99th percentile (14 pg/ml). Results: hsTnT levels greater than 99th percentile were detected in 25% of patients with risk factors only and 44% of patients with established CVD. Overall, elevated hsTnT was associated with an increased risk of CVD (adjusted hazard ratio, HR_{adj} 3.6, $p<0.01$) and myocardial infarction (MI) (HR_{adj} 2.3, $p<0.01$), with similar results in each risk stratum. (Figure, left) There was a stepwise increase in the rates of CV death and MI with higher quartiles of each biomarker. After adjusting for clinical risk factors and biomarkers, elevated concentrations of NT-proBNP and hsTnT remained significantly associated with both CV death and MI, even at lower level elevations. (Figure, right) Similar results were seen for hospitalization for heart failure and MI. Conclusion: In this study of over 12,000 patients with T2DM, regardless of baseline risk, a substantial proportion of stable patients with T2DM have evidence of structural heart disease or hemodynamic stress, which were strongly associated with subsequent risk of CV death and MI.



B-349

Cardiac Troponin Testing Is Over Utilized in Patient Care to Rule In and Rule Out Myocardial Infarction

O. Rodriguez Fraga[1], Y. Sandoval[2], S. Love[2], Z. J. McKinney[2], R. Kohler[2], M. M. Murakami[2], S. W. Smith[2], F. S. Apple[2]. *[1]Hospital Universitario La Paz, Madrid, Spain, [2]Hennepin County Medical Center, Minneapolis, MN*

Background: The primary goal of this study was to determine whether clinicians appropriately utilize cardiac troponin I (cTnI) in the assessment of patients at moderate risk of acute coronary syndrome (ACS) / myocardial infarction (MI). Secondarily, we assessed the cost of excessive cTnI orders.

Methods: Following IRB approval, we retrospectively reviewed medical records (EHR) in 100 consecutive patients who had serial cTnI orders in the cardiac renal (CARE) unit, a unit where patients at moderate risk for MI are evaluated. Patients were adjudicated as MI or no-MI according to the 2012 Third Universal Definition of MI guidelines. A cTnI order set consisted of values obtained at 0, 3, 6 and 9 hours (Ortho-Clinical Diagnostics Vitros ES, 99th percentile 0.034 ug/L). Clinicians were not limited to any number of order sets. "Excessive" cTnI measurements were defined as any cTnI beyond those necessary to diagnose MI or rule out MI, according the 2012 definition. All cTnI results during hospitalization were tabulated.

Results: Of the 100 patients studied, 36 were diagnosed with MI (36%). Nine patients had a type 1 MI (25%) and 27 had a type 2 MI (75%). 5 of 36 MIs underwent percutaneous coronary intervention (PCI), while 31 did not. The remaining 64 patients without MI were primarily evaluated for their underlying medical conditions including shortness of breath, chest pain, heart failure, and renal disease symptoms; none underwent PCI. In the MI group (no PCI), 222 cTnIs were measured, with 107 cTnI values (48%) determined to be excessive (measured after the diagnosis of MI was determined). There were 52 additional cTnI order sets beyond the initial one (0, 3, 6, 9 hour), with an average of 7.16 cTnI values per MI patient. Furthermore, 23% of all cTnI measured were from unnecessary 2nd or 3rd order sets. In the no-MI group, 378 cTnIs were measured, with 150 cTnI values (39.6%) determined to be excessive (measured after the diagnosis of MI was excluded). There were 63 additional cTnI order sets beyond the initial one (0, 3, 6, 9 hour), with an average of 6.0 cTnI values per no-MI patient. Furthermore, 18% of all cTnI measured were from unnecessary 2nd or 3rd order sets. Taking into account nursing time for blood draws as well as laboratory time and supplies (reagents, technical FTE, blood drawing, supplies), we conservatively estimate excessive expenditures of approximately $88,000, based on the 32,000 cTnI tests performed per year at our hospital.

Conclusion: Our data show that in a monitored telemetry unit staffed by attending and resident physicians, a substantially, excessive number of cardiac troponin tests are ordered after establishing the diagnosis of both MI and no-MI. The excessive cTnI testing is wasteful. Better education and monitoring of cTn orders in the diagnosis or exclusion of MI is needed. Laboratorians should take the lead in educating their clinical colleagues on the use of cTn monitoring from the 2012 Third Universal Definition of MI guidelines in patients hospitalized with and without ACS.

B-351

Troponin T Identifies Individuals at Higher Risk for Coronary Heart Disease Within Narrow Blood Pressure Categories in The Atherosclerosis Risk In Communities (ARIC) Study

Y. Pokharel[1], W. Sun[1], G. Taffet[1], S. Virani[1], J. DeLemos[2], C. Ndumele[3], T. Mosley[4], R. Hoogeveen[1], J. Coresh[3], S. Solomon[5], G. Heiss[6], E. Boerwinkle[7], B. Bozkurt[1], C. Ballantyne[1], V. Nambi[1]. *[1]Baylor College of Medicine, Houston, TX, [2]University of Texas Southwestern Medical Center, Dallas, TX, [3]Johns Hopkins University, Baltimore, MD, [4]University of Mississippi Medical Center, Jackson, MS, [5]Harvard Medical School, Boston, MA, [6]University of North Carolina at Chapel Hill, Chapel Hill, NC, [7]University of Texas School of Public Health at Houston, Houston, TX*

Background: Although systolic blood pressure (SBP) is a recognized risk factor for coronary heart disease (CHD), there is variable CHD risk for individuals within narrow SBP ranges, even after accounting for other CHD risk factors. We evaluated whether cardiac troponin T (cTnT) measured using a high sensitivity assay identifies individuals at higher CHD risk within narrow SBP categories among 10,754 ARIC study participants without CHD.

Methods: We measured cTnT (Elecsys Troponin-T, Roche Diagnostics®) on an automated Cobas e411 analyzer with a limit of measurement of 3 ng/L. Incident CHD was defined as hospitalization for myocardial infarction (MI), definite coronary death, MI confirmed by electrocardiogram and coronary revascularization. Hard CHD excluded coronary revascularization procedures. Risks for incident CHD by SBP and cTnT categories were calculated using fully adjusted Cox proportional hazard model (table).

Results: The mean age of the participants was 62.8 years (56% women, 22% blacks). Over a mean follow up of 10.95 years there were 1,377 incident CHD events, which included 857 hard CHD events. Approximately half of the events (53%) occurred in individuals with SBP<140 mmHg and cTnT≥3 ng/L. In fully adjusted models, increasing cTnT was associated with increasing CHD or hard CHD events across most SBP categories (table). Of note, individuals with cTnT≥3 ng/L and SBP<140 mmHg had higher hazards for CHD and hard CHD compared to those with cTnT<3 ng/L and SBP 140-159 mmHg.

Conclusion: Risk for CHD increased significantly with higher cTnT levels within narrow SBP categories. Furthermore, individuals with well controlled SBP but elevated cTnT had increased hazards for CHD compared with those with sub-optimal SBP but undetectable cTnT, suggesting that cTnT is an important marker of cardiovascular health with potential value in identifying individuals at higher CHD risk.

Table. Risk of incident CHD across SBP and cTnT categories

cTnT (ng/L) SBP (mmHg)	<3	3- 5	6-8	9-13	≥14	*P* for trend
SBP <120 mmHg and cTnT <3 ng/L as the reference						
CHD						
<120	reference	1.1 (0.8-1.4)	1.2 (0.9-1.7)	1.5 (1.0-2.1)	2.1 (1.5-3.1)	0.093
120-129	1.1 (0.8-1.6)	1.2 (0.9-1.7)	1.4 (1.0-1.9)	1.5 (1.0-2.1)	2.9 (2.0-4.3)	0.001
130-139	1.3 (0.9-1.8)	1.5 (1.1-2.1)	1.5 (1.0-2.1)	1.8 (1.2-2.6)	2.5 (1.7-3.6)	0.008
140-149	1.4 (0.9-2.1)	1.6 (1.0-2.3)	1.7 (1.1-2.5)	1.9 (1.3-2.9)	2.8 (1.9-4.2)	0.011
150-159	1.1 (0.6-2.0)	1.4 (0.9-2.3)	2.2 (1.3-3.5)	2.1 (1.4-3.4)	3.5 (2.2-5.8)	0.001
≥160	1.6 (0.9-2.7)	1.1 (0.6-2.2)	2.4 (1.6-3.7)	3.5 (2.4-5.1)	3.6 (2.3-5.6)	0.001
Hard CHD						
<120	reference	1.0 (0.7-1.5)	1.1 (0.7-1.6)	1.5 (1.0-2.2)	2.6 (1.7-4.0)	0.014
120-129	1.1 (0.7-1.6)	1.0 (0.6-1.5)	1.5 (1.0-2.2)	1.4 (0.9-2.2)	3.3 (2.1-5.2)	<0.001
130-139	1.1 (0.7-1.7)	1.2 (0.8-1.9)	1.3 (0.8-2.0)	1.6 (0.9-2.6)	2.4 (1.5-3.9)	0.008
140-149	1.0 (0.6-1.8)	1.2 (0.7-2.0)	1.5 (0.9-2.4)	1.3 (0.7-2.2)	3.0 (1.8-4.8)	0.001
150-159	0.8 (0.3-1.8)	1.0 (0.5-2.1)	2.2 (1.2-4.0)	2.1 (1.2-3.7)	4.8 (2.9-8.2)	<0.001
≥160	1.5 (0.8-2.8)	1.7 (0.9-3.3)	1.7 (1.0-3.1)	3.0 (1.8-4.9)	3.4 (2.0-5.8)	0.079
SBP 140-159 mmHg and cTnT <3 ng/L as the reference						
CHD						
<120	0.8 (0.5-1.1)	0.8 (0.6-1.2)	1.0 (0.7-1.4)	1.1 (0.8-1.7)	1.6 (1.1-2.5)	0.093
120-129	0.9 (0.6-1.3)	0.9 (0.6-1.4)	1.1 (0.7-1.5)	1.1 (0.7-1.7)	2.3 (1.5-3.5)	0.001
130-139	1.0 (0.6-1.4)	1.2 (0.8-1.7)	1.1 (0.8-1.7)	1.4 (0.9-2.1)	1.9 (1.2-2.9)	0.008
≥160	1.2 (0.7-2.2)	0.9 (0.4-1.7)	1.9 (1.2-3.0)	2.7 (1.7-4.1)	2.7 (1.7-4.4)	0.001
Hard CHD						
<120	1.1 (0.6-1.7)	1.1 (0.6-1.8)	1.2 (0.7-2.0)	1.5 (0.9-2.7)	2.7 (1.6-4.7)	0.014
120-129	1.1 (0.7-2.0)	1.0 (0.6-1.8)	1.5 (0.9-2.6)	1.5 (0.8-2.6)	3.5 (2.0-6.1)	<0.001
130-139	1.1 (0.6-2.0)	1.3 (0.8-2.3)	1.3 (0.8-2.4)	1.7 (0.9-3.0)	2.6 (1.5-4.6)	0.008
≥160	1.5 (0.7-3.2)	1.8 (0.8-3.8)	1.8 (0.9-3.5)	3.2 (1.8-5.7)	3.6 (2.0-6.7)	0.079

Data presented as hazard ratio and 95% confidence interval as calculated using Cox-proportional hazards model
Model adjusted for age, race and gender, anti-hypertensive medication use, estimated glomerular filtration rate, diabetes, fasting glucose, total/high density lipoprotein cholesterol, body mass index and current cigarette smoking.
P for trend was calculated based on the results of Wald chi-square test on linearity hypothesis of ordered cTnT categories.
Results were similar when the reference was SBP <120 mmHg and cTnT ≤5 ng/L
cTnT: high sensitivity cardiac troponin T, SBP: systolic blood pressure, CHD: coronary heart disease

B-352

Analytical Correlation Between Abbott ARCHITECT High Sensitivity and Contemporary Cardiac Troponin I Assays During Evaluation of Acute Myocardial Infarction within an Unselected Urban Hospital Population

S. A. Love, Y. Sandoval, M. M. Murakami, K. M. Schulz, R. Ler, J. Nicholson, S. W. Smith, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

Background: The Abbott high-sensitivity (hs) cardiac troponin I (cTnI) assay is available for clinical use outside the US, while only the contemporary cTnI assay is used in the US; awaiting FDA clearance. The goal of this study was to examine the correlation between the hs and contemporary cTnI assay results at 1) baseline and 2) over serial measurements during the course of ruling in/out patients suspected of myocardial infarction (MI).

Methods: During the fall of 2013, fresh EDTA samples were collected during the evaluation of patients with symptoms of ACS as clinically indicated. Patients were included if they had at least a baseline and one additional serial sample. Fresh specimens were measured simultaneously for both the contemporary and hs-cTnI Abbott ARCHITECT assays (Abbott ARCHITECT $i1000_{SR}$/$i2000_{SR}$).

Results: 1021 Specimens from 310 patients were analyzed. Correlation data for baseline and all specimens, subdivided by concentration ranges, are shown in the table. For all samples, there was a negative bias with the hs-cTnI assay at baseline, mean -6 ng/L. Focusing at concentrations above and below the 99th percentile values, concordance below 100 ng/L, based on the contemporary cTnI value, was generally poor, 0.77 at baseline and 0.80 for all samples. Slopes varied substantially across the dynamic range of the assays.

Conclusion: Our data show that cTnI results are 1) lower using the hs-cTnI assay, b) show variable correlations depending on specimen concentration range and c) showed poor concordance. Therefore, results are not interchangeable between the contemporary and high-sensitive cTnI Abbott ARCHITECT assays.

Correlation Data				
Specimen	Range	N	Slope	r
Baseline only	all cTnI results	310	0.983	0.98
Baseline only	cTnI < 1000 ng/L	306	0.756	0.94
Baseline only	cTnI < 100 ng/L	271	0.863	0.81
All Specimens	all cTnI results	1021	1.24	0.99
All Specimens	cTnI < 1000 ng/L	967	0.868	0.91
All Specimens	cTnI < 100 ng/L	812	0.829	0.84

B-353

Myocardial injury in cancer patients - are there differences between women and men?

C. Shortt, M. Mansour, S. Dhesy-Thind, S. Hotte, D. Leong, A. Worster, P. A. Kavsak. *McMaster University, Hamilton, ON, Canada*

BACKGROUND: The prevalence of cardiac injury in a general cancer population is not well documented; despite the fact that there are cancer treatments that may elicit cardiotoxicity. Currently, cardiac imaging is the main approach to assess cardiotoxicity; however, laboratory tests may serve as a complementary tool in this regard. New commercially available cardiac troponin assays have obtained regulatory approval (outside of the United States) with preliminary data from healthy individuals suggesting different thresholds in women versus men. These new tests are termed "high-sensitivity cardiac troponin" assays. We conducted an observational study measuring high-sensitivity cardiac troponin I (hs-cTnI) in a convenient non-select group of ambulatory cancer patients assessing whether hs-cTnI differs in male vs female cancer patients.

METHODS: Following ethics board approval, we measured cardiac troponin levels (Abbott hs-cTnI assay) in two cohorts (A and B) attending a regional cancer center with the ordering physicians blinded to the test results. Cohort A consisted of all cancer center patients with clinical lithium heparin plasma samples available for hs-cTnI measurement over a period of 40 consecutive days. Cohort B consisted of cancer center patients whose serum samples collected for measurement of clinical tumor markers and subsequently were measured for hs-cTnI over a period of 20 consecutive days. We used the sex-specific upper limits of normal (i.e., the manufacturer's reported 99th percentiles) to designate myocardial injury (i.e., women >15.6; men >34.2 ng/L) in both cohorts. We used Statsdirect and Medcalc software for non-parametric testing (e.g., Mann-Whitney, spearman correlations) and to compare positivity rates between men and women and correlate with CEA, CA 125, and total PSA in cohort B.

RESULTS: We measured hs-cTnI levels in 4,757 lithium heparin and 285 serum samples. Cohort A comprised 2,272 women of median (IQR) age= 67y (57-76) and 2485 men of median (IQR) age= 62 y (52-70); p<0.01 for the between-sex difference). In cohort A, the prevalence of myocardial injury was higher in females (8.0%; 95%CI:6.9-9.3) compared with males (3.1%; 95%CI:2.5-3.9) (p<0.01). Cohort B comprised 137 women of median (IQR) age= 64 y (54-73) and 148 men of median (IQR) age= 68y (59-76); p=0.03 for the between-sex difference). The prevalence of myocardial injury remained significantly higher in the female population (8.8%; 95%CI:4.5-15) compared with the male population (1.3%; 95%CI:0.2-4.9) (p<0.01) despite the lower age of women in cohort B. There was no difference in hs-cTnI positivity rates in females or males between cohort A and B (p>0.20) nor was hs-cTnI correlated to CEA (rho=0.10 p=0.27), CA 125 (rho=0.12 p=0.33), or total PSA (rho= -0.10 p=0.40) in cohort B (p>0.25).

CONCLUSIONS: Applying sex-specific ULN the prevalence of myocardial injury in female cancer patients is higher than male cancer patients. Additional studies are required to assess what are the contributing causes for this marked difference in this population and importantly, if these differences portend a worse outcome for women.

B-354

On the 99th percentile reference interval determination for the Beckman-Coulter Cardiac Troponin I assays.

D. C. Gaze[1], C. A. Hodges-Savola[2], D. R. Holmes[2], S. A. Faye[2], P. O. Collinson[1]. *[1]St George's Hospital & Medical School, London, United Kingdom, [2]Beckman Coulter, Chaska, MN*

BACKGROUND: The 99th percentile concentration of an apparently healthy population is used define a positive cardiac troponin to aid in the diagnostic and prognostic assessment of chest pain patients with suspected acute coronary syndrome. We sought to determine the 99th percentile upper reference limit of the newly released AccuTnI+3 and pre-commercial hs-cTnI assay.

METHODS: Serum samples (n=1000) were obtained following routine testing from apparently healthy donors. Subjects were selected on the basis of the following criteria: >40 years old with normal urea and electrolytes, liver function tests, glucose and N-terminal pro-B-type natriuretic peptide. Age and gender was also recorded. Serum samples were stored frozen at -70ºC until batch analysis of cTnI using the AccuTnI+3 and pre-commercial high-sensitivity cTnI (hs-cTnI) assay for the Access2 and AccuTnI+3 on the UniCel DxI Immunoassay system (Beckman-Coulter, Chaska, MN). The manufacturers report a total %CV of 5 to 7% in the range 7 to 56,360ng/L

with a European 99th percentile of 40ng/L and a US 99th percentile of 20ng/L for the AccuTnI+3 assay.

RESULTS: Two subjects were removed from the study due to inadequate sample volume leaving a population of 998 comprising 433(43%) males and 565(57%) females. The median age was 59.0 years, interquartile range 57.4 to 60.3 years. There was no significant difference in age between males and females (p=0.4063). There was a good correlation between the AccuTnI+3 concentrations obtained on the two instruments (r=0.90, 95%CI=0.89 to 0.91). However, the 99th percentile upper reference limit were statistically different at 41 and 34ng/L (P=<0.001) for the Access II and DxI AccuTnI+3 respectively. Detectable concentrations were observed in 878 (88%) samples on the Access II but only in 578 (58%) samples on the DxI instrument. Concentrations in males were significantly higher than females using both instruments. Using the prototype assay, the hs-cTnI 99th percentile was 27ng/L on the Access II with detectable concentrations in 98% of subjects. Concentrations of cTnI in males were significantly higher than females using both assay formats.

CONCLUSIONS: The AccuTnI+3 and prototype hs-cTnI 99th percentile concentrations are similar to other contemporary and high sensitive commercial cTnI assays. Differences were seen in the 99th percentile using the AccuTnI+3 on the Access II and UniCel DxI instruments. As gender differences were observed in the reference population, confirming findings for other hs-cTn assays; further prospective studies in chest pain patients are required to assess the clinical utility of gender specific 99th percentile concentrations.

B-355

A step towards D-dimer assays' standardization: Antibodies with Equal Specificity to D-dimer and High Molecular Weight Fibrin Degradation Products

A. Kogan[1], K. Mukharyamova[1], A. Kara[2], A. Bereznikova[1], A. Katrukha[1]. *[1]HyTest LTD, Turku, Finland, [2]School of Biology, Moscow State University, Moscow, Russian Federation*

D-dimer is an acknowledged marker of blood clotting. It has been shown that D-dimer concentration is elevated (higher than 0.5 µg/ml) in plasma of patients with pulmonary thromboembolism, deep vein thrombosis and disseminated intravascular coagulation of different etiology. Despite the long history of D-dimer use in clinical practice, there are some problems concerning its quantitative determination.

D-dimer as well as its precursor - fibrin degradation products (FDPs) are present in blood of patients to varying ratios. Different monoclonal antibodies (Mabs) utilized in different D-dimer assays recognize D-dimer and FDPs with unequal efficiency and that causes significant discrepancy in results obtained by such assays. In addition, differences in Mabs specificities make it impossible to use D-dimer as a standard or a calibrator in current generation of D-dimer assays.

The aim of this work was to obtain monoclonal antibodies with the same specificity to D-dimer and FDPs and to design an assay prototype that equally recognizes D-dimer and FDPs in patient plasma.

Hybridomas producing D-dimer-specific MAbs were generated using standard techniques. Mabs that did not show cross-reactivity with fibrinogen were tested with D-dimer and FDPs in different two-site combinations. To obtain preparations of D-dimer and FDPs with equal concentrations, a fibrin clot was initially partially and then completely digested by plasmin. The partially digested fibrin was used as a source of FDPs, whereas the completely digested product was utilized as a source of D-dimer. Thus, the FDPs and D-dimer preparations contained the same amount of cross-linked fibrin-derived materials. Two-site antibody combination with capture antibody DD189 and detection antibody DD255 (labeled with stable Eu3+-chelate) gave an equal response with FDPs and D-dimer in the range of the antigen concentrations from 20 to 1000 ng/ml and was selected for the further analysis.

DD189-DD255 assay was used to analyze plasma protein profiles obtained by gel filtration on Superdex 200 column. It was shown that plasma samples from different patient groups contained different ratios of D-dimer and FDPs. D-Dimer levels in blood of three patients who had gone through a surgical operation were comparable with the level of FDPs or exceeded it. In contrast, FDPs were the main products of fibrin degradation in samples from two thrombotic patients. In two septic patients' blood different ratios of D-dimer and FDP were observed.

Our results show that the ratio of D-dimer and FDPs varies between patients with different diseases. This fact means that a precise determination of fibrin-derived cross-linked products in patients' blood requires the use of antibodies with equal specificity to D-dimer and FDPs. Use of antibodies with the same reactivity to D-Dimer and FDPs would also allow the use of D-Dimer as a calibrator. This would have a significant impact on standardization of different D-Dimer assays and would benefit number of practicing clinicians who currently need to deal with the variability of different D-Dimer assays.

B-356

An Immunoturbidimetric Assay for the Detection of Thromboxane Metabolites in Urine

I. Muncy, B. Hurley, A. Bamberg, K. R. Pitts. *Corgenix, Inc., Broomfield, CO*

Background: Activated and aggregated platelets play a key role in the pathogenesis of cardiovascular disease. Activated platelets produce thromboxane A2 (TxA2), a potent vasoconstrictor and inducer of platelet aggregation. TxA2 is generated by thromboxane synthase from molecules derived from arachadonic acid by cyclooxygenase-1 (COX-1). TxA2 has a short half-life in plasma and is rapidly hydrolyzed to thromboxane B2 (TxB2). TxB2, in turn, is metabolized to 11-dehydro thromboxane B2 (11dhTxB2), 11-dehydro 2,3 dinor thromboxane B2 (11dh2,3dTxB2, a truncated form of 11dhTxB2), and a number of other minor TxB2 metabolites which are excreted by the kidney. Thus, 11dhTxB2 is a stable metabolite of TxA2 and an *in vivo* indicator of platelet activity. Acetylsalicylic acid (ASA) has been known for many years to have anti-platelet activity. ASA functions by acetylating and irreversibly inhibiting COX-1, thus inhibiting the production of TxA2 and its metabolites. Low dose ASA blocks more than 95% of platelet COX-1 activity. The measurement of stable metabolites of TxA2, such as urinary 11dhTxB2, is a means of quantitating TxA2 production *in vivo* and thus a direct way to analyze ASA's effect post ingestion. Urinary 11dhTxB2 can be quantitated using and ELISA method, but recently an immunoturbidimetric format has been developed. Here we describe the performance and utility of the immunoturbidimetric format (TxBCardio®) on the Selectra ProM chemistry analyzer.

Methods: An R1 reagent/reaction buffer was developed containing a monoclonal antibody specific for 11dhTxB2 and the 2,3 dinor form of 11dhTxB2. The monoclonal antibody facilitates the agglutination reaction with the 11dhTxB2 coated microparticles in a competitive manner based on the amount of 11dhTxB2 present in the urine sample. An R2 reagent/coated polystyrene microparticle buffer was developed containing functionalized polystyrene microparticles coated with 11dhTxB2. These reagents were tested in several iterations to optimize assay performance characteristics specific to the Selectra ProM analyzer.

Results: The linear range was determined to be 225-6000 pg/mL with an LOQ of 400 pg/mL. A 1:10 post-dilution allows the linear range to be extended to 60,000 pg/mL. Within run precision is <10% and total precision is less than <20% at all levels. Control recoveries over 5 days and 2 instruments (n=20) were within labeled ranges. Correlation to an 11dhTxB2 ELISA resulted in an (r) of 0.987, slope of 0.887 and y-intercept of 291 between the two methods. The 2x2 analysis of creatinine normalized results from both assays results in a positive predictive value of 94%, a negative predictive value of 100%, and an overall predictive value of 97%. Of many common urinary components, interference was observed only with high levels of hemoglobin and protein. Reagents are stable for at least 35 days once opened.

Conclusions: The data presented here suggest that the TxBCardio® reagents can be used on the Selectra ProM analyzer to adequately detect levels of **11dhTxB2 in urine samples.**

B-357

Evidence of Inappropriate Utilization Of Cardiac Troponin Order Sets After Initiation Of A Provider Alert Prompt In Electronic Health Record

S. A. Love[1], Z. J. McKinney[2], Y. Sandoval[1], S. W. Smith[1], R. Kohler[1], M. M. Murakami[1], F. S. Apple[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]University of Minnesota, Minneapolis, MN*

Background: Two new, hospital-wide practices were implemented. First, a cardiac troponin I (cTnI) order set was implemented based on serial cTnI testing at 0, 3, 6, and 9 hours, for the diagnosis of myocardial infarction (MI). Second, when any provider placed additional cTnI orders, a pop-up alert was triggered in the electronic health record (EHR) alerting for possible excessive ordering. This study determined the frequency and rationale for providers ordering additional cTnI testing after an initial order set. Further, we determined by patient diagnosis and provider subspecialty the scope of additional cTnI testing beyond the initial order set.

Methods: Over 2 months, data was collected on the alert, triggering provider, provider selected clinical indication, and provider follow up action for each alerts. Providers were not limited to any number of order sets. Alert initiated inclusion of the patient in the study. We reviewed the EHR for patients associated alerts, and collected: patient stay (duration, location), ICD-9 diagnosis, cTnI orders, and timing of cTnI result with measured values (Abbott Architect; 99th percentile 0.025 µg/L).

Results: 1477 alerts were generated by 423 providers. 181 (42%) were resident physicians, of which 42% were 1st year residents, double that for either 2nd or 3rd year

residents (22% each). Alerts were associated with 3045 cTnI results for 702 patients during 833 encounters. 50% of all the cTnI testing (6044 tests) was associated with an alert. Alert-triggering providers acknowledged and overrode the alert 1440 times (97%). For overridden alerts, the provider described the override reason from a pre-determined prompted list or by free text. 929 (65%) alerts were from prompted list, including: 519 (35%) acute coronary syndrome concern (ACS; ST- and non-ST-elevation MI); 249 (17%) demand ischemia; 50 (3%) non-ACS myocardial necrosis. 71% of all free-text overrides gave no indication. The remaining 149 (29%) reasons included: chest pain (N = 23); retiming (N = 11); ACS (N = 10); trauma (N =7); or ED visit (N = 6). In 714 encounters (85%) providers placed a second order set when there were ≤ 2 cTnI results available at the alert time. The most commonly associated primary ICD-9 code was 786.5,chest pain (N = 231, 27%). Using all ICD-9 designations, 1368 alerts (92%) or 779 encounters (93%) were non-ACS related. Alerts were predominately (33%) generated by providers treating in-patients within the cardiac/renal unit (CARE), double the rate for all other units within the hospital. The 52 ACS patients generated 106 alerts.

Conclusion: Our data show that a) visual alerts did not result in a decrease in orders by providers, resulting in excessive cTnI testing after a diagnosis was determined, b) the largest number of ignored alerts was in non-ACS patients, and c) even providers treating patients diagnosed with ACS practiced excessive cTnI ordering. Our observations highlight the need for better education regarding the use and ordering of cTn testing to rule in and rule out the diagnosis of MI.

B-358

Evaluation of standardization capability of current cardiac troponin I (cTnI) assays by a correlation study: results of an IFCC pilot project

J. R. Tate[1], D. M. Bunk[2], R. H. Christenson[3], J. H. Barth[4], A. Katrukha[5], J. E. Noble[6], M. Panteghini[7], H. Schimmel[8], L. Wang[9]. *[1]Pathology Queensland, Royal Brisbane & Women's Hospital, Brisbane, Australia, [2]Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg,, MD, [3]Department of Pathology, University of Maryland School of Medicine, Baltimore, MD, [4]Clinical Biochemistry, Leeds Teaching Hospitals NHS Trust Leeds General Infirmary, Leeds, United Kingdom, [5]HyTest Ltd, Turku, Finland, [6]Analytical Science Group, National Physical Laboratory, Teddington, United Kingdom, [7]Centre for Metrological Traceability in Laboratory Medicine, University of Milan, Milan, Italy, [8]European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium, [9]Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, MD*

Background: The International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Standardization of Cardiac Troponin I (IFCC WG-TNI) performed a pilot study in collaboration with industry to investigate the feasibility of preparing a commutable and stable cTnI reference material (RM). The study aimed to test whether serum pools prepared from patient sera could be used as an RM to standardize cTnI measurement.

Methods: cTnI-positive serum samples from 90 patients presenting to the emergency department with suspected acute myocardial infarction were used to prepare seven pools in the range, 200-10,000 ng/L. Samples and pools were assessed for method comparison, commutability, and stability, and a normal pool was screened for interference from cTnI autoantibodies and heterophile antibodies by 16 cTnI commercial systems according to predefined testing protocols.

Results: Each assay was assessed against median cTnI concentrations measured by 16 systems using Passing-Bablok regression analysis of 79 patient samples with cTnI values above each assay's declared detection limit. An 8- to 9-fold difference in cTnI concentrations was observed among assays, with Pathfast giving lowest values and Immulite 1000 TPI highest. After correction by a mathematical recalculation using slope and y-intercept values, between-assay variation was re-assessed. At 190 ng/L cTnI concentration, average variation of pools reduced from 49% (range, 43-55%) to 16% (range, 14-19%), at medium concentrations (814, 1634 and 1845 ng/L) from 35% (range, 34-36%) to 13% (range, 11-15%), and at high concentrations (4155 and 7517 ng/L) from 25% (range, 24-27%) to 7% (range 7.0-7.4%). For patient samples at low cTnI concentration, average variation reduced from 40% (range, 11-65%) to 22% (range, 11-38%), at medium concentration from 37% (range, 16-63%) to 20% (range, 7-58%), and at high concentration from 29% (range, 13-63%) to 14% (range, 7-42%). Overall, the 16 assays demonstrated negligible bias after realignment; however, a few samples showed substantial positive and/or negative differences for individual cTnI assays that contributed to larger inter-assay variability than for the serum pools.

Conclusion: cTnI values for pooled samples were equivalent within acceptable limits after straightforward assay realignment. This evidence indicates that pools are a viable alternative for providing large volumes of commutable sample for use as a surrogate matrixed RM for cTnI standardization.

B-359

Preliminary Concentration and Density Analyses of Candidate Reference Material SRM 2924 C-Reactive Protein Solution

E. L. Kilpatrick, M. S. Lowenthal. *National Institute of Standards and Technology, Gaithersburg, MD*

The National Institute of Standards and Technology (NIST) has procured recombinant C-reactive protein (CRP) to generate SRM 2924, C-Reactive Protein Solution. This candidate material is intended to serve as a "pure substance" reference material traceable to SI units for use as a calibrant in the analysis of future reference materials containing CRP in biological matrices such as serum. The material was received from the manufacturer in 1200 vials each containing 1 mL of aqueous buffer with a target concentration of 0.49 g/L to 0.51 g/L of CRP. Certification of this candidate material will require the determination of purity, density, molar mass, structure and accurate concentration with homogeneity across the material space. The analysis of concentration will be determined by amino acid analysis (AAA) via liquid chromatography isotope dilution tandem mass spectrometry. A sampling plan was devised to determine concentration homogeneity by analyzing in four independent groups selected by stratified random sampling. AAA was performed on the first group by vapor phase hydrochloric acid hydrolysis (105˚C for 28 hr) of dried samples spiked with isotope labeled free amino acids (isoleucine, leucine, phenylalanine, proline and valine) and calibrated by similarly processed unlabeled amino acids in a five point linear regression curve. NMIJ 6201-b No. 2, C-reactive Protein Solution, was analyzed in triplicate as a control material to assess accuracy. The five calibrants, six samples and three controls were hydrolyzed within the same hydrolysis vessel. Density measurements were determined for the same samples by the Lang-Levy method with a 500 µL pipet calibrated using pure water. The regression coefficient for calibrants of the five individual amino acids ranged from 0.9996 to 0.9999 indicating excellent linearity within the measurement range and produced similar slope and intercept coefficients. The results of the first group (n=6) indicated that the concentration of the material by AAA was 20.0 µmol/kg (CV = 1.7 %). The value for the control material was found to be 39.2 µmol/kg (CV = 0.2 %) matching well with the certified value of 40.0 µmol/kg (1.6 µmol/kg expanded uncertainty). The higher variation of the sample results vs control is expected because the sample values include bottle-to-bottle variation while the control has only within-bottle variability. The density was determined to be 1.0050 g/mL (n = 6) which was used to convert the concentration to a mass/volume basis with a value (0.46 g/L) that is close to that based on UV absorbance as determined by the manufacturer (0.49 g/L). Although the concentration is slightly below expectations, the material remains a candidate for certification. These results do not include the full set of planned sampling and are uncorrected for calibrant purity and water content. However, they do indicate that preliminary concentrations and homogeneity are sufficient for further analysis leading to the future certification of this candidate material as a NIST Standard Reference Material.

B-360

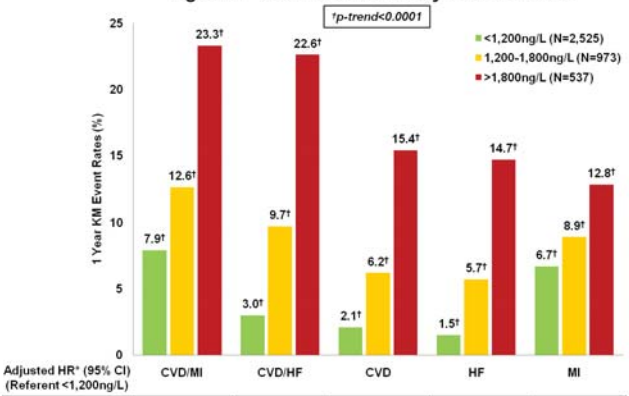
Growth differentiation factor-15 levels predict recurrent cardiovascular events in patients with an acute coronary syndrome in MERLIN-TIMI 36

P. Jarolim, E. A. Bohula May, M. Bonaca, B. Scirica, J. Kuder, S. Murphy, M. Sabatine, D. A. Morrow. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

Background: Growth differentiation factor-15(GDF-15), a marker of myocardial stress, has demonstrated a clear association with mortality. However, the association with myocardial infarction (MI) has been less consistent. We assessed the prognostic performance of GDF-15 for recurrent cardiovascular (CV) events in patients with non-ST elevation acute coronary syndrome (NSTE-ACS). Methods: GDF-15 (R&D Systems) was measured at enrollment in 4,035 subjects from MERLIN-TIMI 36, a randomized, placebo controlled trial of ranolazine in patients with moderate to high risk NSTE-ACS. Patients were classified according to the previously established cutpoints for GDF-15 of <1,200 (n=2,525), 1,200-1,800 (n=973) and >1,800 ng/L (n=537). Endpoints of CV death (CVD), MI and heart failure (HF) were adjudicated by a central blinded events committee. Results: Patients with higher GDF-15 values tended to be older and more likely to have hypertension, diabetes and prior CV disease. The rates of the composite of CVD/MI and CVD/HF, as well as the individual endpoints through one year were higher in patients with higher baseline levels of GDF-15 (p-trend<0.0001 for all endpoints; Figure 1, top). This relationship was

also apparent as early as at 30 days for CVD/MI (3.6% vs 5.5% vs 8.7%, p<0.0001) and CVD/HF (1.2% vs 3.7% vs 10.1%, p<0.0001). Higher GDF-15 levels remained significantly associated with CVD and HF, but not MI, after adjustment for multiple factors (Figure 1, bottom). Conclusions: Following NSTE-ACS, GDF-15 provided prognostic value for CVD and HF, but not MI.

Figure: 1 Year Event Rates by GDF-15 Level



Adjusted HR* (95% CI) (Referent <1,200ng/L)	CVD/MI	CVD/HF	CVD	HF	MI
1,200-1,800	1.1 (0.9, 1.4)	1.9 (1.4, 2.6)	1.7 (1.1, 2.5)	2.3 (1.5, 3.6)	1.0 (0.7, 1.3)
>1,800	1.6 (1.3, 2.1)	3.6 (2.6, 4.9)	3.2 (2.2, 4.8)	4.7 (3.0, 7.2)	1.1 (0.8, 1.5)

*Adjusted for prior HF, CrCl, BNP and TIMI risk score components, including troponin

B-361

The influence of eGFR on high-sensitivity troponins T and I in asymptomatic non-dialysis-dependent patients

T. C. Aw, S. K. Phua. *Changi General Hospital, Singapore, Singapore*

Introduction: Troponins are the preferred cardiac biomarkers. High-sensitivity troponins (hs-cTn) has enabled earlier diagnosis of acute myocardial infarction and better definition of cardiac risk. Troponins are frequently elevated in chronic kidney disease (CKD). CKD is associated with increased risk of cardiovascular morbidity and mortality. It remains unclear if troponin elevation in CKD is consequent to increased troponin release from myocardial injury or due to impaired renal clearance. We decided to investigate the influence of renal dysfunction, as reflected by the estimated glomerular filtration rate (eGFR), on serum hs-cTn in asymptomatic CKD patients not on dialysis.

Methods: We measured serum hs-TnT (Roche) and hs-TnI (Abbott) in 530 ambulatory outpatient subjects (265 women) across a range of eGFR (CKD-EPI) [Stage 1-4 CKD]. Pregnant subjects and those from the emergency, renal and cardiac departments were excluded as were those recently hospitalized (< 30 days). The gender-specific 99th percentile upper reference limits (99PURL) for hs-TnT (Giannitsis. ClinChem2010;56::254) and hs-TnI (Aw. ClinChimActa2013;422:26) were – M: 14.5, 32.7 ng/L and F: 10, 17.9 ng/L respectively. An additional 60 subjects (31 females) with stage 5 CKD (eGFR < 15 ml/min) who were not on dialysis were also studied.

Results: Overall hs-TnT was above the 99PURL in 57.5% (305/530) of subjects with CKD stage 1-4 while hs-TnI was increased in 10.6% (56/530). While hs-TnT and hs-TnI values were 45.2% concordant in the normal range, only 9.6% of patients had both hs-TnT and hs-TnI elevation. eGFR impacts hs-TnT more markedly than hs-TnI (< 60 versus < 45 mL/min respectively) (see Table).

Table. Distribution of Elevated hs-troponin values by CKD stage

CKD stage (eGFR ml/min)	1 (90)	2 (60-89)	3a (45-59)	3b (30-44)	4 (15-29)	5 (<15)	Sub-total
N	104	117	102	100	107	60	590
Hs-TnT elevation - n (%)	23 (22.1)	28 (23.9)	55 (53.9)	80 (80)	99 (92.5)	39 (65)	344
Hs-TnI elevation - n (%)	3 (2.9)	8 (6.8)	8 (7.8)	18 (18)	19 (17.8)	22 (36.7)	78

Conclusion: hs-TnT concentrations are more susceptible to renal dysfunction than hs-TnI. When evaluating patients with chest pain, diagnosis and risk stratification needs to take into account the influence of eGFR especially in those with stage 3 CKD and beyond.

B-362

Magnitude of short- and long-term intra-patient BNP variation in low BNP patients: Implications for more rational BNP utilization

G. S. Cembrowski[1], A. R. Cembrowski[2], A. K. Füzéry[3], D. T. Holmes[4]. *[1]University of Alberta Hospital, Edmonton, AB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Alberta Health Services, Edmonton, AB, Canada, [4]University of British Columbia, Vancouver, BC, Canada*

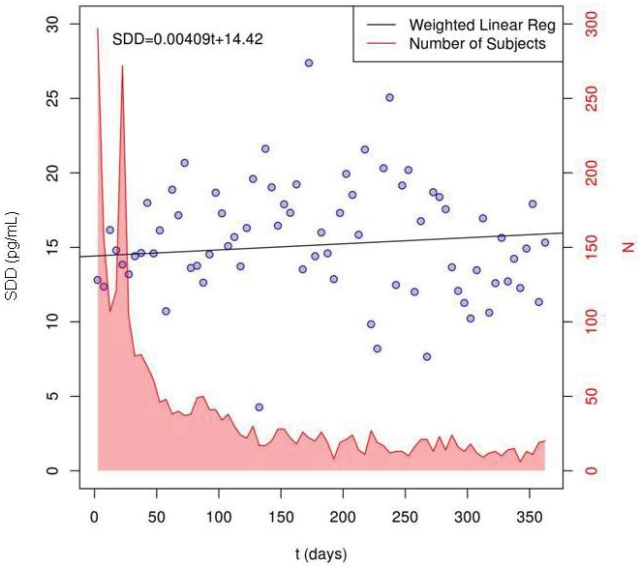
Introduction: Edmonton hospitals have offered BNP testing to the emergency department (ED) for the last 6.5 years and to other wards (nonED) for 5 years. Despite proactive approaches to BNP ordering, BNP usage is increasing in both settings. We used a unique data mining approach to evaluate biologic variation in patients with initial BNP's less than 101 pg/mL (clearly normal). We discovered significant patient repeats as well as very low biologic variation of BNP.

Methods: We tabulated paired intra-patient BNP's repeated within 365 days of each other. The initial BNP had to be under 101 pg/mL and the second BNP could be of any value up to 20,000 pg/mL. We calculated the standard deviation of duplicates (SDD) of the intra-patient pairs grouped in 5 day intervals : 0-5d, 5-10d, 10-15d, and so forth. Using weighted linear regression, the SDDs were regressed against the time intervals. The extrapolation to zero time interval in these regressions represents the sum of the biologic variation (s_b) and the short-term analytic variation (s_a): $\mathbf{y_0}=(s_a^2+s_b^2)^{1/2}$

Results: A total of 2178 patients had initial BNP's that were under 101 pg/mL (1742 ED, 471 nonED). The figure shows SDDs for the intervals of separation between the first and second test. The y-intercept corresponds to a s_b of 14.3 pg/mL (27%) for an initial average BNP of 53.3 pg/mL.

Conclusion: The small biologic variation of patients with initial BNP's under 101 pg/mL implies that such patients have very little risk of congestive heart failure and that repeat testing will result in another low BNP. Therefore, a BNP less than 101 pg/mL should not be followed with a repeat test unless there is a clinical change in the patient.

BNP Results < 101 pg/mL



Wednesday, July 30, 2014

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

Proteins/Enzymes

B-364

Determination of reference value for ALT in our laboratory population

N. Z. Maluf, C. F. d. Pereira, M. L. Garcia, N. Silva, C. C. V. Ricardo, F. S. Fukuoka. *DASA, Barueri, Brazil*

Background: As the reference values informed in the instructions of use (IFU) are just a guidance, we know the ranges can vary from lab to lab and so each lab may have a different range for what´s normal. Thereby, the ranges for ALT (alanine aminotransferase) numbers may differ slightly depending on the technique and protocols used by different laboratories worldwide. However, normal reference ranges are routinely provided by each laboratory in their printed individual patient's reports. This study aims to establish the reference value for ALT in our laboratory population in order to ensure the best follow-up for each patient. The objetive is to evaluate and standardize the reference values of the UF1000 measured parameters.

Methods: 100 serum samples from healthy individuals (based on current IFU Siemens Dimension® ALTI method reference values: 12 - 78 U/L) were tested for ALT in two different instruments: Siemens ADVIA 2400 and Siemens Dimension RxL Max. The Siemens Dimension® ALTI and the Synermed ALT (measured on Siemens ADVIA 2400) are an L-alanine pyridoxal-5-phosphate (P5P) methodology. The reference value for Synermed ALT is from 7 to 35 U/L. We used D'Agostino-Pearson test to establish the reference values for ALT to our hospital´s patients and it represents the central 95% of results determined from a healthy population.

Results: The results are summarized in the table below.

Conclusion: The obtained ALT reference value (5.5 - 45 U/L) is in agreement with the values from the literature. Finding our own reference values is crucial to ensure reliable results and, consequently, improve the patient´s quality of life.

	N	Mean	Median	SD	Lowest value	Highest value	Lower limit	Upper limit
ALT, U/L Dimension ALTI	100	25.24	24.20	9.67	12.2	71.6	5.5	45
ALT, U/L Synermed ALT	100	22	21	7.7	8	54	7	35

B-365

Evaluation of a novel enzymatic HbA1c test on the fully automated system respons®910

A. Lein, H. Mueller, J. Rink, S. Rosenthal, K. Hahne, D. Vendt, A. Grzesista, H. Baethies, T. Maerker, E. Metzmann, M. Grimmler, G. Gorka. *DiaSys Diagnostic Systems, Holzheim, Germany*

Background: Glycated Hemoglobin A1c (HbA1c) is a well established parameter for long-term monitoring and diagnosis of diabetes. Here, we present a novel enzymatic HbA1c test (HbA1c net FS) for highly specific detection of HbA1c, excluding putative interferences by common hemoglobin (Hb) variants. HbA1c net demonstrates excellent precision based on the application type (twin-test). This test links 2 calibrations and 2 detections for Hb and HbA1c in only **one** determination. The test principle is defined by Hb determination after sample hemolysis at 570 nm and H_2O_2 release after oxidative cleavage of fructosylated dipeptides in the same cuvette. H_2O_2 concentration is determined colorimetrically at 660 nm, whereas delta absorbance is proportional to the HbA1c concentration. The aim of this study was to establish a novel enzymatic HbA1c test, with superior performance, convenient handling and optimized workflow compared to other common methods in the market (as immunoassays or HPLC).

Methods: Assay adaption and performance verification have been carried out on respons®910. All reagents,calibrators and controls were from DiaSys Diagnostic Systems GmbH. Method comparisons were performed against HPLC as reference system. Data have been evaluated by using regression analysis according to Passing and Bablok. Inter-and intra-assay imprecision were performed according to the CLSI protocol (EP5-A2). Determination of hemoglobin subtypes was carried out by recovery measurements of IFCC Hb-subtype evaluation samples.

Results: A direct method comparison of HbA1c net values obtained on r910 against HPLC (BioRad Variant II) with 90 native samples demonstrated excellent correlation [r=0.9977; Passing/Bablok: y=1.015 x - 0.23%(DCCT)]. DiaSys HbA1c net FS test is highly precise with an intra-assay precision of CV<0.7% (for HbA1c values from 5.7 to 13.0%) and an inter-assay precision of CV<2.1% (for HbA1c values from 4.4 to 9.9%). High accuracy of HbA1c net FS was demonstrated by recovery IFCC controls (with varying Hb and HbA1c levels) within ± 3% of the target value. Various Hb variants as HbAA, HbAC, HbAD, HbAE, HbAJ, HbAS, HbCC, HbEE, HbSC, HbSS, elevated HbF and b-Thalassemia showed no significant interference with HbA1c net FS.

Conclusion: DiaSys new enzymatic HbA1c assay reveals outstanding specificity and precision. This test highly correlates to HPLC (NGSP/DCCT) but also to IFCC reference material and is unaffected by interferences from common Hb variants. By application of HbA1c net to the fully automated DiaSys system respons910, HbA1c workflow is optimized, due to the implemented on-board hemolysis eliminating error-prone and time-consuming manual preparation.

B-366

Platelet Hyaluronidase-2 as a Potential Novel Biomarker for Both Inflammatory Bowel Disease and Platelet Function.

S. Albeiroti, K. Ayasoufi, C. de la Motte. *Cleveland Clinic, Cleveland, OH*

Background: Inflammatory Bowel Disease (IBD), a group of chronic inflammatory conditions of the intestine, consists of two main types: Crohn's Disease (CD) and Ulcerative Colitis (UC). Different platelet abnormalities are associated with IBD including reactive thrombocytosis, decreased mean platelet volume (MVP) and increased state of activation in the circulation. Our lab has recently reported that platelets contain the enzyme hyaluronidse-2 (HYAL2), which is a GPI-anchored protein that digests hyaluronan (HA). HA is a major polysaccharide component of the extracellular matrix and its deposition is increased during intestinal inflammation. Importantly, HA fragments resulting from HYAL2 digestion can promote inflammatory cytokine production. We therefore hypothesize that platelet HYAL2 plays a role in IBD.

Objective: To compare platelet HYAL2 levels between IBD patients and non-IBD controls and to define normal platelet expression parameters in resting and activated platelets.

Methods: Platelets from IBD patients and non-IBD controls were isolated, washed and lysed (n=12). Protein contents of platelet lysates were measured by Bradford assay and HYAL2 levels were compared using immunoblot assay standardized to protein content (25 µg of total protein). Flow cytometry was used to analyze surface HYAL2 levels of paraformaldehyde fixed platelets from normal donors (n=9). Platelets were activated using thrombin receptor activating peptide (TRAP) and surface P-selectin was used to assess platelet activation state.

Results: 1) Immunoblot analysis of platelets from IBD patients showed an average reduction of 41% in total HYAL2 levels compared to their non-IBD counterparts (p-value < 0.05). 2) Flow cytometric analysis showed that only 6.8 ± 1.3% (±SE) of isolated, normal, non-activated (P-selectin negative) platelets express surface HYAL2. However, after activation the percentage of platelets expressing surface HYAL2 increased to 37.6 ± 6.4% (±SE) (p-value < 0.001).

Conclusion: Platelet HYAL2 levels are significantly lower in patients with IBD compared to non-IBD controls, which demonstrates its potential usefulness as a marker in screening patients with IBD. In addition, we showed that platelet HYAL2 surface expression is significantly higher in activated platelets compared to resting platelets. This reveals that platelet HYAL2, similar to P-selectin, is a potential marker for platelet activation, indicating usefulness in platelet function tests.

B-367

Determination of Fecal Pancreatic Elastase using an Automated Immunoassay Procedure

C. Cruzan, P. Krause, K. Urek, J. A. Maggiore. *Doctor's Data, Inc., Saint Charles, IL*

Background: The clinical laboratory's role in the assessment of chronic digestive disorders and bowel diseases continues to expand. Exocrine pancreatic insufficiency as a contributing source of bowel-related diseases is commonly assessed by measuring

fecal levels of pancreatic Elastase, a serine protease that hydrolyzes dietary proteins. The absence or insufficiency of pancreatic Elastase contributes to incomplete protein digestion leading to varying degrees of malnutrition, and to pathologies ranging from abdominal discomfort to maladies of the large intestine. Manual ELISA techniques to measure fecal pancreatic Elastase are labor-intensive and prone to high variability related to sample handling. An automated sample processing and analytical platform was sought to enhance operating efficiency, reduce variability, and retain analytical accuracy.

Methods: The BIOSERV Diagnostics solid-phase enzyme linked immunosorbent assay (ELISA) is based on a double sandwich technique employing two polyclonal antibodies engineered to recognize several epitopes on human pancreatic Elastase peptide sequences; the first is coated to the assay wells of the microplate, and the second is labeled to biotin which binds to the immobilized Elastase molecule. A streptavidin-labeled horseradish peroxidase binds to the biotin-Elastase complex, which oxidizes the tetramethylbenzidine (TMB) chromogenic substrate to a blue color in the well-characterized reaction. The required sample dilution steps and the procedural sequence of substrate addition, conjugation, washing, incubation, and reading were programmed into a Dynex DSX® analyzer. Analytical precision, linearity, recovery, and accuracy of this automated process were assessed. Further, time studies of technologist direct labor and analytical throughput were undertaken to assess the benefits of automation.

Results: Using the automated procedure on the Dynex DSX, intra-assay precision coefficients of variation (CV) (n=20) at pancreatic Elastase levels of 107 and 459 µg/g were 8.4% and 4.3%, respectively. Inter-assay CV (n=20) at the same pancreatic Elastase levels were 12.0% and 6.4%, respectively. The inter-assay CV and intra-assay CV using the manual ELISA method at a level of 484 µg/g were 4.8% and 8.9%, respectively. Assay linearity was demonstrated between 28 and 500 µg/g, with recovery of expected pancreatic Elastase concentrations ranging from 101.5% to 112.7%. Least-squares regression analysis comparing pancreatic Elastase values from diluted fecal extracts as determined by manual ELISA and automated DSX ELISA processing (n=35, range 35 - 485 µg/g) yielded a correlation coefficient (R^2) of 0.93, y = 1.10x + 58.6; standard error of estimate = 48.5. Time studies (n = 5) indicate that the automated procedure provides an average reduction of 147 minutes of direct, hands-on labor of a technologist processing a 96-well plate of samples, quality controls, and calibrators. Overall, start-to-finish analytical throughput averages 244 minutes using manual processing, compared to 303 minutes using the Dynex DSX program for pancreatic Elastase.

Conclusion: The automated ELISA procedure for fecal pancreatic Elastase using the Dynex DSX has been shown to maintain accuracy and improve analytical precision of the BIOSERV Diagnostics ELISA method. Despite increased overall analytical throughput, the automated procedure provides operating efficiency through the reduction of direct hands-on time.

B-368

Clinical application of urinary protein profiling using cellulose acetate membrane electrophoresis for patients with IgA nephropathy

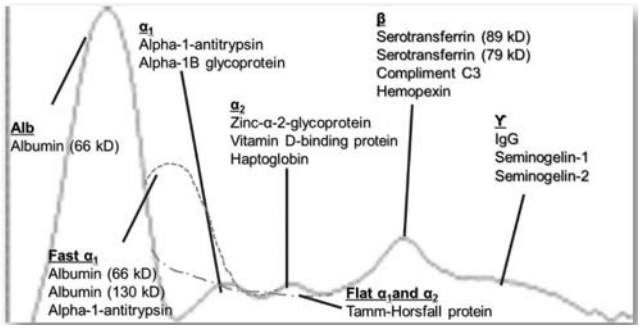
A. Nakayama[1], M. Sakatsume[2], A. Katayama[3], H. Suzuki[3], K. Shiba[1], S. Iijima[1]. *[1]Bunkyo Gakuin University, Tokyo, Japan, [2]Niigata University, Niigata, Japan, [3]Nippon Medical University, Tokyo, Japan*

Background: Urinary protein patterns obtained from cellulose acetate membrane (CAM) electrophoresis are useful for renal damage prediction in patients with glomerulonephritis such as IgA nephropathy (IgAN). To identify the disease-causing proteins in each CAM fraction, we established a protein extraction method for proteomic analysis and developed protein profiles in the patients with IgAN.

Methods: Urine samples from 30 patients with IgAN were each applied onto 10 lanes of a CAM. After electrophoresis, the first and last lanes were cut, silver-stained, and used as a guide for fractions in the unstained region. These sections were fragmented, and proteins were extracted with Laemmle's sample buffer, separated by non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis, silver-stained, and identified by liquid chromatography-tandem mass spectrometry.

Results: Urinary protein profiles for 30 patients with IgAN showed 3 main patterns (types A - C): in type A (13 patients), 5 fractions were similar to the normal serum pattern; in type B (14 patients), mobility of the α1-globulin fraction was relatively fast, similar to that of albumin; and type C (3 patients) showed a lack of α1-, α2-, and γ-globulin fractions. The fast α1-globulin fraction of type B mainly comprised an albumin dimer, which is formed in response to oxidative stress, and α1-antitrypsin. Although there were no bands corresponding to α1- and α2-globulin in type C, a significant amount of Tamm-Horsfall protein, reflecting normal tubular function, was detected. A schematic diagram of the overall urinary protein pattern in IgAN was established (Figure 1) based on proteins identified in more than 50% of subjects of each profile pattern.

Conclusion: The type B urinary protein profile pattern indicates increased oxidative stress compared to type A, whereas type C indicates mild renal impairment compared to types A and B. Our detailed analysis provides a valuable non-invasive tool for predicting the degree of renal damage in IgAN.



B-369

Performance Evaluation of ERM-DA471/IFCC standardized Tina-quant® Cystatin C Generation 2 Assay on Roche Clinical Chemistry Analyzers

C. Schmidt[1], C. M. Cobbaert[2], J. A. Bakker[2], H. P. C. Schippers[2], W. Endlich[2], M. Flodin[3], P. Eliasson[3], D. Hermsen[1]. *[1]University Hospital of the Heinrich-Heine University, Central Institute of Clinical Chemistry and Laboratory Diagnostics, Düsseldorf, Germany, [2]Leiden University Medical Center (LUMC), Department of Clinical Chemistry and Laboratory Medicine, Leiden, Netherlands, [3]University Hospital, Clinical Chemistry and Pharmacology, Uppsala, Sweden*

OBJECTIVE Cystatin C has been proposed as a more sensitive endogenous biomarker regarding the estimation of glomerular filtration rate when compared to serum creatinine. But Cystatin C assays of different manufacturers showed a lack of comparability before the ERM-DA471/IFCC reference material was available. In our study we evaluated the analytical performance of the new ERM-DA471/IFCC standardized Tina-quant® Cystatin C Gen. 2 immunoassay (Roche Diagnostics, Mannheim, Germany).

METHODS AND RESULTS The analytical performance of the new assay was evaluated in three laboratories using Roche/Hitachi **MODULAR *ANALYTICS***, COBAS INTEGRA® 800, **cobas c** 701 and **cobas c** 501 analyzers. Cystatin C concentration was measured turbidimetrically by a particle enhanced immunoassay standardized against the ERM-DA471/IFCC. The analytical performance of the new assay was investigated under routine laboratory conditions using samples covering the entire measuring range of cystatin C (0.4 - 6.8 mg/L).

Within-run imprecision data were collected using three control levels (Roche Diagnostics) and three self prepared pools of human sera (single run, n = 21 replicates per sample) covering a concentration range from 0.75 to 5.02 mg/L. Coefficients of variation (CVs) were determined to be less than 3.3 % for control materials and less than 2.8 % for pooled samples. Between-day imprecision yielded CVs between 1.7 and 7.3 % (one run/day, up to 17 days) using control sera (three levels, Roche Diagnostics). The recovery of target values in different control sera was determined performing triplicate measurements in three independent runs. The recovery of Cystatin levels in control sera from Roche Diagnostics and other manufacturers ranged between 86.0 - 107.8 % and 86.4 - 100.5%, respectively.

All method comparison experiments were designed in compliance with CLSI EP09-A3, performed using > 119 serum samples and analyzed with Passing-Bablok regression. Statistical analysis of method comparison experiments against the Roche Cystatin C Gen.1 yielded correlation coefficients > 0.990, slopes between 0.92 and 1.13 (0.89 - 1.16, 95 % confidence interval) and intercepts from -0.13 to 0.07 (-0.16 - 0.10) mg/L. Method comparison studies against other available ERM-DA471/IFCC standardized Cystatin C immunoassays (Siemens N Latex Cystatin C, Gentian Cystatin C) performed on different analyzer systems (Siemens BN II Nephelometer, Abbott Architect, Roche cobas c502) showed excellent correlation coefficients (r > 0.990). Calculated slopes ranged between 0.93 and 1.04 (0.91-1.06), and intercepts between -0.10 to 0.04 (-0.13 - 0.07) mg/L, respectively, confirming the successful standardization of the new Cystatin C Gen. 2 immunoassay to the ERM-DA471/IFCC reference material.

CONCLUSIONS Our study demonstrated an excellent technical performance of the new Roche Tina-quant® Cystatin C Gen. 2 assay. Method comparison results revealed a high degree of comparability to other available Cystatin C immunoassays that are traceable to ERM-DA471/IFCC. Due to the excellent study results the new Tina-quant® Cystatin C Gen. 2 assay is well-suitable for routine use.

Disclaimer: The study was sponsored by Roche Diagnostics GmbH, Germany. The Roche Tina-quant® Cystatin C Gen.2 assay is not yet cleared for use in the U.S.

COBAS, COBAS C, COBAS INTEGRA, MODULAR and TINA-QUANT are trademarks of Roche.

B-370

Effects of CH3CN-cosolvent and CuBr-catalyst on the synthesis of difluorophosphonates as chemical specialty

H. Tsai. *Department of Chemistry and Material Engineering, Chung Cheng Institute of Technology, National Defense University, Ta-hsi, Tao-yuan, Taiwan*

Background: Fluorophosphonates have found applications as hyperlipidaemic drugs, hormone substitutes, cancer chemotherapy and nervous chemical warfare. However there is generally a conspicuous lack of methods for the preparation of difluoromethanephos phonates. Effects of CH_3CN-cosolvent and CuBr-catalyst on the synthesis of difluoro phosphonates $(EtO)_2P(O)CF_2C(O)R$ were discussed in this research.

Methods: Synthesis of $(EtO)_2P(O)CF_2Br$: ^{19}F NMR: -62.0 ppm (d, J = 90.3); ^{31}P NMR: -0.65 ppm (t, J = 93.7); ^{1}H NMR: 4.42 ppm (4H, q, J = 7.1), 1.42 ppm (6H, t, J = 7.1). Procedure for Synthesis of $(EtO)_2P(O)CF_2C(O)CO_2Et$: (Method A): A three-necked flask was charged with 15.0 mL of a 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$. 42.0 mmols of freshly distilled $ClC(O)CO_2Et$ was added and stirred at rt for 48 hours. The mixture was filtered, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, concentrated by rotary evaporation and vacuum distilled at 94-95°C/0.15 mmHg to give 54 % of the titled compound. ^{1}H NMR : 1.40 (9H, t, J = 7.1), 4.30 (6H, m, J = 7.1). ^{13}CNMR : 13.9 (s), 16.2 (d, J = 5), 63.5 (s), 65.8 (d, J = 7), 115.5 (t, d, J = 275, J = 199), 158.5 (s),181.8 (t, d, J = 16). ^{19}FNMR : -115 (d, J = 95). ^{31}PNMR : 4.5 (t, J = 95). (Method B): 7.5 mL dry CH_3CN, 0.45 mmol CuBr and 42.0 mmols $ClC(O)CO_2Et$ were added to a 15.0 mL of 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$. Stirred at rt for 0.5 hour. 58 % of the titled compound was afforded. (Method C): 0.45 mmols CuBr and 42.0 mmols of $ClC(O)CO_2Et$ were added to a 15.0 mL of 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$, Stirred at rt for 24 hours. The ^{19}F NMR spectrum indicated the presence of $(EtO)_2P(O)CF_2C(O)CO_2Et$, (E,Z)-$(EtO)_2P(O)CF{=}CFP(O)(OEt)_2$ and $(EtO)_2P(O)F$.

Results: Reaction of triethylphosphite with dibromodifluoromethane *via* Michealis-Arbuzov reaction gave 95 % yield of $(EtO)_2P(O)CF_2Br$. Subsequently reacted with acid washed zinc powder in the presence of monoglyme, $(EtO)_2P(O)CF_2ZnBr$ was obtained. It took 48 hours to synthesize $(EtO)_2P(O)CF_2C(O)R$ from the acylation of $(EtO)_2P(O)CF_2ZnBr$ with $ClC(O)R$ (R = CO_2Et, OEt, NEt_2) at rt However, this situation can be easily ameliorated upon addition of a catalytic amount of cuprous bromide and the addition of acetonitrile as cosolvent to the reaction mixture. This reaction is completed within 0.5 hour to yield $(EtO)_2P(O)CF_2C(O)CO_2Et$ in a 58 % isolated yield. Furthermore, if the reaction was carried out in the presence of 1.5 % CuBr-catalyst without the CH_3CN as cosolvent, in addition to the formation of 12 % of $(EtO)_2P(O)CF_2C(O)CO_2Et$, 18 % yield of (E) and (Z)-(1,2-difluoroethylenediyl) bisphosphonate $(EtO)_2P(O)CF{=}CFP(O)(OEt)_2$ and 70 % of $(EtO)_2P(O)F$ at -82 ppm ($J_{P\text{-}F}$ = 972 Hz) were observed in ^{19}FNMR spectrum.

Conclusion: In the presence of CH_3CN-cosolvent, monoglyme solvent and appropriate CuBr-catalyst, acylation of $(EtO)_2P(O)CF_2ZnBr$ with ethyl chloroformate, diethyl carbamoyl chloride or ethyl oxalyl chloride gave good yields of 2-oxo-1,1-difluorophos phonates $(EtO)_2P(O)CF_2C(O)R$ as chemical specialty. However, if these acylations were carried out without acetonitrile as cosolvent, the mixture products of $(EtO)_2P(O)CF_2C(O)CO_2Et$, (E,Z)-$(EtO)_2P(O)CF{=}CFP(O)(OEt)_2$ and $(EtO)_2P(O)F$ were observed.

B-371

Neutrophil Gelatinase-Associated Lipocalin Levels in Patients with Thalassemia and Sickle Cell Disease: Correlation with Renal Injury

I. Papassotiriou[1], E. Voskaridou[2], A. Margeli[1], A. Kattamis[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Thalassemia Center, "Laikon" General Hospital, Athens, Greece, [3]Hematology-Oncology Divison, Department of Pediatrics, University of Athens Medical School, Athens, Greece*

Background and Aims: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily initially found in activated neutrophils, in accordance with its role as an innate antibacterial factor. However, it subsequently was shown that many other types of cells, including in the kidney tubule, may produce NGAL in response to various injuries. The increase in NGAL production and release from tubular cells after harmful stimuli of various kinds may have self-defensive intent based on the activation of specific iron-dependent pathways, which in all probability also represent the mechanism through which NGAL promotes kidney growth and differentiation. NGAL levels clearly correlate with severity of renal impairment, probably expressing the degree of active damage underlying the chronic condition. For all these reasons, NGAL may become one of the most promising next-generation biomarkers in clinical nephrology and beyond. We aimed to investigate the clinical significance of NGAL levels and its correlation with renal function in patients with hemoglobinopathies.

Patients and Methods: 117 adult patients with hemoglobinopathies were included in the study divided in 3 groups. Group A: 30 patients with transfusion-dependent thalassemia major (TM); Group B: 29 patients with thalassemia intermedia (TI) and Group C: 58 patients with HbS/betathalassemia disease, while 20 apparently healthy individuals served as controls (Group D). In patients and controls along with standard blood and urine chemistry, measurements of serum Cystatin C and NGAL were performed. Estimated Glomerular Filtration Rate (eGFR) values were calculated with an unadjusted for body surface Cystatin C based equation: eGFR (mL/min)=77.24(Cys C)exp(-1.2623)

Results: The main results of the study showed that: a) NGAL levels were significantly higher in all the groups of patients compared to controls: Group A 95.0±45.0μg/L, Group B 139.1±86.1μg/L, Group C 117.8±37.3μg/L vs Group D 50.3±11.3μg/L ($p<0.001$), b) Cystatin C levels were significantly higher in patients of Group A 0.94±0.34mg/L and Group C 1.04±0.50mg/L compared to controls 0.75±0.09mg/L (p0.300), c) NGAL levels and eGFR values (Group A: 96.9±39.8, Group B: 117.0±26.0, Group C: 86.2±27.8 and group D: 109.6±15.0mL/min, respectively) correlated significantly in patients of Group A and Group C ($r=-0.739$, $p<0.001$ and $r=-0.735$, $p<0.001$, respectively), while NGAL values are independent from eGFR values in patients of Group B.

Conclusions: These findings illustrate the tubular-glomerular activation feedback mirrored by NGAL in patients with transfusion-dependent thalassemia major and HbS/betathalassemia disease, who suffer from renal injuries, indicating that tubular damage precedes GFR reduction. Upregulation of NGAL in patients with thalassemia intermedia independently of renal injuries may reflect the compensatory, protective role of NGAL in response to diverse cellular stresses, including inflammation and oxidative stress. However, recent reports have implicated NGAL upregulation as a mechanism that contributes to anemia in the setting of chronic low grade inflammation. In experimental models, systemic and medullary NGAL has been demonstrated to induce inhibition of erythropoiesis through induction of apoptosis and arrest of differentiation of erythroid progenitor cells.

B-372

Evaluation of Dried Blood Spots for Use in Isoelectric Focusing Electrophoresis in Deficient Alpha-1-Antitrypsin Phenotype Interpretation

M. M. Duran. *Geonostics, Lincolnshire, IL*

Background: Laboratory diagnostics contribute significantly in the diagnosis of Alpha-1-Antitrypsin (AAT) deficiency, utilizing AAT serum concentration, AAT phenotype determination by isoelectric focusing (IEF) electrophoresis, and genotyping. Dried blood spots (DBS) are a potentially attractive sample type for IEF phenotype analysis on the Sebia Hydrasys because of the ease of sample collection. Previous work demonstrated that the common phenotypes of MM, MS, and MZ, as a DBS sample, were indistinguishable from their companion serum samples. In this current study, we present the novel methodology of DBS for the 3 most prevalent deficient phenotypes: SS, SZ, and ZZ.

Methods: Eighteen whole blood samples from known phenotypes SS, SZ, and ZZ

were spotted on filter paper using 50 uL of sample. The blood spots dried overnight and then the 50 uL dried blood samples were punched and rehydrated with a buffer/deionized water solution. Ten uL of each extracted DBS sample was applied to the IEF comb. Eighteen serum samples of the corresponding DBS phenotypes were also placed on a comb for comparison. IEF was performed on the Sebia Hydrasys using standard protocol and the resultant gel was stained, washed, and digitally scanned.

Results: In Figure 1, phenotype SS is on the left, phenotype SZ is in the center, and phenotype ZZ is on the right. For each phenotype, the DBS sample is on the left and the corresponding serum sample is on the right. All 18 DBS phenotype samples displayed the unique identifiable banding patterns present in the serum samples.

Conclusion: IEF of DBS samples adequately reveal the S and Z alleles, in the absence of the M allele, on the Sebia Hydrasys. Work is underway to validate the 100+ additional rare alleles and to establish AAT protein stability on filter paper

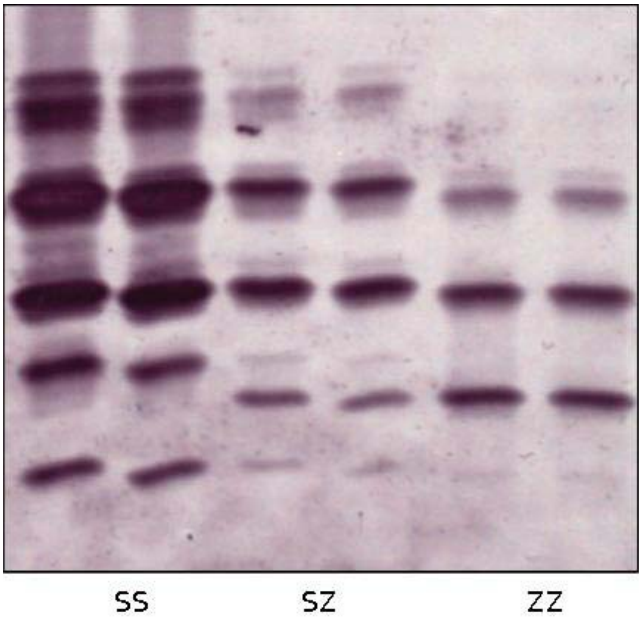


Figure 1

B-373

Evaluation of INNOTEST® β-Amyloid (1-40) immunoassay and comparison with IBL™ Human Amyloid (1-40) immunoassay

J. Oudart, R. Mahmoudi, G. Fiabane, P. Arthuis-Demoulin, J. Novella, L. Ramont. *CHU de Reims, Reims, France*

Background: Combined assay of cerebrospinal fluid (CSF) biomarkers Tau protein, phosphorylated Tau protein and beta Amyloid peptide 1-42 (Aβ42) is commonly used in the diagnosis of Alzheimer's disease (AD). CSF biomarkers are now included in diagnosis criteria in addition to clinical evaluation and medical imaging. In discordant cases where an isolated reduction of Aβ42 peptide is found, the beta Amyloid peptide 1-40 (Aβ40) measurement associated with Aβ42/Aβ40 ratio allows better classification of patients. According to the literature, the clinical cut off for this ratio is less than 0.05 in AD.

The aim of this study was to evaluate the INNOTEST® β-Amyloid (1-40) immunoassay and compare it versus IBL™ Human Amyloid (1-40) immunoassay.

Methods: - Evaluation of Innotest® β-Amyloid (1-40) immunoassay (Fujirebio Europe, Belgium): intra-assay precision studies were evaluated on two CSF samples (5000 and 9800 ng/L respectively), 16 consecutive times. Inter-assay precision studies were evaluated at the 2 same levels of concentrations (n= 10). Dilutional linearity was evaluated by successive dilutions of a CSF sample in the sample buffer.

- Comparison between INNOTEST® β-Amyloid (1-40) immunoassay and IBL™ Human Amyloid (1-40) immunoassay (IBL, Japan): seventy four patients were included in this study. Aβ40 peptide concentration was assessed using the 2 immunoassays for all patients.

Differences between assays were analyzed by Pearson Test and the degree of agreement between measurements was evaluated using Bland-Altman analysis.

Results: For Aβ40 measurement with the INNOTEST® β-Amyloid (1-40) immunoassay, the intra-assay CVs were 1.29% and 2.68% at Aβ40 concentrations of 5000 and 9800 ng/L respectively (n= 16). The inter-assay CVs were 13.94% and 15.66% at the same Aβ40 concentrations (n = 10). The mean SD recovery of CSF Aβ40 immunoassay was 85% (slope: 1.0264; intercept: -328.83; R2= 99.8%).

The correlation coefficient between the 2 immunoassays (INNOTEST® vs IBL™ assay) was R2= 0.735. Interpretation of Aβ42/Aβ40 ratio was concordant between the 2 immunoassays at 92% (68/74) of the cases using a cut-off at 0.05.

Conclusion: This preliminary study showed that INNOTEST® β-Amyloid (1-40) immunoassay's intra-assay CVs, inter-assay CVs and linearity CVs were less than 20%. INNOTEST® β-Amyloid (1-40) immunoassay seems to correlate with IBL™ Human Amyloid (1-40) immunoassay which is commonly used in the laboratories.

B-375

Evaluation of a new liquid UIBC method on Architect c4000 analyzer

M. Gramegna, M. La Motta, R. Lucini. *Sentinel CH. SpA, Milan, Italy*

Objective The objective of this study is to evaluate the analytical and clinical performances of a new liquid UIBC Method on Architect c4000 analyzer.

Relevance: Transferrin is the principal plasma protein for transport of iron. One molecule of TRF binds two ferric ions and an associated anion, usually bicarbonate in vivo. Normally 30% of the iron binding sites of transferrin are occupied by Fe3+, the additional amount of iron that can be bound is the unsaturated iron-binding capacity (UIBC). The sum of serum iron and UIBC represents the total iron-binding capacity (TIBC). UIBC measurement is an iron panel parameter used in the diagnosis and treatment of anemia.

Methodology UIBC is determined directly by saturating the transferrin at an alkaline pH with a known excess amount of iron. The iron that remains free after transferrin saturation is reduced to ferrous state and then complexed by ferene-S to form a stable complex which colour intensity is measured at 580-600 nm. UIBC is therefore determined by subtracting the quantity of unbound iron from the total added quantity. The instrument used for this evaluation was an Architect c4000 analyzer, a random-access analyzer. To perform this evaluation, modified CLSI protocols were adopted. Acceptance criteria as total imprecision were ≤ 5% for samples ≤ 110 µg/dL and ≤ 2% for samples ≥ 250 µg/dL. LOD should be ≤ 41 µg/dL, The method should be linear up to 500 µg/dL. Claimed goal for on board calibration stability was ≥ 7 days and reagent on board stability was 35 days. Comparison to commercial methods had following acceptability: slope 0.90 - 1.10, intercept ≤ 15, r ≥ 0.975.

Validation: Total imprecision (21 days) gave CV% at 103 µg/dL lower than 5%, CV% at 136 µg/dL lower than 4% and at 269 µg/dL lower than 2%. LOD was 13.1 µg/dL. The test was linear from 19 µg/dL up to 500 µg/dL. On board reagent stability was up to 35 days and on board calibration stability was up to 21 days. Compared vs a commercial Ferrozine method (n = 82, samples between 6.5 and 486 µg/dL) linear regression gave y = 1.16x - 9.57 and r = 0.998. Compared versus Sentinel UIBC Liquid REF 17639 (n = 82, samples between 41.5 and 466 µg/dL) linear regression gave y = 1.10x - 10.3 and r = 0.997. Bilirubin (up to 66 mg/dL), hemoglobin (up to 100 mg/dL) and triglycerides (up to 1000 mg/dL) did not interfere.

Conclusions Analytical and clinical performances of new liquid UIBC method on Architect c4000 analyzer meets the acceptance criteria and it shows all the requirements for its use as routine clinical chemistry assay.

B-376

Performance characteristics of a cystatin C immunoassay on the Beckman Coulter AU5800, AU680 and IMMAGE 800 Systems

C. Townsley, T. Nilsen. *Gentian AS, Moss, Norway*

Background: Cystatin C is a biomarker of kidney function. Its measurement can be used to estimate glomerular filtration rate. The objective was to evaluate the performance characteristics of this assay on the Beckman Coulter AU5800, AU680 and IMMAGE 800 systems.

Methods: A cystatin C particle-enhanced turbidimetric immunoassay (Gentian, Norway) with avian antibodies is standardized against the international calibrator standard ERM-DA471/IFCC and holds a current FDA 510k for use on other platforms. Measurement was carried out on the Beckman Coulter AU5800, AU680

and IMMAGE 800 systems at different sites between 2008 and 2013 using a cystatin c immunoassay from Gentian, Norway. Different reagent lots were used and new serum samples and dilution series were made for each site study in the case of precision, linearity, security zone, interference, recovery and limit of quantification. Protocols based on CLSI guidelines were used.

Results: The measuring range is 0.45 - 8.0 mg/L, linearity was proven with a minimum range of 0.45-6.9 mg/L on all instruments with prozone of 32 mg/L. The total C.V. for precision ranged from 1.5 - 6.2% with total C.V. at 1 mg/L of ≤2.7%. LoQ for all instruments was observed to be <0.5 mg/L. Interference studies with potential interferents, Intralipid (≤10 g/L), hemoglobin (≤6 g/L) and bilirubin (≤200 mg/L) showed no interference. Total analysis time was 10 minutes.

Conclusion: The Gentian Cystatin C immunoassay is validated for use on the Beckman Coulter AU5800, AU680 and IMMAGE 800 systems. The assay shows acceptable performance characteristics for measuring cystatin C in human serum and plasma samples on these systems.

Summary of results for studies on AU5800, AU680 and IMMAGE 800

	AU5800	AU680	IMMAGE 800
Linearity (mg/L)	0.45 – 7.07	0.44 - 9.02	0.37 - 6.9
Recovery (%)	96 - 100	98 - 105	100 - 110
Security zone (mg/L)	32	40	40
Precision (total C.V.%)	1.62 - 3.42	1.51 - 2.44	2.7 - 6.2
Bilirubin interference testing	≤400 mg/L	≤200 mg/L	≤800 mg/L
Hemoglobin interference testing	≤6 g/L	≤8 g/L	≤8 g/L
Intralipid interference testing	≤10 g/L	≤16 g/L	≤16 g/L

B-377

Evaluation of new Lipase Color Liquid on Architect c4000 analyzer

M. Gramegna, L. Politi, R. Lucini. *Sentinel CH. SpA, Milan, Italy*

Objective The objective of this study is to evaluate the analytical and clinical performances of new Lipase Color Liquid reagent on Architect c4000 analyzer.

Relevance Lipase enzymes are produced in the pancreas and also secreted in small amounts by the salivary glands as well as by gastric, pulmonary and intestinal mucosa. Determination of lipase is used for diagnosis and treatment of diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct.

Methodology The method for the determination of lipase is based on the cleavage of specific chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid (6'-methylresorufin)-ester emulsified in stabilized micro-particles. In the presence of specific activators of pancreatic lipase as colipase, calcium ions and bile acids, the substrate is converted in 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'-methylresorufin-ester which decomposes spontaneously in glutaric acid and methylresorufin. The increase of absorbance at 580 nm, due to methylresorufin formation, is proportional to the activity of lipase in the sample. For the evaluation of this reagent, modified CLSI protocols were adopted. Acceptance criteria for total imprecision were ≤ 5% for lipase samples. LOD should be ≤ 3.0 U/L. LOQ was defined as the analyte concentration at which the % CV is less than 20%. The method should be linear up to 300 U/L. Method comparison was evaluated by comparing new Lipase Color Liquid reagent on Architect c4000 versus commercial method on Hitachi Modular P.

Validation Total imprecision (during 40 days on 3 level samples) gave CV% at 24 U/L (L1), 67 U/L (L2) and 157 U/L (L3) lower than 5%. LOD was 1.5 U/L. LOQ was 3.0 U/L. The test was linear from 0 U/L up to 600 U/L. On board calibration stability and reagent on board stability were up to 40 days. This lipase test (y) was compared with reference method (x) that uses the same substrate (methylresorufin) and gave the following results: y = 1.07x + 5.78; r = 0.99; n = 74. The test is not affected by the presence of hemoglobin up to 150 mg/dL, bilirubin (unconjugated and coniugated) up to 66 mg/dL. The test is affected by the presence of lipids up to 100 mg/dL.

Conclusions Analytical and clinical performances of new Lipase Color Liquid reagent on Architect c4000 analyzer meets the requirements for its use as colorimetric test to measure lipase in serum. Specificity and better precision make this assay very suitable for the routine measurement of this critical analyte.

B-378

New algorithm for alpha-1-antitrypsin (AAT) deficiency investigation including high resolution capillary zone electrophoresis (CZE-HR) for serum protein electrophoresis (SPE) for screening

J. P. Émond, C. Bergeron, M. Beaulieu. *University of Montréal Hospital Center (CHUM), Montréal, QC, Canada*

Background: AAT inhibits proteases such as neutrophil elastase. AAT deficiency is an underdiagnosed condition, which predisposes individuals to early onset of emphysema. We propose an algorithm in which 1st-step is based on AAT nephelometric measurement in conjunction with high resolution capillary zone electrophoresis (CZE-HR) SPE. CZE-HR SPE allows clear separation and quantification of AAT in an isolated fraction along with assessment of clinical conditions affecting the accuracy of AAT quantification. Genotyping and phenotyping are done in sequence only if required. We aim to review this algorithm performance after 14 month utilisation.

Methods: Implementation of a new algorithm using i) AAT measurement and CZE-HR SPE as screening and interpretation tools, and if required ii) genotyping in 2nd step, iii) addition of phenotyping in 3rd step. AAT concentration decision cut-off is 1.15g/L in normal CZE-HR SPE patients and 2.0g/L in inflamed ones. Integrated interpretation report is always provided. This algorithm replaces individual AAT quantification and/or genotyping requests. CZE-HR SPE was conducted on Sebia Capillarys 2 automated system with high resolution buffer. Retrospective 14 month utilisation study is based on 172 consecutive medical requests for AAT deficiency investigation.

Results: Our population consists of 172 individuals (83 men, 89 women; age (years; mean ± 2SD) of 51.3 ± 34.6 and 52.5 ± 27.4, respectively). CZE-HR SPE allows AAT detection of as low as 0.07 g/L (7 mg/dL). In our population, investigation was conclusive after 1st step in 58% of cases (n=99) where genotyping was not required. Genotyping lead to be conclusive in an additional 25% of patients (n=43), whereas phenotyping was required in 17% (n=30). Overall, detection rate of deficiency was 11%: i) deficiency associated with common variants (SS, SZ, ZZ) was 7.0% (n=12) and ii) additional 7 patients (4.0%) require SERPINA1 gene sequencing for confirmation of rare non-S or non-Z deficiency variants. We identified also 23 heterozygotes carriers (13.4%; 17 patients with S allele and 6 with Z allele). One case previously genotyped as "MS" was retested with the algorithm approach, and was correctly phenotyped as Pi*SNull. Despite adding phenotyping testing in our repertoire, the overall cost of investigation has decreased by 30% when using our new algorithm strategy as compared to our previous one (AAT measurement and genotyping for all).

Conclusion: High resolution automated SPE (CZE-HR) is a powerful tool to assess AAT and to determine many clinical states affecting accuracy of AAT quantification by common techniques. Those conditions may impact screening effectiveness. Implementation of our AAT deficiency screening algorithm has allowed us to increase the efficiency of investigation while eliminating genotyping in majority of cases. Moreover, we were able to perform expensive phenotyping testing in our laboratory repertoire while reducing overall testing cost by 30%. This allows us to selectively require outside sequencing services for only 4.0% of tested patients with unusual deficiency-associated variants.

B-379

Biochip Array Technology Rapidly Identifies a Platelet-Derived Alzheimer's Disease-Specific Phenotype

M. Veitinger[1], R. Oehler[2], E. Umlauf[1], R. Baumgartner[1], C. Gerner[3], R. Babeluk[2], J. Attems[4], G. Mitulovic[5], E. Rappold[1], J. Lamont[6], M. Zellner[1]. *[1]Institute of Physiology, Medical University of Vienna, Vienna, Austria, [2]Surgical Research Laboratories, Medical University of Vienna, Vienna, Austria, [3]Department of Medicine I , Medical University of Vienna, Vienna, Austria, [4]Institute for Ageing and Health, Newcastle University, Newcaste upon Tyne, United Kingdom, [5]Department of Medical and Clinical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria, [6]Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Globally >35 million people have Alzheimer's disease (AD) or a related dementia. Current diagnostic tests use neuropsychological review and brain scan and a number of studies have reported AD-specific cerebrospinal fluid biomarkers but analysis requires invasive lumbar puncture. There is an urgent need to develop a new minimally invasive diagnostic procedure. In this study platelets, which share biochemical features with neurons, were used as a surrogate to characterize AD-modulated proteins. The combination of biochip array technology with a powerful biomarker algorithm is innovative and generates a reliable ante mortem AD test.

Methods: Ethics was granted and 62 patients with clinically suspected AD and 63 age/sex-matched control subjects were enrolled. 2D-DIGE was performed on gel-filtered platelets to resolve alkaline platelet proteins using pH6-9 strips and acidic proteins on pH4-7, subsequently separated in the
second dimension using 11.5%SDS-PAGE. DeCyder software was applied to detect spots and all sample gels were matched with the master gel. Tryptic digest identified peptides using Spectrum Mill MS Proteomics Workbench software and UniProt database. *APOE ε4* and *GSTO1*A140* genotyping was performed. Platelet rich plasma was derived from whole blood (20min, 120 x *g*) and stored at -80^{0}C. After thawing, the platelets were isolated and lysed with SDS before addition of 2%BSA/PBS to bind excess SDS. This lysate was applied to the protein biochip employing immunoassay sandwich principles. The protein biochip was subsequently imaged on the Evidence Investigator analyser.
Results: Monoamine-oxidase-B displayed the most significant increase in spot density in AD patients.The decrease in ApoE spot density was attributed to isoform ε3 and accordingly, this spot exhibited lower detection in *ε4*-positive patients. A second ApoE spot exhibited increased density and was assigned to isoform ε4. The fourth strongest confirmed AD-related biomarker was identified as tropomyosin1 (Tm1) and adjacent spots were also identified as Tm1. Glutathione S-transferase omega1 (GSTO1) spots displayed a strong association with the *APOE*-genotype in AD patients. *APOE*-genotyping revealed significantly more AD *APOE ε4* carriers(66%)than the control group(11%). Genotyping revealed that exclusively two *GSTO1*A140* alleles were present in non-*APOE ε4* AD patients (*n*=20) relative to 38% in controls (30% in non-*APOE ε4* controls) and 32% in *APOE ε4*-positive patients. Biochip determination of the *GSTO1*A140* and *APOE ε4* allele count identified 98% of all samples correctly for the *GSTO1* SNP rs4925 genotype and 100% correct genotyping was achieved for *APOE ε4* by normalization with either ERK2 or panApoE concentrations. Biochip analysis with the cellular loading control replicated also the higher expression of both quantitative markers Tm1 and MaoB in patients relative to controls.
Conclusion: The most powerful AD biomarker algorithm was identified following combinatorial review of the proteins/isoforms that exhibited the most significant fold change. This study has demonstrated the utility of measuring multiple biomarkers from a single platelet preparation to aid the diagnosis of late-onset AD. The combination of biochip array technology with a powerful biomarker algorithm is innovative, generating a reliable ante mortem AD test. It offers the potential to rapidly detect an AD-specific phenotype using routine blood-based clinical screening.

B-380

The Value of Serum Dipeptidyl Peptidase-IV in Early Detection of Mucopolysaccharidosis

I. Kurt[1], E. Sertoglu[2], I. Okur[3], S. Tapan[1], M. Uyanik[1], H. Kayadibi[4], F. Ezgu[3], H. I. Aydin[5], A. Hasanoglu[3]. *[1]Gulhane School of Medicine, Department of Clinical Biochemistry, Ankara, Turkey, [2]Ankara Mevki Military Hospital, Anittepe Dispensary, Biochemistry Laboratory, Ankara, Turkey, [3]Gazi University Faculty of Medicine, Department of Pediatric Metabolism and Nutrition, Ankara, Turkey, [4]Adana Military Hospital, Department of Biochemistry, Adana, Turkey, [5]Gulhane School of Medicine, Department of Pediatric Metabolism, Ankara, Turkey*

Background/Aim: The mucopolysaccharidosis (MPS) are a group of eleven lysosomal storage disorders (LSDs) that result from the absence or malfunctioning of enzymes required for the breakdown of glycosaminoglycans (GAGs). Early recognition of MPS allows for immediate treatment, before the onset of irreversible impairment, and, in many cases, prevention of catastrophic health outcomes, including death. We aimed to investigate the diagnostic utility of serum dipeptidyl peptidase-IV (DPP-IV) enzyme activity, urinary GAG/Cre ratio, total adenosine deaminase (ADA) and ADA-1 isoenzyme activity in the diagnosis of MPS.

Material and Method: 31 MPS patients which were previously diagnosed by clinical and enzymatic analysis were included in the study along with 31 healthy controls matched with patients for age and gender (8 (4 - 14) years, 61% male for patients and 8 (5 - 11) years, 58% for controls). Serum DPP-IV enzyme activity was measured according to Beesley et al and reported as nmol/min/mL of plasma. Serum total ADA and ADA-1 isoenzyme activity was measured according to Guisty G, Galanti B colorimetric method and reported as U/L. Urinary GAG concentration was measured by dimethylmethylene blue method, creatinine was measured by the modified Jaffe method and results were reported as mg GAGs/mmol creatinine. All of the statistical analyses were carried out by using the SPSS (V15) package for Windows.

Results: Serum DPP-IV enzyme activity, urinary GAG/Cre ratio, total ADA and ADA-1 isoenzyme activity were significantly higher in patients than in controls ($p< 0.001$, $p< 0.001$, $p= 0.038$ and $p= 0.006$, respectively). There were significant correlations between serum DPP-IV enzyme activity and urinary GAG/Cre ratios, ADA-1 activity, ADA-1/total ADA ($r= 0.498$, $p< 0.001$; $r= 0.348$, $p= 0.006$; $r= 0.270$, $p= 0.034$, respectively). Area under ROC curve for DPP-IV enzyme activity was 0.988, $p< 0.001$ and for urinary GAG/Cre ratio was 0.986, $p< 0.001$. DPP-IV enzyme activity and urinary GAG/Cre ratio were the most significant parameters according to the univariate logistic regression analysis ($p= 0.001$ and $p< 0.001$, respectively).

Conclusion: According to our results, measurement of plasma DPP-IV activity can be used as a biomarker for MPS screening. However, no prominent diagnostic value emerged (so far) for the soluble plasma DPP-IV, although methods for plasma DPP-IV activity measurements are available since the 1980s. Although many cases are needed to decide more precisely, the results of this study indicate that the measurement of serum DPP-IV activity can be used as first-line screening test complementary to urinary GAG/Cre ratio for MPS screening.

B-381

Macrophage Inflammatory Protein (MIP-1α), an Early Biomarker of Chronic Kidney Disease

M. Summers, T. McFadden, E. Healy, C. Richardson, J. Lamont, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Chronic kidney disease (CKD) describes abnormal kidney function and/or structure. It is frequently unrecognised and presents with progressive decline of renal function leading to end-stage renal failure and death. The Modification of Diet in Renal Disease (MDRD) classification of renal disease describes the progressive stages of the disease (stages 1-5) with respect to the estimated glomerular filtration rate (eGFR). The complexity in diagnosing a patient with CKD at an early stage of disease has led to most patients not receiving a diagnosis until the disease has progressed to an advanced stage. Currently there are no reliable methods for assessing early stage kidney disease. Inflammation plays a key role in the developmental progression of CKD and identification of inflammatory biomarkers in patients suspected of having CKD would aid early diagnosis. This study aimed to investigate the potential of MIP-1α as an early biomarker of CKD, which would aid the diagnosis of early stage CKD.

Methods: MIP-1α was assessed in serum samples from a total of 202 subjects: 152 CKD patients (51 Stage 1, 50 Stage 2 and 51 Stage 3) and 50 healthy controls. The analysis was performed with a biochip based immunoassay applied to the Evidence Investigator analyser. Statistical analysis was performed using MedCalc v12.5, all data represented as median [95% CI].
Results: Differences in concentration of MIP-1α across the disease groups and controls were initially assessed using the Kruskal-Wallis test and MIP-1α displayed significant difference between the diseased subjects and the healthy controls (significance determined as $p<0.05$). Post-hoc analysis was performed comparing CKD groups with controls using Mann-Whitney (with Bonferroni correction). This showed that MIP-1α displayed significantly higher median concentrations at all CKD stages (Stage 1-3) compared to control. (10.39 [9.0-13.77], 8.7 [7.29-11.24], 11.9 [9.89-14.23] pg/ml respectively; $p<0.0001$ for all) compared to control (5.36 [4.89-5.81] pg/ml). Receiver Operating Characteristic (ROC) curve analysis was conducted to assess diagnostic performance of diseased versus healthy subjects. Area under the curve was determined as 0.856(95% CI: 0.800-0.901).
Conclusion: The results indicate increased median concentration of MIP-1α in the serum of CKD patients (stages1-3) compared to controls. This study highlights the potential utility of MIP-1α in diagnosing early stage CKD. Early identification will enable effective clinical management and prevent progression to a later stage.

B-382

FIA-MS/MS multiplex enzyme assay for screening oligosaccharidoses

K. K. Nickander[1], S. E. Hofherr[2], M. J. Magera[1], D. Matern[1]. *[1]Mayo Clinic, Rochester, MN, [2]Children's National Medical Center, Washington, DC*

Background: Oligosaccharidoses are autosomal recessive lysosomal storage disorders of glycoprotein catabolism. Current clinical testing for oligosaccharidoses is performed by a thin layer chromatography (TLC) screen lacking sensitivity and specificity. A positive TLC screen is followed by individual fluorometric enzyme assays in leukocytes or fibroblasts, which although specific, address only one enzyme at a time. We addressed this widespread deficiency by developing a multiplexed enzyme assay to screen for several oligosaccharidoses, including α-mannosidosis, β-mannosidosis, Schindler disease, sialidosis, galactosialidosis, GM1 gangliosidosis, Morquio B disease, fucosidosis, and mucolipidoses II α/β, III α/β, and III γ, utilizing flow injection analysis and tandem mass spectrometry (FIA-MS/MS).

Methods: Leukocytes or fibroblast lysates (100 µL) prepared in 0.1 M citrate

phosphate buffer, pH 4.5, are incubated overnight with 3 different substrate mixes (10 µL) then extracted using solid phase extraction and FIA-MS/MS (API 3200; AB Sciex, Framingham, MA). Substrate mix 1 was prepared with 0.2 mM 4-methylumbelliferyl α-D-mannopyranoside, 0.8 mM 4-nitrophenyl β-D-mannopyranoside, and 0.8 mM 4-methylumbelliferyl N-acetyl-α-D-galactosaminide, substrate mix 2 was prepared with 0.4 mM 4-methylumbelliferyl-N-acetyl-α-D-neuraminic acid sodium salt hydrate and substrate mix 3 was prepared with 0.4 mM 4-methylumbelliferyl-β-D-galactopyranoside and 0.4 mM 4-methylumbelliferyl α-L-fucopyranoside. All mixes also included 0.2 mM umbelliferone as an internal control. Linearity was assessed by 1:2 dilutions of substrate starting at 4 times the normal starting concentration and processed with heat inactivated matrix. Reference intervals were determined from 157 leukocyte and 253 fibroblast specimens. Accuracy was determined from 15 known deficient specimens.

Results: Precision (inter-assay and intra-assay) performance was within acceptable limits (≤20% CV). Method response for the individual substrates demonstrated acceptable linearity from 6.25-400% the normal starting concentration, with R^2 values ranging 0.982-0.999. Acceptable clinical utility was demonstrated by correct identification of 100% (N=15) of specimens with decreased or minimal residual enzyme activity.

Conclusions: The FIA-MS/MS method provides a reliable alternative to TLC, improving sensitivity and specificity without the need for follow-up assays with individual enzymes. Six different enzymes are assayed screening for 9 different oligosaccharidoses.

B-383

Assessment of Albuminuria using High Performance Liquid Chromatography in Diabetic and Nondiabetic Patients

R. Prusa, M. Fortova, E. Klapkova, K. Kotaska. *Faculty Hospital Motol, Prague 5, Czech Republic*

Background: Many studies have found higher values of urinary albumin reported using high performance liquid chromatography (HPLC) in comparison with immunochemical methods. Two opposite hypotheses explaining the difference between these methods are as follows: 1) the presence of immunounreactive albumin in urine that is not detected using immunochemical methods, 2) the presence of co-eluting proteins that falsely overestimate the results on HPLC. The aim of our study was the implementation of HPLC method for albuminuria, testing the hypothesis about co-eluting proteins, and comparison of albuminuria assessed using HPLC and immunoturbidimetric methods in diabetic and nondiabetic patient samples.

Methods: We developed the HPLC method for detection of albuminuria under these chromatographic conditions: multilinear gradient consisting of water (solvent A), acetonitrile (solvent B), 100 mM NaH_2PO_4 (solvent C), 1 % trifluoracetic acid (solvent D), flow rate 2 mL/min, temperature 22 °C, UV detection in the wavelength 280 nm, chromatographic column Zorbax 300 SB-C3, liquid chromatograph Agilent 1200 (Agilent Technologies, USA). We analyzed two mixtures of urine. The first one was prepared from 30 patient urine samples with immunoturbidimetrically physiological albuminuria, to which we added albumin standard. The second mixture was prepared from 30 patient urine samples with mild albuminuria. We compared albuminuria assessed using HPLC with the immunoturbidimetric method (automatic analyser Cobas Integra 400, Roche Diagnostics GmbH) in two patient groups: 636 diabetics [345 males and 291 females, mean age 50.1 years (1-97)] and 456 nondiabetics [250 males and 206 females, mean age 33.9 years (0.1-91)].

Results: Transferrin, α-1-acid glycoprotein, α-1-antitrypsin, α-1-antichymotrypsin and hemopexin do not interfere with albumin in HPLC method. The elution curve of prealbumin splits into several peaks, of which a few interfere with albumin. This interference has no clinical importance. In mixture 1 we did not find a significant difference between the albuminuria assessed using both methods (79 mg/L vs. 82 mg/L), while in mixture 2 we measured over 26 % higher albuminuria using HPLC (79 mg/L vs. 100 mg/L). These results suggest the existence of immunounreactive albumin. We found a statistically significant difference between the methods in both patient groups (14.6 ± 19.3 mg/L immunoturbidimetrically vs. 25.3 ± 21.1 mg/L HPLC in diabetics, 30.1 ± 23.6 mg/L immunoturbidimetrically vs. 41.2 ± 27.6 mg/L HPLC in nondiabetics, median ± standard error of the mean, $p < 0.0001$, Mann-Whitney test).

Conclusion: Our results prove that the HPLC method for albumin detection is more sensitive than immunoturbidimetry. We did not confirm nonspecificity of the HPLC method. We found statistically significant higher concentrations of urinary albumin using HPLC in both diabetic and nondiabetic patients.

Acknowledgment: Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague,Czech Republic).

B-384

Performance Evaluation of the AnshLite Myelin Basic Protein Chemiluminescent Immunoassay Using Cerebrospinal Fluid

J. Lu[1], D. G. Grenache[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Myelin is the insulating sheath surrounding neurons. Myelin basic protein (MBP) accounts for about one-third of total central nervous system (CNS) myelin protein. The cerebrospinal fluid (CSF) concentration of MBP increases in response to neuronal damage allowing the measurement of CSF MBP to be used clinically as a nonspecific marker of CNS inflammation. The objective of this study was to evaluate the performance characteristics of the AnshLite™ MBP chemiluminescent immunoassay (CLIA) for the quantitative determination of MBP in CSF.

Methods: MBP was measured using the AnshLite MBP CLIA (Ansh Labs, Webster, TX, USA). Performance characteristics including precision, linearity, analytical sensitivity, recovery, method comparison, MBP stability, the effects of freeze/thaw cycles, and the reference were evaluated using residual CSF specimens sent to ARUP Laboratories. The University of Utah's Institutional Review Board approved the project.

Results: Precision was determined using CSF pools with MBP concentrations of 3.51 and 1.54 ng/mL assayed in three replicates once each day for ten days. Within-run and total CVs were 9.6 and 14.8%, respectively. Linearity was determined by serially diluting a high-MBP CSF sample with the zero calibrator to create six samples each tested in two replicates. The assay was linear within the measuring range to 11.25 ng/mL (linear regression $y=0.98(x)+0.05$, $R^2=1.00$). The limit of blank (LOB) and limit of detection (LOD) were determined by testing the zero calibrator ten times and 0.45 ng/mL calibrator seven times. The LOB was 0.01 ng/mL calculated as the mean concentration added to 3 SD. The LOD was 0.08 ng/mL calculated as the LOB added to 3 SD. Accuracy was determined by recovery studies performed by adding volumes of one of two calibrators (6 and 14 ng/mL) to two CSF samples with MBP concentrations of 1.18 and 0.34 ng/mL. Calculated recovery ranged from 86 to 109%. A method comparison using 42 samples with an MBP concentration range of 0.30-58.57 ng/mL was performed using the Beckman MBP ELISA (Beckman Coulter, Inc., Brea, CA) as the comparator method. Deming regression yielded $y=2.75(x)-2.68$, $R^2=0.95$, $Sx/y=4.78$. MBP stability was determined by storing two CSF specimens with MBP concentrations of 6.77 and 1.49 ng/mL at room temperature for two days, 4°C for two weeks, and -20°C for three weeks. MBP decreased <12% relative to time zero under all conditions. The effects of up to three freeze/thaw cycles were evaluated by testing two CSF samples with MBP concentrations of 7.02 and 1.59 ng/mL after each freeze/thaw cycle. The MBP concentration changed by -12.6, +6.94, and -18.0% relative to time zero after each cycle, respectively. The reference interval was established using 130 CSF specimens that were negative for oligoclonal bands. Using non-parametric analysis, this was determined to be 0.0 to 5.5 ng/mL.

Conclusions: The AnshLite™ MBP CLIA demonstrates acceptable performance characteristics for quantifying MBP in CSF. MBP is stable for two days at room temperature, two weeks at 4°C and three weeks at -20°C. Freeze/thaw cycles of CSF samples for MBP testing should be avoided.

B-385

Rationalising not rationing: the case of serum protein electrophoresis (SPE)

R. M. Dorizzi, V. Zanardi, P. De Vita, R. Agnoletti, A. Alberelli, S. Babini. *Corelab, Laboratorio AVR, Pievesestina di Cesena, Italy*

Background Serum protein electrophoresis (SPE) is highly requested in Italy. The AVR laboratory, which serves 1,125,000 inhabitants, runs about 300,000 tests/year while e.g. in Kent 10,557 SPEs are requested in the same period for a catchment area of 759,000 inhabitants. Since research/monitoring of monoclonal components is the only appropriate indication for this test, the AVR Laboratory carried since 2010 several actions to increase the appropriateness of the requests: adoption of automated alerts in the CPOE, benchmarking of the requesting modes of GPs, frequent meetings with GPs, changes in the report format. Since October 1st 2013 the report contains the various fractions only when a monoclonal component (MC) is present; in the other cases only the comment "Monoclonal components are not noticeable" is reported. In January 2014 we carried out the first audit of the effect of this action in the fourth trimester after its implementation.

Materials and methods We extracted the results of the SPEs carried in the fourth quarter of the years 2009-2013 stored in the LIS (Noemalife, Bologna, Italy). All the SPEs were performed using Capillarys (Sebia, Marcy l'Etoile, France). We assessed

the tests carried out in inpatients and outpatients and the percentage of detected MCs.

Results The results summarized in the table shows a steady decline of the demand for SPE since 2009 and a constant increase in the percentage of detected MCs consistent with a progressive increase of the appropriateness of the request. This trend is more evident in inpatients and it could be suggested that a MC is more frequently detected in patients with symptoms requiring hospitalization and undergoing more accurate investigation.

Conclusions 1) The laboratory can and should promote the appropriateness of laboratory tests; 2) appropriateness can improve in relatively a short time; 3) effectiveness of the actions can be measured with simple tools.

OUTPATIENTS			
Trimester 4	Tot. SPE	Detected MCs	MCs %
2009	63,474	3,333	5.3
2010	62,076	3,608	5.8
2011	54,182	3,602	6.6
2012	53,296	3,718	7.0
2013	45,736	3,542	7.7
INPATIENTS			
Trimester 4	Tot. SPE	Detected MCs	MCs %
2009	16,924	1,612	9.5
2010	14,123	1,365	9.7
2011	12,760	1,232	9.7
2012	10,356	1,055	10.2
2013	8,606	1,091	12.7

B-386

Performance Evaluation of a Polyclonal Based Fecal Alpha-1-Antitrypsin ELISA

J. A. Erickson[1], R. A. Jensen[2], D. G. Grenache[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, [2]Parasitology and Fecal Testing Technical Section, ARUP Laboratories, Salt Lake City, UT, [3]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

Background: Alpha-1-antitrypsin (A1A) is a serum protease inhibitor that inhibits a wide range of proteases. A1A resists degradation by digestive enzymes and therefore, can be used to detect the presence of serum proteins in the gastrointestinal tract. The primary causes of protein-losing enteropathy can be divided into erosive gastrointestinal disorders, non-erosive gastrointestinal disorders, and disorders involving increased central venous pressure or mesenteric lymphatic obstruction. The diagnosis of protein-losing enteropathy is most commonly based on the determination of fecal A1A clearance. The purpose of this study was to assess the performance characteristics of a polyclonal antibody based A1A ELISA for the measurement of A1A in stool and to determine reference limits for A1A in stool and A1A clearance.

Methods: Stool samples included deidentified residual random stool specimens sent to ARUP Laboratories for routine testing, and timed stool specimens and paired serum obtained from healthy volunteers. A1A was extracted from stool and measured using the ImmuChrom Human A1A ELISA (ImmuChrom, GmbH, Heppenheim, Germany) according to the manufacturer's instructions. Serum A1A was measured using the Tina-quant® A1A assay (ver.2) on a cobas® 8000 modular analyzer (Roche Diagnostics, Indianapolis, IN). The performance characteristics evaluated for the stool ELISA were analytical sensitivity, linearity, accuracy, precision, and A1A stability in stool. The University of Utah's Internal Review Board approved the project.

Results: The analytical sensitivity was 0.14 ng/mL (0.002 mg/g stool) determined from 23 replicates of the sample diluent (mean + 3SD). Linearity was evaluated using diluted stool extracts with elevated A1A concentrations (range, 1.5 - 90.0 ng/mL). Linear regression produced results of $y = 1.02x - 3.15$ ($r^2 = 0.986$). Accuracy was determined from analyte recovery studies by adding sera of known A1A concentration into previously measured stool extracts. Recovery (measured ng A1A / expected ng A1A) ranged from 95.2 to 118.4%. Precision was determined from ten stool extracts obtained from each of two random stool specimens, with one extract analyzed in five replicates each day for ten days. Repeatability and within-laboratory CVs were 5.4 and 6.5% at 17.3 ng/mL (0.21 mg/g) and 13.8 and 14.5% at 66.9 ng/mL (0.84 mg/g), respectively. A1A was stable in stool for a minimum of 2 days, 7 days and 3 months at room temperature, 4 - 8 °C and -20 °C, respectively. A1A measured in timed stool obtained from 45 healthy volunteers (21 males, 24 females, ages 21 - 61) ranged from <0.002 to 0.59 mg/g stool. Using a robust skewed method, the reference limits for A1A in stool and A1A clearance were ≤0.47 mg/g and ≤45 mL/day, respectively.

Conclusions: The ImmuChrom Human A1A ELISA demonstrates acceptable performance for quantifying A1A in stool. The assay can also be used in conjunction with the Roche Diagnostics Tina-quant A1A (ver.2) assay for assessing A1A clearance.

B-387

Lipolysis Suppresses Insulin Signaling and Glucose Uptake

G. R. Mullins, L. Wang, T. E. Harris. *University of Virginia, Charlottesville, VA*

Background: Acute hyperglycemia often develops after trauma or major surgery, particularly surgery within the abdominal cavity such as coronary bypass procedures. This condition, termed stress-induced hyperglycemia, contributes to mortality and delays healing in post-surgery and ICU patients and is a result of systemic insulin resistance (IR) caused by the adaptive stress response. Although intensive insulin treatment reduces hyperglycemia and mortality in some patients, insulin therapy after surgery can result in hypoglycemic incidents, emphasizing the need to develop new treatment strategies. Despite its clinical significance, the pathophysiology of post-surgery acute IR is largely unknown. Understanding the contributing molecular mechanisms will provide new insights in developing therapeutic approaches to improve patient outcomes after major surgery. It is known that β-adrenergic stimulation by catecholamines will inhibit insulin stimulated glucose uptake in adipose tissue. This catabolic signaling leads to the release of stored fat through lipolysis, which may play a role in the observed stress-induced IR and hyperglycemia. Our objective was to investigate a possible mechanistic link between lipolysis and catecholamine-induced IR in adipose tissue, which would likely contribute to stress-induced IR after surgery.

Methods/Results: We have used biochemical assays to investigate the effect of catecholamine-induced lipolysis on insulin signaling and glucose uptake in adipocytes. Activation of the cyclic AMP/Protein Kinase A pathway acutely inhibits insulin signaling by disrupting mammalian Target of Rapamycin (mTOR) complexes 1 and 2 in adipocytes. Activation of lipolysis is required for this PKA-induced inhibition of mTOR. The effect of lipolysis is also transferrable in vitro, suggesting a lipid product of lipolysis may mediate mTOR complex dissociation. In addition, the signaling lipid, referred to as the mTOR Dissociative Factor (mDF) is downstream of Adipose Triglyceride Lipase (ATGL) and depleted by Diacylglycerol Lipase (DAGL), suggesting it is likely a diacylglycerol released during lipolysis. Here we summarize our progress in our effort to isolate and identify the mDF through reverse-phase high-performance liquid chromatography (RP-HPLC). We show that activation of the PKA pathway inhibits mTOR activity and adipocyte glucose uptake by 40-50%, as measured by phosphor-specific immunoblots and radiometric glucose uptake assays, respectively. Although significant, this inhibition is not seen when lipolysis is genetically or pharmacologically blocked. This suggests that inhibition of mTOR by lipolysis is a potential mechanism of dampened glucose uptake during catecholaminergic signaling and provides new insight into stress-induced hyperglycemia.

Conclusion: This study demonstrates a novel intracellular signaling mechanism where activation of lipolysis controls the PI3K-Akt-mTOR pathway to inhibit insulin signaling. Understanding this mechanism provides new insight into insulin resistance that is concurrent with elevated lipolysis found during obesity or after major surgery. Understanding the molecular mechanism of stress-induced insulin resistance will lay the groundwork for future therapeutic treatments and improve patient outcomes after surgery. In addition, identification of the mDF may provide a new biomarker for predicting the risk of insulin resistance and hyperglycemia.

Wednesday, July 30, 2014

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

TDM/Toxicology/DAU

B-389

Point of Care PK Quantitation Device for Pharmacokinetic Guided Dosing of Paclitaxel as a Companion Diagnostic Device

C. Lee, C. Park, C. Hsiao, A. Trieu, S. Lee. *Autotelic Inc, Fountain Valley, CA*

Background: Paclitaxel chemotherapy is the cornerstone of most anti-cancer regimens due to its potent cytoxic activity against tumor cells. The variability of paclitaxel dosing can be as high as 10X across patients, therefore, dosing at a fixed dose even when adjusted for body weight will leave a significant portion of the patients underdosed - getting no benefit from the treatment- and another portion overdosed- getting undue toxicity. Therefore, paclitaxel therapy would benefit from TDM guided dosing. A full pharmacokinetic (PK) study is required to determine whether patients are getting the appropriate and efficient dosage. However, at the current state, a full pharmacokinetic requires not only multiple high-volume blood draws and extended stays in the hospital but also testing method by LC/MS/MS, which are both time-consuming and expensive. Here we describe the development of Point of Care TDM (Therapeutic Drug Monitoring) device for PK guided dosing of paclitaxel as a companion diagnostic device.

Methods: The 8A10 and 3C6 mAbs (mAbs against paclitaxel) were purified from the antibody-rich harvested medium using MabSelect (GE Healthcare, Pittsburgh, PA). Commercial antibodies (29B7B3C and 69E4A8E; Santa Cruz Biotechnology Inc.) were also tested. To synthesize BSA-paclitaxel, we used the method of J-G Leu et al. as described in Cancer Res. (1993) 53:1388-1391. BSA-paclitaxel and mAbs were labeled with colloidal gold using the gold labeling kit from BioAssay Works, LLC, Ijamsville, MD.

Results: Rapid test for paclitaxel based on the lateral flow system was developed. Of the mAbs tested, only 8A10 and 3C6 were useful. The assay requires the unique configuration of immobilizing the mAb against paclitaxel onto the membrane followed by flowing the BSA-paclitaxel-colloidal gold through in presence of test analyte. This configuration seems more effective than the traditional configuration where BSA-paclitaxel is immobilized on the membrane and colloidal-gold labeled anti-paclitaxel mAb as flow through. This resulted in a competitive assay for paclitaxel where the signal decreased as the concentration of paclitaxel analyte in blood increased. Coupled with the current lateral reader technology - especially the one developed by Qiagen- a rapid quantitative assay for paclitaxel is possible. The assay demonstrated good linearity and range suitable for paclitaxel TDM. The application of this assay in a preclinical pharmacokinetic study yield reasonably comparable result to LC/MS method.

Conclusions: A quantitative lateral flow platform coupled to a reader was developed to easily detect the paclitaxel concentrations in small amount of blood samples. Individual pharmacokinetic profiles can be obtained and used to determine the suitable treatments. In addition, the lateral flow PK quantitative assay can be deployed at point-of care (in home, doctor's office or central lab).

B-390

Comparison of Roche Methadone Screening Assay with DRI Methadone Metabolite (EDDP) Screening Assay

M. R. Sneidman, H. E. Yu. *Geisinger Health System, Danville, PA*

Geisinger Medical Laboratories offer immunoassay based screening. Methadone, one of the drug classes we test, is a synthetic opioid used for narcotic addiction and pain management. Urine drug testing is used to monitor medication compliance for these patients. We observed significant number of false positive methadone screens (Roche). In a five-month period, 88 urine samples were screened positive for methadone, but 27% were negative by GC-MS. Because of the high rate of false positive results, we evaluated an alternative screening assay "DRI Methadone Metabolite (EDDP)". The following results were obtained:

EDDP Screening (DRI)	Methadone Screening (Roche)	GC-MS Confirmation
POS (n=29)	POS (n=24)	POS (n=24)
		NEG (n=0)
	NEG (n=5)	POS (n=4, false NEG methadone)
		NEG (n=1, false POS EDDP)
NEG (n=25)	POS (n=10)	POS (n=0)
		NEG (n=10, false POS methadone)
	NEG (n=15)	POS (n=0)
		NEG (n=15)

The EDDP screening assay (DRI) was more accurate with no false negative and only 1 false positive out of 54 urine samples tested. We also noted that many false positive methadone screens were from patients who were taking tramadol and suspected that is the interfering substance causing the false positive results. We therefore replaced the Roche methadone screening assay with the DRI methadone metabolite (EDDP) screening assay.

B-391

Light-Sensitivity of Chlordiazepoxide

T. V. Hartman, M. Dallman, L. J. Langman, P. Jannetto. *Mayo Clinic, Rochester, MN*

Objective Due to conflicting information in the literature, this study was conducted to determine if Chlordiazepoxide (CDP) is light sensitive at a variety of sample storage conditions.

Relevance If CDP patient specimens are not protected from light for greater than 24 hours, clinical results could be inaccurate leading to erroneous clinical results. Furthermore, the light-sensitivity of CDP is not sufficiently addressed in the current literature.

Methodology This study utilized High Performance Liquid Chromatography with Ultra-Violet Visible Spectroscopy (HPLC/UV-Vis) to compare three identical serum samples at various concentrations throughout the analytical measurement range (0.5 µg/mL - 12.0 µg/mL). Briefly, the three samples were split into 4 aliquots and stored protected and unprotected from light at both ambient and refrigerated temperatures for analysis on days 1, 3, 7, 16, 21, and 28.

Validation After 28 days, the three aliquots stored protected from light yielded an average degradation of 20.7% at ambient storage and essentially negligible degradation at refrigerated storage while the three aliquots not protected from light yielded an average degradation of 93.5% at ambient storage and 22.8% at refrigerated storage.

Conclusions Some degradation of CDP found at ambient temperatures was determined to be due to the storage temperature; however, by comparing the light-protected samples vs. the non-light protected samples at the ambient storage or refrigerated storage temperatures, the data clearly shows significant degradation of CDP due to the exposure to direct light which could significantly affect patient results.

B-392

Modified HPLC-UV Method without Evaporation Step for the Determination of Serum Ribavarin Level in Patients with Hepatitis C

A. Khan[1], I. Altraif[1], P. Marquet[2], A. Al-Othaim[1], K. M. Khan[3], N. Al Hussain[1], W. Tamimi[1]. *[1]King Fahad National Guard Hospital, King Saud Bin Abdulaziz Univresity for Health Sciences, Riyadh, Saudi Arabia, [2]Université de Limoges, faculte de medecine, Limoges, France, [3]University of Karachi, Karachi, Pakistan*

Bcakground: The measurement of serum concentration of anti-viral drug ribavarin is indicated for some patients with hepatitis C, for therapeutic drug monitoring. A simple and fast high performance liquid chromatographic method for the determination of ribavarin in serum sample is developed and validated according to FDA method validation guidelines without the evaporation step. Method: Serum sample (500 μL), internal standard (50 μL), and 20 mM ammonium acetate buffer of pH =8.5 (500 μL) were mixed and allowed to centrifuge for 5 minutes. After centrifugation, the supernatant was transferred into phenyl boronic acid cartridges (previously conditioned with 1mL of 3% formic acid and then with 1mL of 20mM ammonium acetate buffer pH 8.5) for solid phase extraction followed by washing step with 20 mM ammonium acetate buffer (1 mL) under vacuum not exceeding 10 psi and discarded. ribavarin and internal standard were eluted with 3% formic acid (300 μL) and was injected (90µL) into HPLC system. Serum samples from 37 patients were run simitnutenosly on both methods and compared. Results: A linearity between 0.1 - 6.25 mg/L with 0.06mg/l as limit of detection was obtained. The correlation coefficient was 0.950 with p value of 0.92 showing a good reproducibility of results. The mean

accuracy was checked at three different concentrations and found to be 108% for each level. The mean recoveries (extraction efficiency) of ribavarin and internal standard from serum were found to be 65.5 % and 71.2% respectively at concentrations ranging from 0.1 to 6.22 mg/L for ribavarin and 50mg/L for internal standard. The intra assay precision were determined at 0.5, 1.2, & 2.2 mg/L and % CV were found to be 7.1%, 5.1% % 7.4% respectively whereas injection reproducibility were calculated 5.6%, 0.7% and 0.6% at three levels. Maximum 1hour and 30 minutes was consumed to complete this assay whereas it takes about 3 hours and 30 minutes if the step of evaporation and gravity elution was used in the older method. Conclusion: The newly developed HPLC method was faster, accurate and sensitive. It may be applied for the estimation of ribavarin level in serum samples.

B-393

A HPLC-High Resolution Accurate Mass Spectrometric Method for the Quantification of Doxorubicin and Doxorubicinol

D. Sartori, A. Breaud, W. Clarke. *Johns Hopkins University School of Medicine, Baltimore, MD*

Background: While chemotherapeutic agents have been commonly used in the treatment in a majority of cancers, these drugs are typically associated with significant adverse effects. Although chemotherapeutic regimens reduce mortality, morbidity may be high due to significant drug-induced toxicities. The anthracycline anti-neoplastic agent doxorubicin is a common treatment modality for hematologic malignancies, as well as a variety of solid tumors. Doxorubicin, and its active metabolite, doxorubicinol, is associated with severe cardiotoxicity; therefore, it is important to generate tools to measure doxorubicin concentrations following intravenous administration to minimize drug-mediated toxicity. Both invasive (blood) and non-invasive (saliva) collection schemes have been pursued for other drugs, and this is largely due to ease of collection, particularly in pediatric populations. Here, we present a liquid chromatographic-high resolution mass spectrometric (LC-HRMS) method for the dual quantification of doxorubicin and its metabolite in both serum and saliva.

Methods: Standard solutions of doxorubicin and its active metabolite, doxorubicinol, as well isotopically-labeled internal standards for each, were prepared in water and spiked into drug-free human serum and saliva. Following protein precipitation, a 10 µl aliquot was injected onto our LC-HRMS system. Chromatographic separation was achieved using a Thermo Scientific PFP HPLC column (50 × 2.1 mm, 5 µm particle size) and eluted under a gradient elution containing acetonitrile with 0.1% formic acid. Both doxorubicin and doxorubicinol were detected over 5 minutes using a Q-Exactive hybrid quadrupole-orbitrap mass analyzer (Thermo Scientific). A HESI (heated electrospray ionization) source was used and the instrument was operated in full scan (*m/z* 250-750) mode with a resolution of 70,000 at m/z 200.

Results: The high resolution Q-Exactive mass analyzer was able to detect both doxorubicin (m/z 544.1813) and doxorubicinol (m/z 546.1970) in serum and saliva with a mass tolerance of 5 ppm from theoretical mass. Assay development and subsequent validation was performed following the recommendations of the FDA for bioanalytical method validation. The analytical measuring range of the assay for both doxorubicin and doxorubicinol was 5 to 1000 ng/ml. Calibrator solutions were evaluated at drug concentrations of 5 ng/ml, 15 ng/ml, 50 ng/ml, 75 ng/ml, 125 ng/ml, 250 ng/ml, 500 ng/ml and 1000 ng/ml. Linearity was assessed as the average slope of $1/x^2$ weighted linear regression analysis. Across five independent injections from serum-extracted specimens, the average slope for doxorubicin and doxorubicinol were 0.953 and 0.951, respectively. Quality control solutions were prepared in serum with theoretical concentrations of 30 ng/ml and 400 ng/ml for both drugs. Intra-assay precision across five injections were determined to be 2.9% and 2.5% for the parent and metabolite drug, respectively.

Conclusion: A LC-HRMS assay has been developed and validated for the simultaneous quantification of doxorubicin and its active metabolite in both serum and saliva specimen sources.

B-394

Rapid Enzyme Hydrolysis by a Novel Recombinant Beta-Glucuronidase in Benzodiazepine Urinalysis

A. A. Morris, S. A. Chester, E. C. Strickland, G. L. McIntire. *Ameritox, Ltd, Greensboro, NC*

Background: Benzodiazepines are widely prescribed drugs that are readily abused for their sedative effects and as such are very frequently targeted in therapeutic drug monitoring. Only trace amounts of parent drug are present in urine due to extensive metabolism and conjugation of benzodiazepines and their glucuronides are major urinary species. Hydrolysis to cleave the glucuronides prior to analysis is necessary for improved detection. For benzodiazepine analysis, hydrolysis by enzyme is preferred over acid because the latter produces benzophenones, which convolute result interpretation. Enzyme hydrolysis can be costly and time-consuming, with reported incubation times ranging from 0.5 to 20 hours. The assessment and application of a novel recombinant beta-glucuronidase for rapid hydrolysis in benzodiazepine urinalysis is presented.

Methods:IMCSzyme™ recombinant beta-glucuronidase was buffered to recommended optimum pH and evaluated. Aliquots of drug-free urine fortified separately with glucuronides of oxazepam, lorazepam, temazepam at 2500 ng/mL were hydrolyzed with the enzyme in triplicate. The hydrolysis efficiency was assessed at 55◦C at incubation times of 0, 15, 30 and 60 mins and at room temperature at incubation times of 0, 5 and 10 mins. The optimized enzyme hydrolysis was applied to 20 randomly selected positive authentic urine samples for each analyte and compared to hydrolysis by commonly used beta-glucuronidase from abalone under its validated optimized conditions. Hydrolysis efficiency for alpha-hydroxyalprazolam glucuronide was evaluated solely with patient samples positive for that compound. Hydrolyzed urine samples were analyzed on a TLX-4 Multiplexed HPLC with Agilent 1200 Series Binary Pumps coupled to a Thermo Scientific™ TSQ Quantum Ultra™ Triple-Stage Quadrupole Mass Spectrometer using a previously validated method. Analytical column performance was monitored.

Results: The validated benzodiazepines liquid chromatography tandem mass spectrometry (LC/MS/MS) method had a linear range of 20-5000 ng/mL for oxazepam, lorazepam, temazepam and alpha-hydroxyalprazolam. At 0 mins (immediately run after addition), mean analyte recovery >92% from all hydrolyzed glucuronide controls was observed at both 55◦C and room temperature. Complete hydrolysis of the glucuronide controls (mean analyte recovery ≥ 100%) was observed at 15 mins (shortest incubation time tested) at 55◦C and at 10 mins at room temperature. This was considerably faster than the optimized incubation time of 30 mins for the abalone beta-glucuronidase. Hydrolysis at room temperature was also more convenient, eliminating heat activation. Mean analyte recovery changed by no more than -2% at longer incubation times at both temperatures. In patient samples, total oxazepam, lorazepam and temazepam compared well between the two enzyme sources. Recovery of alpha-hydroxyalprazolam was >30% higher using the recombinant beta-glucuronidase versus that from abalone, highlighting the differential hydrolysis of alphahydroxyalprazolam by the abalone enzyme under conditions optimal for the other analytes. The IMCSzyme™ recombinant beta-glucuronidase appeared to be a cleaner extract and the maximum number of analytical column injections seen with abalone-treated samples was exceeded with this recombinant source.

Conclusion: The superiority of the IMCSzyme™ recombinant beta-glucuronidase was demonstrated with fast benzodiazepine hydrolysis at room temperature. The use of this enzyme decreases processing time due to reduced incubation time, requires no heat activation and affords improved column life.

B-395

Comparison of paired immunosuppressant levels in venous and dried blood spot levels in post-transplant patients

J. A. Dickerson, K. Sadilkova, R. Jack. *Seattle Children's Hospital, Seattle, WA*

Background: Transplant patients are routinely and chronically monitored with laboratory testing to assess risk of rejection, infection, and to optimize therapeutic drug doses. Therapeutic drug monitoring of tacrolimus and sirolimus plays a significant role in the clinical follow-up of transplant patients receiving immunosuppressant (IMS) therapy. Success of transplant and favorable patient outcome relies on maintaining adequate therapeutic drug levels. Drug dosing must be individualized due to the narrow therapeutic range of these drugs, significant inter-individual variability, and intra-individual variability in absorption and metabolism. We developed, validated, and implemented a clinical liquid chromatography-mass spectrometry (LC-MS/MS) assay for simultaneous quantitation of tacrolimus and sirolimus in dried blood spots (DBS) collected remotely by patients and mailed into the laboratory. The purpose of this research is to assess the clinical utility of remote collection of dried blood spots for immunosuppressant monitoring, and compare the IMS level in paired collections of venous whole blood and dried blood spots.

Methods: The validation of the LC-MS/MS assay in dried blood spots was described previously, and the assay correlated well with our routine whole blood assay. To clinically correlate sirolimus and tacrolimus levels in capillary blood collected from a finger poke with venous whole blood, pediatric, post- transplant patients were asked to provide up to three paired collections of DBS and venous whole blood (total of 25 collections per drug). To be eligible, Seattle Children's patients needed to be

> 1 yr old, status post a heart, liver, or kidney transplant, and currently monitored for sirolimus or tacrolimus levels. Thirty-one patients consented and completed at least one paired collection. The phlebotomist only ordered the clinical whole blood immunosuppressant level, but collected both the venous and the blood spot. The participant took the dried blood spot card home with them with a pre-addressed, postage-paid envelope and mailed it back to the lab. The concentration and the turnaround times of the dried blood spot were compared with the whole blood sample. The recorded data include the unique study ID number, age of the patient, organ transplanted, immunosuppressant therapy, date and time of collection, whole blood level, date and time DBS was received, date and time DBS was analyzed, and DBS level.

Results: Tacrolimus in DBS correlated well with venous levels ($y = 1.03x + 0.84$, $R^2 = 0.93$, n=25). Overall, a small, but statistically significant negative bias was observed (-0.6 ng/mL, $p = 0.0013$). A chart review was performed to assess if clinical management would have changed, and none of the cases revealed a clinically significant change. Sirolimus in DBS also correlated with venous levels ($y = 0.83x + 1.17$, $R^2 = 0.84$, n=23). Overall, a small, negative bias was observed which was not statistically significant (-0.5 ng/mL, $p = 0.09$). Turnaround time from collection to receipt of DBS cards was on average 5.8 days, and ranged from 3 to 18 days.

Conclusions: In summary, analysis of IMS levels in DBS is possible, and the difference noted between capillary and venous blood is within the clinically acceptable limits.

B-396

A fast and sensitive high performance liquid chromatographic method for measuring 6 tricyclic antidepressants in human plasma

C. Yuan, M. Burgyan, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Monitoring certain tricyclic antidepressants (TCA) in blood is needed due to the large inter- and intra-individual variations. The objective of this work was to develop a high performance liquid chromatography (HPLC) method to measure 6 commonly prescribed TCA drugs, namely amitriptyline, imipramine, doxepin, nortriptyline, desipramine, and nordoxepin in plasma. **Methods:** Five hundreds µL of plasma samples and 50 µL internal standard solution (5 µg/mL of trimipramine and protriptyline in 0.1N HCl) were vortex mixed, followed by solid phase extraction using C18 cartridges. Chromatographic separation was achieved on a cyanopropyl bonded phase column (4.6x150 mm, 5 µm) with an isocratic elution of 22:18:60 mix of 10mM phosphate buffer (pH 7.0):methanol: acetonitrile at 1.8 mL/min. Detection was by UV at 214nm and quantification was based on peak height ratios using 5 level calibration. This method was compared with a separate HPLC-UV method using left-over patient specimens and spiked samples. **Results:** Baseline separation of all 8 compounds was achieved within 8.0 min. The obtained linearity ranges, mostly 20-500 ng/mL, fully encompassed the therapeutical ranges (within 50-300 ng/mL) of all the analytes. Precision was assessed at three different levels, and intra-assay and total CVs were <7.8%. No interference was found from lipemic, hemolytic, icteric and uremic plasma samples. Agreeable results were obtained in the method comparison study (Table). **Conclusion:** This newly developed HPLC-UV method offers fast and sensitive quantification of 6 common TCA drugs in human plasma.

Table 1. Method Comparison.

	n	Slope (95% CI)*	Intercept (ng/mL)*	Correlation Coefficient	Standard Error of Estimate (ng/mL)	Bias (%)
Amitriptyline	35	1.006 (0.968-1.044)	-5.3 (-13.1-2.4)	0.9943	14.1	5.5
Imipramine	32	0.939 (0.885-0.993)	2.1 (-7.9-12.1)	0.9880	15.7	4.9
Doxepin	22	1.046 (0.995-1.097)	-5.7 (-153.7-4.3)	0.9945	11.7	-0.4
Nortriptyline	33	1.032 (0.973-1.091)	-7.8 (-19.5-3.8)	0.9878	20.2	3.2
Desipramine	32	0.963 (0.930-0.989)	1.1 (-4.0-6.2)	0.9967	8.2	3.3
Nordoxepin	23	0.967 (0.943-0.990)	0.9 (-3.6-5.5)	0.9986	5.7	1.5

*Numbers in parenthesis indicate 95% confidence intervals.

B-397

Quantitation of (-)-Δ9-Tetrahydrocannabinol(THC) in Dried Blood Spots Using LC-MS/MS

J. Ye, H. Qiao, E. Majdi, L. Cousins. *IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada*

Background: Tetrahydrocannabinol (THC) is the major psychoactive ingredient in the cannabis plant. It has been shown that THC has complex effects on the central nervous system, correlated with the development of anxiety and psychotic disorders. After smoke inhaling of cannabis, THC is absorbed and incorporated into the bloodstream. THC is detectable for many hours after consumption and is a good indicator of recent cannabis consumption. LC-MS/MS is considered a useful tool for assessing the THC level in blood. The aim of the present study was to develop and validate a much simplifier and efficient high-throughput LC-MS/MS approach for the rapid quantification of THC in dry blood spot (DBS) samples.

Methods: The DBS was created by spotting 25uL spiked whole blood onto blank Whatman 903 paper and allowing a full 24 hours to dry at room temperature. A 3 mm punch was placed into a 1.5 mL vial with 100 uL exaction solution (acetonitrile with 10 mM ammonium acetate). The vial was vortexed for 10 minutes and soaked for 60 minutes. Then centrifuged and the supernatant transferred to an LC for injection. No SPE or evaporation step was required. A Shimadzu UFLC XR system was used. Sample was loaded onto Chromolith-RP18E column (100X3 mm, 3 um) held at 40^{0}C. A gradient LC method was created at a flow rate of 600 ul/min and a total LC run time of 4 minutes. Mobile phase A is 0.1% formic acid in 100% H2O and B is 0.1% formic acid in 100% ACN. An IONICS 3Q 220 mass spectrometer system was used which is equipped with heated coaxial flow ion source and a "Hot Source-Induced Desolvation" (HSID™) interface was used.

Results: The Calibration curve of the neat THC solution showed a good linearity over a range of 0.05-100 ng/mL with correlation value of R^2=0.994. At LLOQ of 0.05 ng/mL, the accuracy is 96% and CV is less than 10%. For DBS sample, no matrix interference was observed. Six calibration curves were generated with single injection over the range of 1-100 ng/mL. Good linearity with 1/x weighting was obtained for each curve with correlation value R^2 >0.99. The average accuracies for the six sets of THC spiked DBS samples at 1 ng/mL, 10 ng/mL, and 100 ng/mL are 100.9%, 99%, and 99.4% respectively. And the CVs at 1 ng/mL, 10 ng/mL, and 100 ng/mL are 4.2%, 4.7% and 4%, respectively.

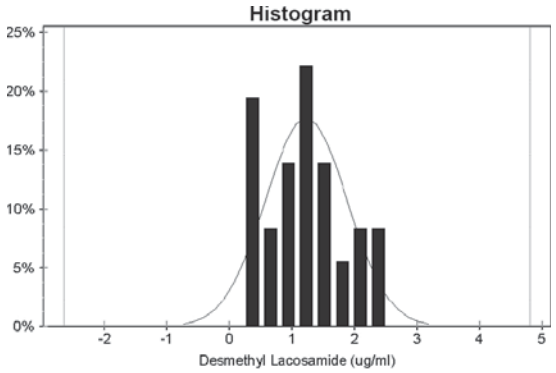
Conclusion: A fast, accurate, and precise LC-MS/MS method with IONICS 3Q 220 mass spectrometer was developed for direct measurement of THC in DBS. Significant time can be saved in the absence of SPE or LLE sample preparation. In the neat standard THC solution, an LLOQ of 0.05 ng/mL was achieved with accuracy of 96% and CV of 3.6%. Six sets of single injection calibration curves for DBS extraction showed good linearity over a range of 1 to 100 ng/mL. Averaged accuracy was between 99 and 101%, and the CVs were < 5%. This LC-MS/MS method confirms its clinical applicability for THC level monitoring in DBS.

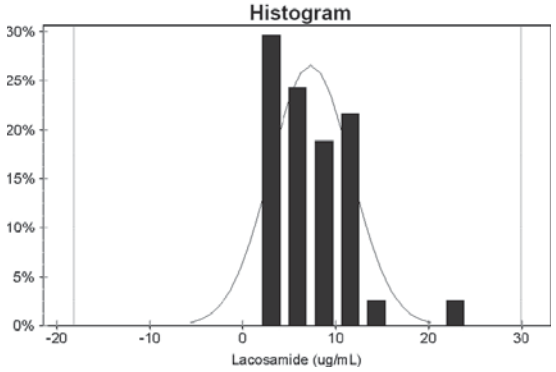
B-398

Establishment of Expected Ranges for the Random Serum Concentration of Lacosamide and Desmethyl Lacosamide

D. A. Payto, N. Foldvary-Schaefer, N. So, M. Bruton, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Lacosamide (LCM) is an antiepileptic drug approved by the Food and Drug Administration for adjunctive treatment of partial onset seizures. LCM has a novel mode of action by selectively enhancing the slow deactivation of the sodium gated channels. LCM reaches peak concentration within 1-4 hours when taken orally and has a half-life of 13 hours. In humans the major metabolite is O-desmethy lacosamide (ODL). Theraputic drug monitoring (TDM) of LCM and ODL is used to help optimize therapeutic dosing, while limiting adverse effects. TDM is also used for establishing an individualized therapeutic range and to assess compliance to therapy. **Method:** Random serum samples (n=45) were obtained from adult subjects taking LCM adjunctive therapy for partial onset seizers as part of a clinical trial. The subjects consisted of 7 males and 16 females. These subjects were on either a 200mg/day or 400mg/day dose of Lacosamide. The random serum samples were analyzed on a previously validated LC-MS/MS method for LCM and ODL. **Results:** A statistical analysis was performed, in Excel, using the central 95% criteria to establish an expected concentration range. For subjects taking 200mg/day of LCM the random serum concentration of LCM and ODL was found to be 2.2 to 9.8 µg/mL and up to 1.6 µg/mL respectively. For subjects taking 400mg/day LCM the random serum concentration of LCM and ODL was found to 3.1 to 19.8 µg/mL and 0.5 to 2.5 µg/mL respectively. Histograms, see figure, were constructed using EP Evaluator to show distribution. **Conclusion:** The expected random concentration of LCM and ODL for patients taking 200-400mg/day of LCM is 2.2 to 19.8 µg/mL and up to 2.5 µg/mL respectively.





B-400

13-Panel toxicological drug screen of umbilical cord tissues with enzyme-linked immunosorbent assays (ELISAs)

I. Shu, M. Pilkington, C. A. Plate, J. Jones, D. Lewis. *United States Drug Testing Laboratories, Inc, Des Plaines, IL*

Relevance: Current best practice for detecting *in utero* drug exposure is to test newborn's meconium samples. However, a meconium sample is not available in 8-20% of births in the United States. Umbilical cord is readily available for collection in every birth. Previous studies have shown that an umbilical cord drug test performs equivalently well as a meconium drug test. The detection of drugs requires high sensitivity and fast turn-around for further interventions. Our **objective** is to develop and validate a multiplex 13-panel ELISA drug screen that provides equivalent sensitivities as the chromatography-mass spectrometry (GLC-MS) based confirmatory methods.

Method: An aliquot of approximately 0.5g of umbilical cord was homogenized in 3.0mL of acetone followed by centrifugation. The supernatant was filtered, 0.2% succinic acid in acetone was added, the sample taken to dryness, and finally reconstituted with 700 micro-liter buffer. Laboratory determined volumes of the extracts were aliquoted to each of thirteen ELISA plates using automated liquid handling devices for development according to drug assay manufacturer package inserts. The assay principle is homogeneous-competitive immunoassay, where the intensity of the developed color is inversely proportional to the sample's drug concentration. The absorbance of each sample well was normalized to that of the negative controls (B/B_0) of the same ELISA plate. Validation was performed according to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. We set the cut-off levels (decision points) listed below: 0.1ng/g for Tetrahydrocannabinoids; 0.5ng/g for Cocaine, Opiates, Oxycodone, and Buprenorphine; 1.0ng/g for Barbiturates; 2.0ng/g for Phencyclidine, Benzodiazepines, Methadone, and Meperidine; 4.0ng/g for Propoxyphene and Tramadol; and 5.0ng/g for Methamphetamine.

Results: Five controls with concentrations ranging between 50-150% of cut-off levels were prepared for analysis of precision and linearity. Coefficients of variation (CV%) were 0.9-12.4% within-run (n = 4) and 2.9-19.4% between-run (5 runs, n=20). The mean ± 2 standard deviations (SDs) for levels at ±50% of cut-off did not overlap with the decision points. Correlation coefficients (R^2) of B/B_0 versus concentrations (expressed in logarithm) were 0.9567-0.9977, demonstrating acceptable linearity. The mean $B_0-3.3$SDs determined lower limits of detection ranging 3.0-41% of cut-off levels. The immunoassays did not present hook effect and carry-over at least at 100 times of cut-off levels. The assays did not show interference from common over-the-counter or prescription drugs at 1000 ng/g, except dihydrocodeine for Opiates and Oxycodone assays. ELISA screened positive samples were subsequently quantitated using previously validated confirmatory methods to detect determinant drugs and metabolites of each class at the same cut-off. We found that 83.6-98.0% of the ELISA screened positive samples were eventually confirmed positive.

Conclusion: Fetus exposure to drugs results in adverse effects on newborn health, and early intervention is crucial. Although GLC-MS is a preferred methodology to detect minute amounts of harmful substances deposited into the umbilical cord, solely depending on this technology is operationally difficult to support such critical care. We validated and determined the 13-panel multiplex ELISA screen method to be appropriate for implementation and equivalently sensitive as the more time-consuming drug-class specific GLC-MS methods.

B-401

Evaluation of a liquid chromatography tandem mass spectrometry analytical method for the quantification of a panel of antiepileptic drugs and their metabolites in human plasma

C. De Nardi[1], A. Morando[2], A. Del Plato[2]. *[1]Thermo Fisher Scientific GMBH, Dreieich, Germany, [2]Ospedale "La Colletta", Arenzano, Italy*

Background: the evaluation of an analytical method for the quantification of 17 different antiepileptic drugs and metabolites in human plasma is reported. The panel includes levetiracetam, theophylline, felbamate, lacosamide, rufinamide, carbamazepine, oxcarbazepine, carbamazepine diol, carbamazepine epoxide, 10-hydroxycarbamazepine, PEMA, primidone, phenytoin, stiripentol, zonisamide, phenobarbital and valproic acid. The method is based on liquid chromatography tandem mass spectrometry performed on a Transcend™ LC system combined with a TSQ Quantum™ Access MAX triple stage quadrupole mass spectrometer, both from Thermo Scientific™. The MassTox® TDM Series A kit for antiepileptics from Chromsystems™ was used for the scope; the kit included mobile phases, an analytical column, calibrators, controls and extraction, precipitation and dilution buffers. The calibration range covered the therapeutic window of concentrations in plasma for each analyte.

Methods: following the instructions provided by the kit supplier, lyophilized calibrators (on three levels) and controls (on two levels) were resuspended with distilled water, protein precipitated using the precipitation buffer (containing 12 different internal standards), vortex-mixed, centrifuged and the supernatant diluted with the dilution buffer prior to injection. Blank plasma samples from different donors were also added to the batch. Each calibrator and control sample was prepared in duplicate. The analytes of interest were divided into three groups and analyzed using three different gradient elutions, with runtimes of 5.0, 3.8 and 2.2 minutes. A heated electrospray source was used for sample ionization in both positive and negative mode and detection was performed by Single Reaction Monitoring (SRM). Data acquisition and processing was performed using Xcalibur™ software. Specificity, linearity and accuracy of the method were evaluated for each analyte. Specificity was tested by checking the absence of interfering peaks in plasma samples from different donors not taking any antiepileptic drug. Linearity in the calibration range set by the kit supplier was evaluated using a linear interpolation with 1/x weighting for all the analytes. The percentage bias between nominal and experimental concentration for both calibrators and control samples was used to assess the accuracy of the method; the adopted acceptance criterion for accuracy was: bias within ±15% for calibrators and within ±20% for control samples.

Results: the analytical method proved to be specific and accurate, with no interfering peaks and a maximum percentage bias within the acceptance criteria for both calibrators and control samples. Linear calibration curves were obtained for all the analytes of interest, with a correlation coefficient (R^2) always above 0.99.

Conclusion: the reported method can be successfully applied to the quantification of a large panel of antiepileptic drugs and metabolites in human plasma by liquid chromatography tandem mass spectrometry using a TSQ Quantum Access MAX.

B-402

Quantitation of Pentobarbital in Serum Using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS)

V. Ricchiuti, E. Chaffin, F. Lucas. *University of Cincinnati Medical Center, Cincinnati, OH*

Background. Pentobarbital is a central nervous system depressant with sedative and hypnotic properties. It is crucial to monitor levels with an acceptable turn-around time. Gas Chromatography Flame Ionization Detection (GC/FID) is a commonly used

method for the analysis of barbiturates, but can be labor intensive. Our aim was to validate a quantitative pentobarbital analytical method by LC/MS-MS which will be used as a routine method in the clinical laboratory with a faster turn around time (TAT) and will be less labor intensive than GC/FID.

Methods. Pentobarbital present in serum is extracted using a methanol/acetonitrile protein precipitation, followed by dilution and analysis by Shimadzu Prominence 20A Liquid Chromatograph, followed by the AB SCIEX QTRAP® 4500 Mass Spectrometry System (LC/MS-MS). A 20 uL specimen is mixed with a methanolic, deuterated internal standard (pentobarbital-D5), which initiates protein precipitation. The proteins are then further precipitated with the addition of acetonitrile. A portion of the supernatant is transferred and diluted with methanol:water (50:50) for injection into the LC/MS-MS. Qualitative identification is based on the presence of the specific MRM transitions for pentobarbital at the correct retention time. Quantitative measurement is accomplished by normalization of the peak area with the area of the internal standard for each specimen, including matrix specific calibrators, and quality control (QC) materials. Each sample is separately processed by the instrument software. The program automatically constructs a calibration curve, using the peak abundance data from the calibrator samples. QC are extracted and analyzed with patient samples. All data is subjected to analyst and technical QC review, prior to acceptance for reporting of results.

Results. The relative intra-laboratory reproducibility standard deviations were, in general, better than 5% at concentrations in the therapeutic range. The estimated lowest limit of detection (LOD) was zero 0.09 ug/mL and lowest limit of quantitation (LOQ) was 0.29 ug/mL. LOQ for serum pentobarbital using CV<20% was <0.5 ug/mL and was used as clinical cut-off. The mean true recoveries for samples in the analytical measurement range 0.5-100 ug/mL were between 96-106%. Correlation with GC-FID was excellent (y=0.9x1.1, r=0.99, p<0.05, n=21). Sample recoveries (n=3) following five freeze/thaw cycles were 98-111%. TAT was approximately 2 hours from receiving of specimen to reporting result to physician.

Conclusion. The quantitative pentobarbital method by LC/MS-MS is an easy and user-friendly method with an excellent analytical sensitivity. It requires one-step for extraction of pentobarbital from serum, and then a direct injection into the LC/MS-MS system. We were able to achieve excellent TAT with our method, and were able to meet our institution's goal for TAT.

B-403

Drug monitoring and toxicology: simultaneous determination of total mycophenolic acid and its glucuronide by HPLC-UV

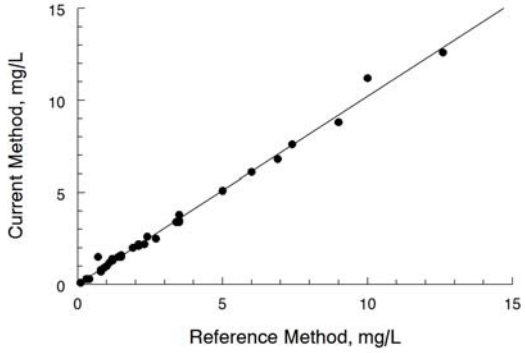
P. H. Tang. *Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH*

Background: Mycophenolic acid (MPA) is a potent immunosuppressant. MPA is primary metabolized to an inactive glucuronide MPAG, which is transported from liver into bile. Biliary MPAG then enters the GI tract, where it is converted back to MPA, which is then recycled into the bloodstream *via* the enterohepatic circulation pathway. Several studies have documented that variation in MPA plasma concentrations are unpredictable; variability in plasma concentration of MPA both within and between individuals is high. Therapeutic drug monitoring (TDM) of MPA plasma concentrations can help the clinician to develop personalized therapy strategies to avoid toxicity and maintain efficacy. To support the clinical studies of mycophenolate mofetil and to provide the clinical service for the TDM of MPA and MPAG ordered by the researchers and physicians, a simple and rapid HPLC method using UV detection has been developed.

Methods: The analytical procedures include single dilution step, protein precipitation, ultracentrifugation and gradient elution chromatography. Separation of MPA, MPAG and internal standard clonazepam was achieved by using a 5-μm Microsorb-MV C18 column (150 x 4.6 mm). These compounds were quantified by UV absorbance at 306 nm.

Results: Linearity was verified over ranges of 0.1-20 mg/L and 1-200 mg/L for MPA and MPAG, respectively. Recoveries of MPA and MPAG ranged between 93 and 105%. Both within-run (n = 6) and between-run (n = 30) precisions were lower than 10%. Figure 1 illustrates a comparison between the current method and reference method for MPA. The linear regression statistics indicated an r value of 0.993 (P < 0.0001). The linear regression equation for correlation was $y = 1.018\ x + 0.031$; where y, the current method and x, the reference method. No interferences with other common drugs were observed.

Conclusion: The analytical method is very suitable for both routine clinical use and pharmacokinetic studies.



B-404

Detection of 13 Opioids by LC-MS/MS from dried urine spots

J. M. Boyd[1], V. Simon[2], J. Bacani[3], V. Dias[1], S. M. H. Sadrzadeh[1]. *[1]Department of Pathology and Laboratory Medicine, University of Calgary and Calgary Laboratory Services, Calgary, AB, Canada, [2]Calgary Laboratory Services, Calgary, AB, Canada, [3]Faculty of Medicine, University of Calgary, Calgary, AB, Canada*

Introduction: Dried specimens offer a convenient and economical approach for collection, storage and transport of clinical specimens. Although, dried blood spots have been used as the specimen of choice in clinical laboratories (Biochemical Genetics) for years, dried urine spots (DUS) have not been established for routine use in clinical laboratories. Traditionally, drugs of abuse in clinical laboratories are detected by immunoassay (IA) and confirmed by GC-MS, which is time consuming and expensive. LC-MS/MS can offer a one-step detection and confirmation method for detection and measurement of drugs.

Objectives: To develop a LC-MS/MS method to detect multiple drugs in DUS in one-step.

Methods: 13 opioids (morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, heroin, 6- mono-acetyl morphine (6-MAM), fentanyl, norfentanyl, naloxone, tramadol, and meperidine) were analyzed in dried urine spot extraction. Briefly, 15 µL of de-identified urine samples containing the above drugs was spotted onto Whatman 903 Protein Saver Cards (GE Healthcare Biosciences). Drugs were extracted using a mixture of methanol, acetonitrile and water (4:4:1). Extracts, evaporated at RT, reconstituted in 5% acetonitrile before injecting into the LC-MS/MS. Urine samples were spiked by standards ranging from 100-2000 ng/mL. HPLC: Agilent 1290 (Palo Alto, CA) with a Restek Ultra Biphenyl column (100mm x 2.1 mm x 5µm). Mobile phase A was 1 mM ammonium formate and 0.1% formic acid in water; Mobile phase B was 0.1% formic acid in acetonitrile. Separation was achieved using a gradient elution program starting at 99% A for 1 minute, decreasing to 95% A for one minute, and then decreasing to 50% A until 13.6 minutes, holding at 50% A until 15.6 minutes, and returning to 99% an over 0.1 minutes. Flow rate was 0.5 mL/min and the injection volume was 10 µL.

Mass spectrometric detection was performed using an Agilent 6460 triple quadrupole mass spectrometer. Source parameters were optimized as follows: Electrospray voltage +2500V, Sheath gas temperature 380 °C, sheath gas flow 11 L/min, Nebulizer gas was 30 psi, Source gas temperature was 300 °C and the gas flow was 9 L/min. MRM transitions for all analytes were selected and optimized using the Agilent Optimizer software using 1000 ng/mL of each analyte in methanol. Data analysis was performed using Agilent MassHunter Quantitative analysis software

Results:

Our preliminary recovery results from DUS by LC/MSMS were: Morphine, 97%; Oxymorphone, 97%, hydromorphone, 100%; naloxone, 100%; codeine, 100%; 6-MAM, 99%; hydrocodone, 99%; oxycodone, 100%; heroin, 98%; fentanyl, 97%; norfentanyl, 97%; tramadol, 100%; and meperidine, 97%. These results matched those generated from identical liquid urine same by traditional immunoassay followed by GC/MS.

Conclusions: We modified a LC-MS/MS method to detect 13 opioids from DUS, simultaneously. DUS offers a convenient and economical approach for specimen collection, transportation and storage. This one-step LC-MS/MS detection confirmation method eliminates the need for the traditional multi-step approach of immunoassay and GC-MS and can greatly reduce the operational cost. More drugs of abuse are being tested.

B-405

Effectiveness of Afternoon Dosing Policy on Digoxin Level Monitoring

Y. R. Hsu, G. S. Cembrowski, D. F. LeGatt. *University of Alberta, Edmonton, AB, Canada*

Background: Determining serum or plasma digoxin levels is helpful in titrating drug dosage as well as assessing compliance and toxicity. However, inappropriately timed sample collection in relation to dose may result in level misinterpretation with the risk of over or under dosing. To circumvent inappropriately timed collections, the policy of afternoon dosed digoxin (PM dosing) in all inpatient care facilities in Alberta Health Services Edmonton Zone was instituted on November 5, 2008. This policy was also adopted in other but not all Northern Alberta health care facilities. The purpose of this study was to evaluate the effect of the policy change on digoxin dosing time by comparing pre- and post-implementation periods.

Method: The evaluation was conducted by comparing the time periods of approximately four years before and four years after policy implementation (November 1, 2004 to October 31, 2012). All digoxin levels during this period were included. Those with available dosing information were grouped into AM (0001h to 1159h) and PM (1200h to 2400h) dosing groups as well as into inpatients and outpatients, which also included collection at emergency departments.

Results: A total of 58,236 digoxin levels were performed in Northern Alberta from November 1, 2004 to October 31, 2012. There was a steady decline in the number of tests done annually (45.8% decline over eight years), likely reflecting the decreased prescription of digoxin in recent practice. Of the 58,236 requests, 23,324 (40%) had dosing information provided on the requisition forms. Within this latter group, the proportion where dosing occurred PM was significantly higher after policy implementation (mean 47±2.1% vs 28±0.73%, $p < 0.05$). For Edmonton Zone inpatient facilities, the proportion of requests with PM dosing increased significantly in the four year period after policy implementation (mean 56±3.4% vs 27±1.5%, $p < 0.05$). The proportion of digoxin levels at increased risk of toxicity (level >2.0 nmol/L) was significantly higher pre-policy implementation (mean 13±2.5% vs 5.5±0.58%, $p < 0.05$); whereas, the proportion of samples below target digoxin level was higher post-policy (mean 32±6.3% vs 16±2.9%, $p < 0.05$). The proportion of samples within the target range (0.6 to 1.2 nmol/L for heart failure) was similar pre- and post-policy (mean 42±2.6% vs 46±4.8%, $p > 0.05$).

Conclusion: This study demonstrates the sustained effect of increased compliance to PM dosing since the institution of this policy in Alberta Health Services Edmonton Zone in November 2008. However, other intervention strategies are required to further improve policy adherence, hence minimizing the chance of inappropriately timed collections. The lack of dosing information on the requisition forms is another issue that precludes a complete assessment.

B-406

Development of a High-throughput LC-MS/MS Assay for Pain Management Panel from Urine

H. Qiao, J. Ye, E. Majdi, L. Cousins. *IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada*

Background: The widespread use and the potential abuse of opiates, sedatives, and stimulants drugs have increased the need and in some cases the requirement to screen patients on a routine basis. Pain panels continue to grow in complexity as more prescription and non-prescription compounds are added. This has made the job of toxicological analysis even more challenging. To fulfill these requirements, a fast, reliable, and accurate LC-MS/MS method has been created for the analysis of a pain panel comprised of 30 drugs on an IONICS 3Q 120 triple quadrupole mass spectrometer.

Methods: First, the mixed drug standard solution was spiked into the urine matrix, then diluted with the mobile phase A (100% H2O, 0.1% formic acid) to make a series of concentrations ranging from 0.016 to 16 ng/mL. The internal standard concentration used was 10 ng/mL. The calibrator solutions will be directly injected without further treatment. IONICS 3Q 120 mass spectrometer equipped with a heated coaxial flow ion source and "Hot Source-Induced Desolvation" interface was used. The time-managed MRM in Molana™ software was used to optimize the dwell time for each MRM transition based on the retention times and the number of MRM transitions within given experiments. The separation was performed on a Shimadzu Prominence LC system. A 10 uL sample was loaded onto a Restek Ultra II Biphenyl column (50 x 2.1 mm, 5um) kept at 40 °C. A gradient method was created with a flow rate of 600 uL/min and a total LC cycle time of 7.5 minutes. Solvent B was composed of 0.1% formic acid in 100% methanol.

Results: A total of 57 MRM transitions were used to monitor 30 drugs including internal standards. No matrix interferences were observed. LC system carryover was checked to ensure the validity of the data. An overlay of the extracted chromatograms of 30 drugs in a 7.5 minute LC run showed that all of the anlaytes were clearly separated. The calibration curves showed good linearity for all the analytes across the whole concentration range with a coefficient $R^2>0.99$. All calibration curves used a linear weighting regression of 1/x. The LLOQs for the 30 drugs were in the range of 0.032 to 2 ng/mL. At LLOQs, the accuracy was between 84-114%, and CVs were < 10% for all analytes.

Conclusion: The results in this study show that in a 7.5-minute LC run, this LC-MS/MS method can effectively separate the 30 pain panel drugs. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 30 drugs is in the range of 0.032 to 2 ng/mL, which is 2 to 3 orders lower than the typical screening cutoff concentration (300 ng/mL), and much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of the drugs of abuse. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is an effective combination for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

B-407

Development of Abbott Phenytoin Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

J. Donaldson[1], N. Pham[1], A. Kong[1], S. Hoang[1], S. Shaw[2], N. Pham[2], O. Ndimbie[2], C. Kasal[2], L. Ye[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Abbott Laboratories, Irving, TX*

Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of phenytoin in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of phenytoin overdose and in monitoring levels of phenytoin to help ensure appropriate therapy.

Methods: The Abbott Phenytoin Assay is a liquid ready-to-use, homogeneous enzyme immunoassay. The two reagent kit uses specific antibodies to detect phenytoin in the sample, with minimal cross-reactivity to various over-the counter, structurally unrelated compounds. The method is based on the competition for a fixed amount of specific antibody binding sites between enzyme [glucose-6-phosphate dehydrogenase (G6PDH)]-labeled phenytoin, and phenytoin contained in the sample. In the absence of phenytoin from the sample, the specific antibody binds the G6PDH-labeled phenytoin and causes a decrease in enzyme activity. If phenytoin is present in the sample, it occupies the antibody binding sites, which allows the G6PDH-labeled phenytoin to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the phenytoin concentration in sample and enzyme activity. By measuring the enzyme's ability to convert nicotinamide adenine dinucleotide (NAD) to NADH, its activity is determined spectrophotometrically at 340 nm.

Results: The performance of the Abbott Phenytoin Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of $\leq$ 1.8 µg/mL using inter-assay precision $\leq$ 7% CV or $\leq$ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 1.8 to 40.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing phenytoin at concentrations ranging from 3.9 µg/mL to 36.1 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.6 %CV to 3.3 %CV for Within-Run and 2.0 %CV to 4.1 %CV for Total-Run. The assay accurately recovered spiked phenytoin at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with phenytoin derivatives and metabolites at the concentrations tested with the exception of fosphenytoin. Abbott ARCHITECT Phenytoin patient correlation studies: new vs. current on-market assay yielded a regression equation of $y=1.11x + 0.12$ and a correlation coefficient of 0.99. The new reagent has an onboard stability of 40 days and calibration curve stability of 7 days.

Conclusion: The Abbott Phenytoin Assay enables measurement of phenytoin in human serum or plasma with high precision across the linear range. The ability to monitor levels of phenytoin with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-408

Urine drug screening: using GC-MS/MS to augment LC-MS/MS screens

J. M. Colby, A. M. Gordon, A. H. B. Wu, K. L. Lynch. *University of California, San Francisco, San Francisco, CA*

Background & Objective Clinical toxicology services, including the detection of pharmaceuticals and drugs of abuse in biological samples, are routinely offered by many hospital laboratories and can play an important role in patient management. In addition to immunoassay screening for routine drugs of abuse, our laboratory offers a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to screen patient urine samples for a large number of clinically relevant compounds. Immunoassays are not available for many of the compounds in our method, which makes it a challenge to verify results by two methods. Here we report the development and validation of a gas chromatography tandem mass spectrometry (GC-MS/MS) method for comprehensive urine drug screening with method performance evaluated in part by concordance with our LC-MS/MS method.

Methods GC-MS/MS was performed on a Bruker ScionTQ mass spectrometer with an EI source and a Bruker 436 GC with a 30m BR-5ms column. Chomatographic runs began at 80°C and ramped to 295°C over 20 minutes. Retention times (RT) were established and two multiple reaction monitoring (MRM) transitions were developed for each of the approximately 150 compounds in the method. Compounds were identified by RT and the presence/ratio of the two MRM transitions. For method validation analytes were spiked into 0.5 mL urine, isolated using solid phase extraction (SPE), eluted in either the acid/neutral or basic fractions, and evaporated to dryness at 40° under N_2 flow. Extracts were reconstituted in 50:50 methanol:acetonitrile and 1 µL was injected. Our validation included determining the lower limit of detection (LLOD), matrix effects, and SPE recovery for each compound. At least 30 patient samples were analyzed by both GC-MS/MS and LC-MS/MS.

LC-MS/MS was performed on an Agilent HPLC with an ABSciex 3200 QTRAP mass spectrometer in positive-ESI mode. Compounds were identified by a combination of RT, one MRM transition and a match between the collected product ion spectrum and our in-house-built spectral library.

Results Overall the GC-MS/MS method performed quite well. LLODs ranged from 5 ng/mL to 250 ng/mL. A small fraction of compounds were not observed in any validation samples, likely due to extremely low volatility. Method validation included determining matrix effects using two donor urine matrices and evaluating the recovery of our solid phase extraction method for acid/neutral and basic drugs. Comparison of patient samples between LC-MS/MS and GC-MS/MS showed good concordance. As expected, many of the compounds that were missed by GC-MS/MS were large and/or quite polar, for example glucuronide conjugates.

Conclusions GC-MS/MS has many advantages for identification of drugs in biological matrices. GC-MS/MS instruments are generally less expensive than LC-MS/MS instruments and can analyze some compounds that LC cannot and GC-MS/MS can offer lower limits of detection than GC-MS systems. We have recently had cases involving pentobarbital and 1,4-butanediol, neither were detected using our LC-MS/MS but were easily observed with our GC-MS/MS. Use of both technologies in our laboratory allows us to screen patient urines for a wider variety of compounds with a greater assurance of accuracy.

B-409

Ultrafast Quantitative Analysis of Illicit Drugs and Benzodiazepines in Urine Using High-throughput SPE/MS/MS

F. Mbeunkui, B. Marshall, S. Sullivan, R. Dixon. *Physicians Choice Laboratory Services, Rock Hill, SC*

Introduction Pathologists, employers and law enforcement officials use drug testing extensively today. Drug screening typically involved immunoassay analysis followed by a confirmatory test by GC/MS or LC/MS detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. The RapidFire (RF) platform provides an automated solid-phase extraction (SPE) system that gives a throughput of approximately 12 seconds per sample to the mass spectrometer.

In the present study, we evaluated the ability of the RF/MS/MS system to quantitatively measure a panel of illicit drugs or benzodiazepines in urine. Imprecision, accuracy and linearity results achieved with this ultrafast RF/MS/MS system were comparable to LC/MS/MS.

Methods Blank matrix containing internal standards was spiked with the analytes of interest in a range of concentration to prepare the calibration curve. The illicit drug panel consisted of 6-monoacetylmorphine, benzoylecgonine and phencyclidine. This panel was subjected to dilution (1/10) with an aqueous solution containing the internal standards prior to online SPE. The benzodiazepine panel consisted of temazepam, 7-amino clonazepam, nordiazepam, alpha hydroxy alprazolam, lorazepam, alprazolam and oxazepam. Benzodiazepine samples were subjected to enzymatic hydrolysis followed by centrifugation and then diluted (1/50) prior to online SPE. Online SPE methods were optimized for each panel. Analysis of all samples was performed at a rate of <12 seconds per sample using a RapidFire high-throughput system coupled to a triple quadrupole mass spectrometer.

Results The analytes in the illicit panel had good linearity with R2 values >0.995 within the measurement range of 2.5-500 ng/mL for 6-monoacetylmorphine, 5-1000 ng/mL for phencyclidine and 25-5000 ng/mL for benzoylecgonine. The average intra- and interday accuracy at LLOQ was 99% for 6-monoacetylmorphine, 105% for phencyclidine and 102% for benzoylecgonine. The average intra- and interday imprecision at LLOQ was 17% for 6-monoacethylmorphine, 7% for phencyclidine and 2% for benzoylecgonine. The standard curve of benzodiazepine analytes had excellent linearity within the measurement range (50-5000 ng/mL) with R2 values >0.999. The average intra- and interday accuracy and imprecision at LLOQ for all compounds were 103% and 8% respectively. No significant carry-over was detected for any illicit and benzodiazepine analytes. The linearity, accuracy and imprecision results from SPE/MS/MS analysis were comparable to those from traditional LC/MS/MS. The SPE/MS/MS analysis was more than 20 times faster than LC/MS/MS.

Conclusions Illicit drugs and benzodiazepines can accurately, precisely and rapidly be quantified using an ultrafast SPE/MS/MS system. This system is an alternative to traditional LC/MS/MS, combining efficiency and speed.

B-410

Compliance Rates In Chronic Cancer Pain Patients.

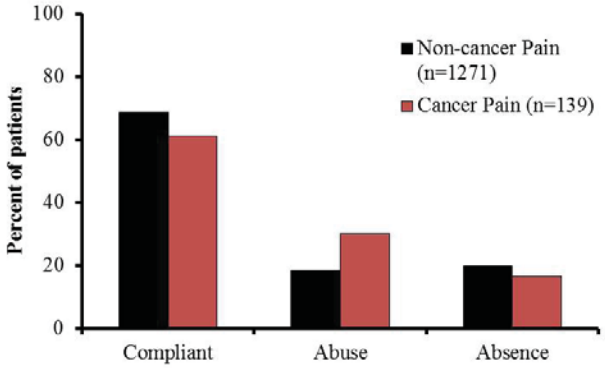
J. A. Hayden, P. Mathias, A. Hoofnagle, G. Baird. *University of Washington, Seattle, WA*

Background: A distinction is often made between patients on prescription opioid therapy for chronic cancer and chronic non-cancer pain. This distinction can have wide reaching implications, such as impacting which patients are and are not subject to new legislation. No evidence exists to suggest that these two patient populations differ in terms of the prevalence of opioid misuse.

Methods: This retrospective cohort study included 1,271 non-cancer and 139 cancer patients on prescription opioids who submitted urine samples to our laboratories between May of 2012 and April of 2013. All patients were subject to chart review as part of normal clinical operations by a single reviewer. Chronic cancer pain was based on cancer or cancer therapy related pain being the primary diagnosis in the chart. Urine samples were analyzed for the presence or absence of prescription opioids (oxycodone, hydrocodone, methadone, morphine, hydromorphone, fentanyl, buprenorphine, meperidine, propoxyphene) and the heroin-specific metabolite 6-monoacetylmorphine using a clinically validated, laboratory-developed liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. Cocaine abuse was determined by an immunoassay (Syvia EMIT II) and methamphetamine was determined with a separate validated LC-MS/MS assay. Benzodiazepines and other analgesics (tramadol,carisoprodol, etc) not detected in our assays were not considered.

Results: Cancer patients showed a compliance rate statistically indistinguishable from that of non-cancer patients (61% and 68%, respectively). The patterns of abused drugs were not substantially different between these two populations. Both populations were three times more likely to abuse prescription opioids than illicits (heroin, cocaine and methamphetamine).

Conclusion: Chronic cancer pain patients are equally likely to misuse prescription opioids, and in particular to use non-prescribed prescription opioids. This misuse puts them at risk for adverse outcomes and clinicians should be aware of these dangers when managing chronic cancer pain.



B-412

Rapid measurement of tacrolimus, cyclosporin A and sirolimus in blood by paper spray-tandem mass spectrometry (PS-MS/MS)

R. Shi[1], E. M. El Gierari[2], N. E. Manicke[3], J. D. Faix[1]. *[1]Stanford University School of Medicine, Stanford, CA, [2]Stanford Hospital & Clinics, Stanford, CA, [3]Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN*

Background: therapeutic drug monitoring (TDM) of immunosuppressant(s) is critical in preventing organ rejection after transplant. Automated immunoassays provide quick turnaround time but are costly and lacking standardization. Conventional tandem mass spectrometry (MS/MS) requires a liquid chromatography (LC) system and assay requires pre-analytical manipulation which is not amenable to random access testing. Paper spray (PS) ionization is a technique that generates gas phase analyte ions directly from dried blood spots for quantitative MS/MS analysis, without complex sample preparation, nor a LC system. We planned to evaluate PS-MS/MS for simultaneous tacrolimus, cyclosporin A and sirolimus TDM in a clinical diagnostic laboratory, by examining assay precision, accuracy, and analytical measurement range (AMR), as well as assay specificity and possible analyte ion suppression phenomenon.

Methods: 200 µL of whole blood, calibrators, or quality control material were mixed with 50 µL of stable isotope labeled internal standard mixture ([^{13}C, $^{2}H_2$]-FK506, [$^{2}H_4$]-cyclosporin A, and [$^{2}H_3$]-rapamycin). The blood mixture (10 µL) was spotted onto triangular shaped card paper contained in disposable cartridges and dried at 40°C. Cartridges were analyzed using an automated paper spray ion source coupled to a triple quadrupole MS/MS (TSQ Vantage™; Thermo Scientific). Small amount (up to 120 µL) of methanol and chloroform mixture with added sodium acetate was delivered by the ion source to blood containing paper in cartridge before a voltage of 3.5 kV was applied to generate ion spray. Sodium adduct ions of each immunosuppressant, along with corresponding internal standard ions were detected in the selected reaction monitoring (SRM) mode and quantitated by the area under the curve of collected 50 scan within 1.0 min, with an average analysis time of 3 min per sample. Each analyte result was confirmed by using a second SRM.

Results: the PS-MS/MS method has acceptable AMR and precision, and results correlate well with those of a FDA approved immunoassay currently used in clinical labs. Patient samples may be analyzed in batches by PS-MS/MS, or analyzed by adding on to batch throughout the day, much like in a random access fashion.

Conclusions: PS-MS/MS is much simpler in comparison to a conventional LC-MS/MS system. It simultaneously provides accurate results for tacrolimus, cyclosporin A and sirolimus, with fast turnaround time amenable to random access testing protocols.

B-413

Development of Abbott Theophylline Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

J. Donaldson[1], N. Pham[1], A. Kong[1], S. Hoang[1], S. Shaw[2], N. Pham[2], O. Ndimbie[2], C. Kasal[2], L. Ye[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Abbott Laboratories, Irving, TX*

Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of theophylline in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of theophylline overdose and in monitoring levels of theophylline to help ensure appropriate therapy.

Methods: The Abbott Theophylline Assay is a liquid ready-to-use, homogeneous enzyme immunoassay. The two reagent kit uses specific antibodies to detect theophylline in the sample, with minimal cross-reactivity to various over-the counter, structurally unrelated compounds. The method is based on the competition for a fixed amount of specific antibody binding sites between enzyme [glucose-6-phosphate dehydrogenase (G6PDH)]-labeled theophylline, and theophylline contained in the sample. In the absence of theophylline from the sample, the specific antibody binds the G6PDH-labeled theophylline and causes a decrease in enzyme activity. If theophylline is present in the sample, it occupies the antibody binding sites, which allows the G6PDH-labeled theophylline to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the theophylline concentration in sample and enzyme activity. By measuring the enzyme's ability to convert nicotinamide adenine dinucleotide (NAD) to NADH, its activity is determined spectrophotometrically at 340 nm.

Results: The performance of the Abbott Theophylline Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 2.0 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 2.0 to 40.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing theophylline at concentrations ranging from 3.5 µg/mL to 37.0 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.4 %CV to 2.3 %CV for Within-Run and 1.9 %CV to 3.3 %CV for Total-Run. The assay accurately recovered spiked theophylline at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with theophylline derivatives and metabolites at the concentrations tested with the exception of caffeine. Abbott ARCHITECT Theophylline patient correlation studies: new vs. current on-market assay yielded a regression equation of y=1.07x - 0.07 and a correlation coefficient of 1.0. The new reagent has an onboard stability of 40 days and calibration curve stability of 7 days.

Conclusion: The Abbott Theophylline Assay enables measurement of theophylline in human serum or plasma with high precision across the linear range. The ability to monitor levels of theophylline with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-414

Isotope Dilution Gas Chromatography-Mass Spectrometry (GC/MS) Method for the Analysis of Hydroxyurea

U. Garg, D. Scott, C. Frazee, G. Kearns, K. Neville. *Children's Mercy Hospitals and Clinics, Kansas City, MO*

Background: Hydroxyurea (HU) is used in the treatment of various malignancies such as chronic myelogenous leukemia, squamous cell carcinomas, polycythemia vera and essential thrombocytosis. It is also used in the treatment of sickle cell disease where it is shown to decrease painful crisis and reduce number of transfusions. There are limited studies on the pharmacokinetics of hydroxyurea. An accurate, precise and sensitive method is needed to support such studies and also the monitoring of therapeutic adherence. Current methods for measurement of HU include colorimetry, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). While available GC-MS methods are sensitive and specific, they involve use of internal standards that are structurally different from HU and prone to error due to poor (e.g., < 3%) extraction efficiency. Here we describe a novel GC-MS method for the determination of HU that involves stable labeled HU as an internal standard.

Materials and Methods: HU was purchased from Sigma-Aldrich and the internal standard, HU [13C] 15N2 was custom synthesized from Toronto Research, Canada. The calibrators and controls were prepared in drug-free plasma. To 0.5 mL plasma, 1 mL of phosphate buffer (pH 6.0) and 0.1 mL of internal standard were added. HU was extracted with ethylacetate. The extract was dried and trimethylsilyl derivatives of HU were prepared. 1 µL of the derivatization mixture was injected into the GC-MS. Selected ion monitoring was used for identification and quantification of HU. The monitored m/z ions were: HU (quantification ion 277, qualifier ions - 292 and 249), HU [13C] 15N2 (quantification ion 280, qualifier ion - 295).

Results and Conclusion: The method was evaluated for reportable range, accuracy, within and between-run imprecision, and limit of quantification. Reportable range (linearity) of the method was from 0.1 to 100 µg/mL. All results were accurate within allowable error of <10% or 0.03 µg/mL. Within-run and between-run imprecision

were <5 and <10% respectively. Lower limit of quantification, at inaccuracy of <10% or <0.03 µg/mL, was 0.1 µg/mL. To check specificity and selectivity of the method, 20 negative samples were analyzed. All the drug free samples quantified less than the lower limit of quantification and failed qualifier ion ratios. Samples were stable for at least 4 h, 2 months and 6 months at room temperature, -20°C and -70°C respectively. Samples were also stable after 3 freeze/thaw cycles. Extraction efficiency for 1, 5, 10 and 50 µg/mL samples averaged 2.2%, 1.8%, 1.6% and 1.4% respectively. These data demonstrate that our new, isotope dilution GC/MS method for analysis of HU is accurate, sensitive, precise and robust.

B-415

Bupropion Exposure in an Infant: A Case Report

S. Delaney[1], G. Neuman[2], S. Ito[2], D. A. Colantonio[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada,*

Background: The last two decades has seen a substantial increase in the rates of women breastfeeding their infants. However, it has been reported that 66 - 80% of nursing women are on medication. While many drugs are safely taken by nursing mothers, there is accumulating evidence of toxicity in some breastfed infants. Information on drug excretion into milk is lacking for most drugs, and early phase drug studies exclude breastfeeding women. This uncertainty in the risk of drug exposure causes maternal non-adherence to therapy or avoidance of breastfeeding. This is a clinical problem in drug safety and is an important women's health issue, uniquely affecting both mother and infant. The objective of this study is to investigate the risk of drug exposure in nursing infants. We present a proof-of-principle case of infant bupropion exposure highlighing the importance and clinical applicability of this study.

Methods: To investigate infant drug exposure through breast milk, we established a drug safety monitoring program, Drugs in Lactation Analysis Consortium (DLAC), to measure several drugs commonly used by women breastfeeding. Breast milk is a complex lipid- and protein- rich matrix, with drugs partitioning to either the aqueous or lipid phase, thus requiring meticulous sample processing before analysis. We first investigated whether drugs partition to the aqueous or lipid phase of breast milk. We then worked to create a simplified drug extraction method using hexane, methanol and acetonitrile to facilitate efficient drug extraction from breast milk. Extraction efficiency and stability were determined. Methods were then developed to measure these drugs using LC-MS/MS. This process was followed in the case presented below.

Results/Case Report: A 6.5 month old previously healthy infant presented to SickKids Hospital with vomiting and seizures. She was exclusively breastfed; her mother was taking escitalopram daily for several months, with the recent addition of bupropion. The usual clinical workup was negative. As we suspected this might be a case of drug exposure, the infant's urine was tested and was positive for bupropion and escitalopram. Serial breast milk and serum samples were obtained and bupropion and its major metabolite, hydroxybuproion, were measured using HPLC-MS/MS. Using a pharmacokinetic model, we determined that the bupropion levels were significant around the time of the event. Average milk bupropion and hydroxybupropion levels were 20.3 ng/mL and 68 ng/mL, respectively. Predicted bupropion steady state in the infant around the time of the event was 0.12 ng/mL. The average observed infant serum hydroxybupropion level was 11.2 ng/mL. Discharge diagnosis was bupropion-induced seizures.

CONCLUSION: This case highlights the importance of the DLAC study. Given the increasing use of medication in nursing women, there is urgency to investigate the potential adverse events associated with infant drug exposure through breast milk. Future investigations will focus on population pharmacokinetic modeling to simulate and estimate infant drug exposure and assess potential risks associated with drugs and breastfeeding. The data generated from this study will help guide decisions for drug use in nursing mothers.

B-416

Rapid confirm and determination of d and l methamphetamine in human urine by liquid chromatography tandem mass spectrometry

S. Y. Wu, F. Hassouna. *Confirmatrix Clinical Laboratory, Lawrenceville,, GA,*

Background: Prescription methamphetamine (Desoxyn) is composed entirely of the d isomer. Over-The-Counter cold medications contain l isomer (Vicker's inhaler). Street-methamphetamine is either d-isomer or racemic mixture with d-isomer of 20-100%. In order to narrow down the potential sources of the methamphetamine, clinical toxicology laboratories need to accurately differentiate between d and the l isomer forms of methamphetamine. Because of the identical mass spectrum of d and l isomers, directly identifying specific isomer relies on the chromatographic separation of isomers in the racemic mixture. We therefore developed an analytical method to simultaneously confirm and determine d and l methamphetamine isomers in urine samples by LCMSMS.

Method: 100 ul urine from prescreened positive sample was mixed with 100 ul internal standard of isotope d and l methamphetamine isomers. Samples were concentrated by using one step liquid-liquid extraction with diethyl ether and ethyl acetate (1:1). At least two small amounts of concentrated standards were added to the sample. The overlay chromatograms of sample and added standards combining with the retention time of isomer of isotope internal standard were used to confirm the expected isomer. This separation of isomers was achieved with LC/MS/MS mass spectrometer with macrocyclic glycopeptide-based 10cm x2.1 mm chiral column and mobile phase of consisting 100% methanol with1% formic acid and 0.01% NaOH. The x-intercept of the standard addition calibration curve corresponds to the concentration of analyte. A query of instrument data processing software was written to perform the calculation for determination and conversion of d and l isomer concentrations to percentage of total racemic mixture of methamphetamine.

Results: The analytical curve for d and l methamphetamine was linear over the range of 40 ng/ml (40% of cutoff 100 ng/ml) to 800 ng/ml with correlation coefficient of 0.9988. The precision of method was evaluated based on three different concentrations and racemic mixture ratios of d and l methamphetamine over two weeks of intra runs. CVs (n=25) of 4.2 %, 3.0% and 2.0% were obtained. Limit of quantitation was determined as 40 ng/ml. Limit of detection was 10 ng/ml with retention time within ±2% of isotope internal standard. Recoveries ranged between 95% and 106% for spiked and pooled samples.

Conclusion : This analytical method was rapid, simple and specific. The accuracy, precision, repeatability and robustness were found to be within the acceptable limit.

B-417

Development of Abbott Carbamazepine Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

J. Donaldson[1], N. Pham[1], A. Kong[1], S. Hoang[1], S. Shaw[2], N. Pham[2], O. Ndimbie[2], C. Kasal[2], L. Ye[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Abbott Laboratories, Irving, TX,*

Introduction: The study objective is to develop a sensitive immunoassay intended for the quantitative measurement of carbamazepine in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in monitoring carbamazepine levels to help ensure appropriate therapy. Since carbamazepine concentrations correlate better with pharmacologic activity than dosage, monitoring of blood levels can increase efficacy and safety.

Methods: The Abbott Carbamazepine Assay is a liquid stable, particle-enhanced turbidimetric inhibition immunoassay used for the analysis of carbamazepine in serum or plasma. The assay consists of two reagents and is based on competition between carbamazepine in the sample and carbamazepine coated onto a micro-particle for anti- carbamazepine antibody binding sites. In samples lacking carbamazepine, the carbamazepine -coated micro-particles rapidly agglutinate in the presence of anti-carbamazepine antibodies. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of particle agglutination. In samples containing carbamazepine, the agglutination reaction is partially inhibited, slowing the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with maximum rate of agglutination at the lowest carbamazepine concentration and the lowest agglutination rate at the highest carbamazepine concentration.

Results: Performance of the Abbott Carbamazepine Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 1.9 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.3 µg/mL SD and bias within 10% or 0.4 µg/mL over an extended period. The assay is linear from 1.9 to 20.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and five human serum samples containing carbamazepine at concentrations ranging from 1.9 µg/mL to 18.1 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. Precision ranged from 1.0 %CV to 3.0 %CV for Within-Run and 1.9 %CV to 6.3 %CV for Total-Run. The assay accurately recovered spiked carbamazepine at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. The assay did not exhibit obvious cross reactivity with carbamazepine derivatives and metabolites at

the concentrations tested with the exception of carbamazepine-10, 11-epoxide. Abbott ARCHITECT Carbamazepine patient correlation studies: new vs. current on-market assay yielded a regression equation of y=0.91x + 0.56 and a correlation coefficient of 0.97. New assay vs. HPLC yielded y=1.09x + 0.37 and a correlation coefficient of 0.96. The new reagent has a 45 day onboard stability and 7 day calibration curve stability.

Conclusion: The Abbott Carbamazepine Assay enables measurement of carbamazepine in human serum or plasma with high precision across the linear range. The ability to monitor levels of carbamazepine with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-418

Chemotherapy with pharmacokinetic (PK) guided exposure optimization: US based experience with 5-fluorouracil (5-FU) in colorectal cancer (CRC) patients

I. Baburina[1], Y. Li[1], J. B. Courtney[1], M. P. Duda[2], S. Diamond[2], M. C. Miller[1], F. Braiteh[3], S. J. Salamone[1]. *[1]Saladax Biomedical, Inc., Bethlehem, PA, [2]Saladax Biomedical Laboratories, Inc., Bethlehem, PA, [3]Comprehensive Cancer Centers of Nevada, Las Vegas, NV,*

Background: 5-FU is the backbone of colorectal cancer chemotherapy. Numerous studies over the last 30 years have demonstrated wide pharmacokinetic variability of 5-FU, which can lead to undue toxicity and suboptimal treatment. Exposure optimization of 5-FU based on PK-guided dose adjustment has been tested in multiple studies, demonstrating improved response rates and reduced toxicity compared to body surface area (BSA) based dosing. Here we present the experience of a US based laboratory evaluating 5-FU plasma levels to optimize systemic exposure in CRC patients of US based oncologists. **Methods:** Between June 5, 2013 and January 17, 2014, 5-FU concentrations were determined in 631 patient samples at Saladax Biomedical Laboratories (a CLIA-certified lab) using a laboratory developed immunoassay (My5-FU). Systemic 5-FU exposure [the area under the concentration curve (AUC)] was calculated from the 5-FU level determined in a steady state sample collected 24±6Hr after the start of 5-FU continuous infusion from 240 CRC patients (n=250 therapy lines). The calculated AUC and dose adjustment recommendations to achieve the target range AUC (20 – 30 mg*hr/L) were provided in the laboratory report. Differences between cycles and the comparison between dose adjustment recommendations and actual dose changes were made between evaluable cycle pairs (defined as two consecutive cycles with AUC results). Actual vs. target AUC, recommended vs. actual dose adjustment, and ability to adjust exposure to target range were evaluated. **Results:** The majority of AUC results (62%) were outside the target range in the first sample from each therapy line where exposure was determined, irrespective of the 5-FU dosing or regimen used. For the 250 samples collected during the first cycle of therapy, 48% had AUCs below the target range, 38% were within the target range, and 14% were above the target range. In patients dosed at the standard of 2400 mg/m^2 (57% of doses), the majority of AUC results were still out of range. At this dose, 44% had exposures below the target range, 42% were within the target range, and 14% were above the target range (n=144). In 288 cycle pairs, 201 (69%) were outside the target range: doses were decreased in 48% of the 40 above the target range and doses were increased in 51% of the 161 below the target range. No dose change was made in 89% of the 87 within the target range. In 101 cycle pairs out of target range where a dose adjustment consistent with recommendation was made, 42% of these patients achieved exposure in the target range. **Conclusions:** 5-FU exposure optimization is feasible in the US clinical setting. Consistent with other reports, body surface area based 5-FU dosing resulted in frequent under dosing (48%). The majority (62%) of dose adjustment recommendations were followed in subsequent cycles. When AUCs were out of range and dose adjustments were made, 5FU exposure was optimized in almost half the patients with a single dose adjustment. PK-guided dose adjustment is a practical approach to personalize and optimize 5-FU exposure.

B-419

Evaluation of Tacrolimus QMS assay by using Indiko and AU680 analyzers and comparison to Architect

S. Wong[1], K. Garrison[2], M. Hinsdale[2]. *[1]Wake Forest School of Medicine and Wake Forest Baptist Health, Wisnton-Salem, NC, [2]Wake Forest Baptist Health, Wisnton-Salem, NC,*

Background: Tacrolimus has been a widely used calcineurin inhibitor immunosuppressant for renal, liver and other transplants. Therapeutic ranges are: 10-15 ng/mL for post-transplant and 5 -10 ng/mL for maintenace, with more recent studies suggesting minimization range to be 3-7 ng/mL. According to CAP surveys, tacrolimus may be monitored by 4 immunoassays/analyzers, and by LC-MS-MS and Mass Spectrometry. More recently, the QMS turbidimetric immunoassay was developed for tacrolimus.

Objective: This study initially established the clinical efficacy of the QMS tacrolimus assays by using AU 680 and Indiko, followed by comparison to clinically used assay by using Architect.

Methods: QMS tacrolimus is a turbidimetric immunoassay. Sample preparation included mixing 200 microliter of samples - patient whole blood with 200 microliter of the extraction reagent. After vortexing and centrifugation, the supernatant was transferred to sample cups. Drug in the supernatant and drug coated on microparticle underwent competitive binding for a limited number of antibody binding sites. If tacrolimus was absent, tacrolimus-coated microparticle was agglutinated in the presence of antibody reagent. If tacrolimjus was present, agglutination was partially inhibited depending on tacrolimus concentration. Thus, agglutination rate was inversely proportional to everolimus concentrations, and was measured photometrically. Six calibrators ranged from 0 to 30 ng/mL.

Results: Precision studies of control samples showed the following mean concentrations and CVs: n= 10, AU 680, 4.14 ng/mL and CV = 2.6%, and Indigo, 3.96 ng/mL and 11.6%., and n =20, AU 680, 9.67 ng/mL and 2.4%., Indiko, 9.79 ng/mL and 2.3% respectively. Calibration stabilities for both AU680 and Indiko were shown to be 10 days. Comparison studies of the three analyzers for kidney transplant samples with concentration ranging from <2.0 to 25.8 ng/mL showed the following slopes, intercepts and correlation: Arch. vs AU 680 for n = 95, 1.224, 0.62 and 0.976., Arch. vs Indiko n = 95, 1.204, 0.59, and 0.965., and AU 680 vs Indiko for n = 97, 0.979, 0.03, and 0.982.

Conclusions: Tacrolimus may be monitored by the QMS assay using two autoanalyzers with adequate sensitivity and acceptable precision. While both QMS assays offered comparable tacrolimus determination suitable for monitoring renal transplant patient, the concentrations were about 20 to 22% higher than those obtained by the clinically used comparison method using Architect.

B-421

An Immunoassay for Methotrexate in Blood on ARCHITECT *i* System

R. Smalley[1], R. Picard[1], B. Burkhart[1], R. Frescatore[1], C. Glover[1], L. Zhu[1], E. Roessner[1], K. Majnesjö[2], A. Öhrvik[2], K. He[1], S. Raju[1], R. Radwan[1], D. Dickson[1], Z. Li[1], T. Kettlety[1]. *[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]Fujirebio Diagnostics AB, Gothenburg, Sweden,*

Background: Methotrexate (MTX) is a cancer therapeutic drug for leukemia, osteosarcoma, non-Hodgkin's lymphoma and others. MTX levels in blood are monitored in patients to ensure appropriate therapy and determine when to intervene with the counter-acting 'rescue' therapy. An assay for measurement of MTX in serum and plasma on the ARCHITECT *i* System (ARCHITECT Methotrexate) is presented.
Methods: ARCHITECT Methotrexate is a competitive immunoassay. First, the instrument mixes and incubates a sample (calibrators, controls, sera or plasma) with anti-MTX antibody-coated magnetic microparticles, biotinylated anti-MTX antibody and acridinium-conjugated MTX. Then, the instrument washes the microparticles and adds pre-trigger and trigger solutions to initiate the chemiluminescence reaction. Signals obtained as relative luminescent units are inversely proportional to the amount of MTX in the sample.
Results: The limit of quantitation was ≤ 0.040 µmol/L MTX. The direct measuring range was from 0.04 to 1.5 µmol/L and up to 12000 µmol/L with specimen dilution. The 20-day imprecision study showed a total CV ≤ 7.1% within the range of 0.056 to 1705.433 µmol/L MTX with 6 controls and 5 panels on 4 instruments using 3 lots of reagents (n = 80). Deviations from linearity were -4.1% to 7.2% within the range of 1.881 to 0.012 µmol/L MTX. Spike recovery ranged from 92% - 94% for samples at 0.045, 0.909 and 9.090 µmol/L of MTX. In the interference studies, the average levels of MTX in the individual interfering substance-spiked samples (10 endogenous substances including human anti-mouse antibody and rheumatoid factor, and 21 therapeutic drugs) were within 90% - 110% of that in the unspiked control samples. Method comparison of ARCHITECT Methotrexate to TDx/TDxFLx Methotrexate II (TDx) generated a Passing-Bablok correlation as [ARCHITECT] = 0.00 + 1.01 [TDx] for samples within the range of 0.040 to 1.415 µmol/L (n = 92) and [ARCHITECT] = 0.00 + 1.04 [TDx] for samples within the range of 0.040 to 1624.760 µmol/L MTX, n = 142). Specimen storage study denoted that specimens could be stored at room temperature for 24 hours or at 2-8°C for 48 hours since the MTX values of the stored specimens described above were within ± 10% deviation from that of the baseline control concentrations. MTX concentrations in specimens collected in K2-EDTA, sodium heparin, and lithium heparin tubes were within ± 10% deviation from that collected in serum tubes across the range of 0.040 to 10.00 µmol/L MTX. Reagent on-board stability showed that the ARCHITECT Methotrexate reagents could remain

on the analyzer for a minimum of 30 days with no more than 10% shift from baseline. **Conclusion:** The ARCHITECT Methotrexate assay under development was demonstrated to be an accurate, precise, sensitive and robust assay for the measurement of methotrexate in human serum and plasma.

B-422

Monitoring Rivaroxaban Anticoagulation Therapy

H. Ketha, E. W. Korman, J. I. Tange, R. K. Pruthi, S. S. Ketha, R. D. McBane, P. Jannetto, L. J. Langman. *Mayo Clinic, Rochester, MN,*

Background Currently, laboratory monitoring of direct acting anticoagulants, dabigatran (direct thrombin inhibitor), rivaroxaban and apixaban (factor Xa inhibitors) is not being performed based on their predictable pharmacokinetic profile and a lack of FDA-approved clinical assays to measure drug levels.

Relevance Measurement of drug levels may be useful to ensure drug clearance (e.g., in patients with active bleeding or those undergoing invasive procedures with high bleeding risk), as well as to ensure compliance in patients who develop thrombosis. Furthermore, the lack of any antidote also supports monitoring of patients.

Objective The first objective was to develop and validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for measuring serum rivaroxaban concentration. The second objective was to monitor serum concentrations in patients receiving rivaroxaban.

Methods An LC-MS/MS assay for measuring serum rivaroxaban concentration was developed. Briefly, 200 µL of serum was added to 700 µL methanol containing 15 ng rivaroxaban-d4 (Toronto Research Chemicals, Toronto, Canada). The sample was vortexed for 30 seconds, centrifuged at 3,000 rpm for 10 minutes and the supernatant transferred to a test tube. The supernatant was dried under a stream of nitrogen at 50ºC for 45 minutes with the subsequent residue reconstituted in 100 µL acetonitrile: water (30:70). 30 µL of the reconstituted residue was then injected onto a C-18 50mm x 3mm column (Hypersil Gold, Thermo Scientific), and separated using an acetonitrile/0.01% formic acid and water/10mM ammonium formate/0.01% formic acid gradient over 8.0 minutes at 40ºC and analyzed on a mass spectrometer (Agilent 6460, Agilent Technologies, Santa Clara, CA) using multiple reaction monitoring mode (MRM). One and two MRM transitions were used as quantifier (m/z 436.1>144.9) and qualifiers (m/z 436.1>231.1 and 436>73.0) respectively. Rivaroxaban calibrators (2.5, 5, 10, 25, 50, 100, 200 and 500 ng/mL) and quality control samples were prepared in bovine serum. Thirteen serum samples from a total of twelve patients who were receiving rivaroxaban were also analyzed.

Results The LC-MS/MS assay was linear across an analytical measurement range of 2.5-500 ng/mL with a slope of 1.004 and a correlation coefficient (r^2) 0.998. The intra assay imprecision was 2.3 % at 2.9 ng/mL (n=10), 2.9 % at 25 ng/mL (n=20), 1.2% at 36 ng/mL (n=20), and 2.0% at 217 ng/mL (n=20). The inter assay imprecision ranged from 2.7-7.4%. The limit of detection was determined to be 0.5 ng/mL and the limit of quantitation was set at 2.5 ng/mL. The rivaroxaban concentration in the patient samples ranged between 3.0-220 ng/mL.

Conclusion We have developed an LC-MS/MS assay for measuring rivaroxaban concentration in serum. In our initial cross sectional study, rivaroxaban could be reliably quantitated in samples from patients receiving the drug. Measuring serum concentration of direct acting anticoagulants is of clinical value, particularly for managing active bleeding events and in patients undergoing elective procedures. In the future, developing LC-MS/MS based anticoagulant drug panels are an attractive option for clinical laboratories to facilitate an improved management of patients receiving anticoagulation therapy by novel anticoagulants.

B-423

Development of Abbott Phenobarbital Assay for the ARCHITECT *c*Systems Automated Clinical Chemistry Analyzers

J. Donaldson[1], N. Pham[1], A. Kong[1], S. Hoang[1], S. Shaw[2], N. Pham[2], O. Ndimbie[2], C. Kasal[2], L. Ye[1]. *[1]Thermo Fisher Scientific, Fremont, CA, [2]Abbott Laboratories, Irving, TX,*

Introduction: The objective of this study is to develop a sensitive immunoassay intended for the in vitro quantitative measurement of phenobarbital in human serum or plasma on the ARCHITECT *c*Systems. The measurements obtained are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of phenobarbital to help ensure appropriate therapy. Monitoring serum phenobarbital concentrations has been shown to improve patient therapy by providing physicians with a tool for adjusting dosage.

Methods: The Abbott Phenobarbital Assay is a liquid stable, homogenous particle-enhanced turbidimetric inhibition immunoassay used for the analysis of phenobarbital in serum or plasma. The assay consists of two reagents and is based on competition between phenobarbital in the sample and phenobarbital coated onto a micro-particle for anti- phenobarbital antibody binding sites. In samples lacking phenobarbital, the phenobarbital -coated micro-particles rapidly agglutinate in the presence of anti-phenobarbital antibodies. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of particle agglutination. In samples containing phenobarbital, the agglutination reaction is partially inhibited, slowing the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained, with maximum rate of agglutination at the lowest phenobarbital concentration and the lowest agglutination rate at the highest phenobarbital concentration.

Results: The performance of the Abbott Phenobarbital Assay was evaluated on the Abbott ARCHITECT *c*8000 analyzer. Based on guidance from Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, the assay demonstrates a Limit of Quantitation of ≤ 2.0 µg/mL using inter-assay precision ≤ 7% CV or ≤ 0.7 µg/mL SD and bias within 10% or 1.0 µg/mL over an extended period. The assay is linear from 2.0 to 80.0 µg/mL using guidance from CLSI protocol EP6-A. Assay precision was evaluated using CLSI guideline EP5-A2. A tri-level commercial control and six human serum samples containing phenobarbital at concentrations ranging from 2.5 µg/mL to 77.2 µg/mL were tested. Each sample was assayed in duplicate twice a day for 20 days with at least two hours between runs. The precision ranged from 1.1 %CV to 3.3 %CV for Within-Run and 1.7 %CV to 6.7 %CV for Total-Run. The assay accurately recovered spiked phenobarbital at levels representing sub-therapeutic, therapeutic, and toxic samples. No significant interference was observed with various endogenous substances or compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity. Abbott ARCHITECT Phenobarbital patient correlation studies: new vs. current on-market assay yielded a regression equation of y=1.00x + 0.42 and a correlation coefficient of 1.00. New assay vs. HPLC yielded y= 0.93x + 0.68 and a correlation coefficient of 0.99. The new reagent has an onboard stability of 40 days and calibration curve stability of 14 days.

Conclusion: The Abbott Phenobarbital Assay enables measurement of phenobarbital in human serum or plasma with high precision across the linear range. The ability to monitor levels of phenobarbital with high accuracy can help ensure appropriate therapy. The assay has applications on the ARCHITECT *c*16000, *c*8000, and *c*4000.

B-424

Development and Validation of a Robust Tandem LC-MS/MS Method for the Quantification of Antidepressants in Serum

A. K. Petrides, J. Moskowitz, W. Clarke, M. A. Marzinke. *The Johns Hopkins University School of Medicine, Baltimore, MD,*

Background: Depression is a rapidly growing issue in the United States, with more than 11% of Americans taking antidepressant medications, allowing for antidepressants to become the most commonly prescribed drug in the United States. There are many drug classes that may be used to treat depression, including the selective serotonin-reuptake inhibitors (SSRIs) such as citalopram (Celexa) and sertraline (Zoloft), as well as the dopamine-reuptake inhibitor bupropion (Wellbutrin). However, treatment success may be variable and can either result in lack of efficacy due to suboptimal drug administration or non-compliance, as well as potential adverse side effects. Success is further complicated by environmental and genetic factors, and the delayed access to an optimal treatment regimen can have deleterious effects. Thus, methods for drug quantification can become important tools in the assessment of drug efficacy to optimize treatment regimens, particularly in scenarios where therapeutic regimens are frequently altered. Here, we present a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for the robust, simultaneous quantification of the commonly prescribed antidepressants citalopram, sertraline and bupropion, and its active metabolite, hydroxybupropion, following FDA bioanalytical guidelines.
Methods: Drug-free human serum was spiked with citalopram, sertraline, bupropion and hydroxybupropion, along with their corresponding isotopically-labeled internal standards. Following protein precipitation, samples were injected onto the Prelude turbulent-flow-liquid chromatography system (Thermo Fisher Scientific), consisting of a Cyclone-P column for on-line solid phase extraction and a Hypersil Gold C18 column for chromatographic separation. Sample elution was achieved using acetonitrile (ACN) containing 0.1% formic acid. All four compounds were detected over 4 minutes using a TSQ Vantage Quadrupole mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization (HESI) source in positive ionization mode operated in selected reaction monitoring (SRM) mode. Assay validation followed bioanalytical guidelines, and included precision (intra-assay) and accuracy, linearity, and functional sensitivity studies. **Results:** The analytical measuring range

of the assay for each analyte was 5-1000 ng/ml. Linearity was assessed from the slope of a $1/x^2$ weighted least squares-fitted linear regression analysis. Three independently prepared and analyzed calibration curves were used to assess linearity. Average slopes for citalopram, sertraline, bupropion and hydroxybupropion were 1.033, 1.036, 1.011, and 1.042, respectively. Further, recoveries across the aforementioned analytical measuring range for citalopram, sertraline, bupropion, and hydroxybupropion were 94.7-104.1%, 94.9-104.2%, 92.5-103.3%, and 92-105.2%, respectively. To assess precision, quality control solutions were prepared at 5 ng/ml (lower limit of quantification), 25 ng/ml (low), 125 ng/ml (mid) and 850 ng/ml (high) levels. Intra-assay precision was determined through analysis of six replicates of each level from a calibration curve. Intra-assay precision ranged from 2.6-3.6% for citalopram, 3.8-11.4% for sertraline, 5.4-9.2% for bupropion and 3.0-6.8% for hydroxyburporion. The functional sensitivity of the assay, which is the computational extrapolation of drug concentration with a %CV=20%, was 0.05 ng/ml, 0.57 ng/ml, 1.54 ng/ml, and 1.4 ng/ml using this method for citalopram, sertraline, bupropion and hydroxybupropion, respectively.

Conclusion: The development and validation of this LC-MS/MS method allows for the robust and high-throughput quantification of antidepressants commonly prescribed to our patient population.

B-425

Detection of 55 Drugs and Pain Management Analytes in Urine Using a Quantitative Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS). An All-In-One Screening and Confirmatory Method.

V. Ricchiuti, E. Chaffin, E. Eve, F. Lucas. *University of Cincinnati Medical Center, Cincinnati, OH,*

Background. Screening drug tests are qualitative, and are conducted to identify classes of drugs present in the urine using immunoassay-based methods. They rely on a threshold above which a positive result is produced, and do not detect lower concentrations of a drug. Confirmatory tests are used to verify a positive screening and identify a specific drug. They use gas chromatography-mass spectrometry (GC-MS) or high performance liquid chromatography-tandem MS (LC-MS/MS) methods. Our goal was to validate an all-in-one screening and confirmatory method by LC-MS/MS for 55 drugs and metabolites panel with lower limits of detection than immunoassays and GC-MS.

Methods. 70 urine samples were screened using QuickTox® Drug Dipcards (Branan Medical Corporation, Irvine, CA). Confirmation by GC-MS was a send-out to a large reference laboratory. LC-MS/MS was performed in-house using the Shimadzu (Shimadzu Corporation, Kyoto, Japan) Prominence 20A Liquid Chromatograph, followed by the AB SCIEX QTRAP® 4500 Mass Spectrometry System (AB SCIEX, Framingham, MA). Amphetamines, benzodiazepines, cannabinoids, cocaine, opiates, opioids, oxycodone, fentanyl and analogues and buprenorphine were analyzed in positive mode using the TurboIonSpray® ion source (Electrospray ionization). Barbiturates and marijuana metabolite were analyzed using the negative ion mode for maximum sensitivity. 50 µL of urine was mixed with 20 µL of combined internal standard (IS) solution to each tube. Hydrolysis was performed by adding 10 µL of the β-glucuronidase solution (100,000 units/mL) to each tube and incubation for two hours at 55°C. 110 µL of the curve diluent was added to each tube, mixed and centrifuged to separate any proteins and the supernatant. Samples were then analyzed by LC-MS/MS based on the presence of the specific MRM transitions for each analyte at the correct retention time. Quantitative measurement was accomplished by normalization of the peak area with the area of the IS for each specimen, including matrix specific calibrators, and quality control materials. The instrument software program automatically constructs a calibration curve, using the peak abundance data from the calibrator samples.

Results. Analytical performance of LC-MS/MS was excellent. CVs were <5% within the analytical measurement range. Among all the drugs analyzed, fentanyl showed the lowest estimated limit of detection (LOD) and limit of quantitation (LOQ), 0.003 ng/mL and 0.009 ng/mL, respectively. LOQ for fentanyl at CV<20% was <0.05 ng/mL and was used as clinical cut-off. LC-MS/MS clinical cut-offs ranged from 0.05 (fentanyl) to 20 ng/ml (barbiturates). Recoveries within the analytical measurement range were between 95-105%. Qualitative method comparison between GC-MS and LC-MS/MS using GC-MS cut-offs showed 91% agreement. Quantitative correlation between GC-MS and LC-MS/MS including all drugs was excellent (slope=1.04, r=0.99, p<0.05, n=21). 42% of initial drugs screened by immunoassay were confirmed by GC-MS, however 80% were confirmed by LC-MS/MS using our clinical cut-offs. Turn-around-time (TAT) was between 24-48 hours from receiving the specimen.

Conclusion. The quantitative drug analysis method by LC/MS-MS is easy and user-friendly with excellent analytical sensitivities. It requires one-step for extraction of drugs from urine and a direct injection into the LC/MS-MS system. Low volume of urine needed as well as analytical sensitivity is ideal for the Neonate Abstinence Syndrome program.

B-426

Formation of 6-monoacetylmorphine in urine specimens with high morphine concentrations during enzymatic hydrolysis

C. Heideloff[1], J. Gabler[2], C. Yuan[1], L. Zhang[3], S. Wang[1]. *[1]Cleveland Clinic, Cleveland, OH, [2]Thermo Fisher Scientific, West Palm Beach, FL, [3]Cleveland State University, Cleveland, OH,*

Background: 6-monoacetylmorphine (6-MAM), a unique metabolite of heroin, is known as a definitive indicator of heroin intake. 6-MAM in urine is measured by GC-MS or LC-MS methods. Due to variable glucuronide conjugation rates between individuals, some laboratories employ enzymatic hydrolysis using glucuronidase during sample preparation to improve detection consistency. Acetate buffer is the primary choice for preparing enzymatic hydrolysis solution. In our routine LC-MS/MS analysis, we observed a number of low levels of 6-MAM in patient urine specimens with morphine at high concentrations. We hypothesized that the acetate buffer used for enzymatic hydrolysis serves as an acetylating agent leading to the formation of 6-MAM in urine specimens with high morphine concentrations. **Design:** Two sets of studies were performed. In one set, morphine standard and the major morphine metabolite, morphine-3-glucuronide (M3G), were spiked into morphine negative urine samples (n=2) to create five levels of morphine specimens (10,000- 50,000- 100,000- 150,000- 200,000 ng/ml) and M3G specimens (16,250- 81,250- 162,500- 243,750- 325,000 ng/ml) respectively. Internal standards (6-MAM-D3 and Morphine-D3) were added to the samples and the mixtures were incubated with 1M sodium acetate buffer (pH 4.5) at 60°C. Aliquots were taken at timed intervals (0, 2, 4, 6, 18 hours). In the 2nd set, leftover urine specimens (n=4) with elevated concentrations of morphine were incubated with 1M sodium acetate buffer (pH 4.5) at 60 °C for 0 and 18 hours. An additional study was performed to optimize the enzymatic hydrolysis procedure using an alternate buffer (1M citrate buffer, pH 4.5) to determine if 6-MAM would still be formed. The urine specimens used in the 2nd study, as well as the 200,000 ng/mL morphine and 325,000 ng/mL M3G spiked samples from the 1st study were incubated with 1M citrate buffer (pH 4.5) at 0 and 18 hours. Analysis was performed by an LC-MS/MS method. **Results:** Urine samples with elevated levels of both free morphine and M3G (>100,000 ng/mL) incubated for 18 hours using acetate buffer formed measurable amounts (≥ 5 ng/mL) of 6-MAM. All samples with <100,000 ng/mL and all samples incubated <18 hours did not form measurable amounts of 6-MAM. In all samples using citrate buffer, no 6-MAM was at the measurable level. **Conclusion:** False positive identification of heroin may be possible in urine specimens with extremely high levels (>100,000 ng/mL) of morphine when using acetate buffer for enzymatic hydrolysis.

B-427

Development of a Homogenous Enzyme Immunoassay for the Screening of Synthetic Cannabinoids Applicable to Automated Chemistry Analysers

S. Smyth, V. Anderson, J. Darragh, P. Ratcliffe, S. Smillie, L. Long, M. Benchikh, R. McConnell, S. FitzGerald. *Randox Toxicology Limited, Crumlin, United Kingdom,*

Background. Synthetic cannabinoids are chemical compounds that mimic the effects of tetrahydrocannabinol, the main active ingredient of cannabis. Originally sold under the brand name “Spice”, this brand name has become a generic term to include the entire class of “legal” smoking blends sold on the internet.The most common of the first wave of synthetic cannabinoid compounds available was JWH018. As these designer drugs continue to be sold there is a need for screening tests, which facilitate the detection process. Immunoassays are antibody-based tests that provide high throughput screening. The application of these tests to automated chemistry analysers is advantageous in clinical laboratory testing settings as it increases the screening capacity. This study reports the development of a homogeneous enzyme immunoassay for the screening of JWH018 and related metabolites in urine, applicable to a variety of automated systems.

Methods. The assay is based on competition between drug in the sample and drug labelled with glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent. In the absence of drug in the sample, JWH018-labelled G6PDH conjugate is bound to antibody, and the

enzyme activity is inhibited. However, when free drug is present in the sample, the antibody binds to free drug and the unbound JWH018-labelled G6PDH then exhibits maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340nm. The assay is qualitative utilising a 15ng/ml cut-off and in this evaluation the RX daytona analyser was used.

Results. The assay was standardised to the major urinary metabolite JWH018 N

pentanoic acid, 55 synthetic cannabinoidswere also detected with %cross-reactivity (%CR) values ≥5%, including the 5 hydroxypentyl metabolite and 4 hydroxypentyl metabolite of JWH018 (%CR: 62% and 24% respectively) and AM2201 N- 4-hydroxypentyl metabolite (%CR: 114%). The limit of detection in urine (n=20) was 5.2ng/ml (measuring range 0-20ng/ml). Inter-assay precision (n=20) was calculated as 3.07% at 10ng/ml, 1.89% at 15ng/ml and 2.06% at 20ng/ml. Total assay precision was assessed by running a negative (10ng/ml) and positive (20ng/ml) spiked urine samples, 2 replicates per run, 2 runs per day over 20 days. All replicates were correctly reported as negative and positive (n=80). Recovery was assessed at 10, 15 and 20ng/ml and all samples (n=20) showed recovery of between 115-124%. 101 samples were assessed against LC/MS and exhibited 92% agreement. When 245 samples were assessed with this assay and a commercially available biochip based immunoassay an agreement of 95% was obtained.
Conclusion. The results show that the developed homogeneous enzyme immunoassay for the screening of synthetic cannabinoids, exhibits optimal analytical. The assay presents a broad cross-reactivity profile, which increases the screening capacity. Moreover, the application to automated chemistry analysers improves the screening for designer drugs in clinical laboratories.

B-428

ARK™ Voriconazole Assay for the Roche/Hitachi Modular P Automated Clinical Chemistry Analyzer

S. J. Oh, K. Pham, B. Moon, J. J. Valdez. *ARK Diagnostics, inc, Fremont, CA,*

Background: Despite the availability of newer antifungal agents, invasive fungal diseases remain a leading cause of morbidity and mortality in immunocompromised patients. Voriconazole (VRZ) is an extended-spectrum triazole indicated for treatment of invasive fungal diseases. Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of VRZ is described.

Methods: The ARK Voriconazole Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.00, 1.00, 2.00, 4.00, 8.00 and 16.00 µg/mL) and 3 controls (1.50, 5.00 and 10.00 µg/mL). The performance of the ARK assay was evaluated on the Roche/Hitachi Modular P analyzer. Limit of quantitation, linearity, precision, recovery, specificity and method comparison were studied.

Results: The assay measured concentrations as low as 0.50 µg/mL and was linear to 16.00 µg/mL. Total precision ranged 5.1% to 6.3%CV and within-run precision ranged 4.3% to 4.8%CV in a 20-day study (CLSI guideline EP15-A2) that evaluated precision for the 3 quality controls in a synthetic proteinaceous matrix and for similar concentrations in spiked serum samples. The assay accurately recovered spiked VRZ samples throughout the assay range. The assay did not crossreact with the antifungal fluconazole, itraconazole, and posaconazole. Voriconazole N-oxide (major metabolite) was tested at 10 µg/mL and no crossreactivity was observed. No interference from endogenous substances, anticoagulants and potentially co-administered drugs occurred at the elevated concentrations studied. Seventy-four specimens at concentrations throughout the range (0.5 to 8.1 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to LC/MS/MS values: ARK = 1.01 LC/MS/MS + 0.07 (r2=0.98).

Conclusions: The ARK Voriconazole Assay measures VRZ in human serum or plasma with excellent precision at very low concentrations which is essential for long-term monitoring of patients. Ability to measure trough levels of VRZ with high accuracy and fast turn-around time makes this method clinically useful for VRZ TDM.

B-429

Ultrafast, high-throughput quantitative analysis of Carboxy-THC in urine using Laser Diode Thermal Desorption coupled to tandem mass spectrometry

A. Birsan, S. Auger, P. Picard, J. Lacoursiere. *Phytronix, Quebec, QC, Canada,*

Background: The 11-Nor-9-Carboxy-THC (THCC) is a major metabolite of THC in urine. The confirmation of THCC in patients is often conducted in urine as a non-invasive procedure to determine the presence of drugs of abuse. The detection and quantification of THCC are traditionally performed by gas (GC) or liquid (LC) chromatography coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) methods.

The laser diode thermal desorption (LDTD) ion source uses an infrared laser diode to thermally desorb neutral species of THCC molecules from a dried sample. These neutral species are carried into a corona discharge region, where they undergo efficient protonation and are directed into the mass spectrometer. The total analysis time is performed very rapidly (9 seconds).

The objective of this work is to validate this analysis method and test different real samples using the LDTD-MS/MS. A cross validation study against the LC-MS/MS approach for the analysis of THCC was done in order to evaluate the performance of the alternative LDTD-MS/MS developed method.

Methods: A calibration curve and quality control materials were prepared in blank urine samples. To 500 uL of calibrators, QC and patient specimens, 50 µL of internal standard (THCC-d9, 5ug/mL in MeOH) and 200 uL KOH (3N) were added. The mixture was vortex-mixed and incubated at 38°C for 15 minutes for the glucuronide hydrolysis. A liquid-liquid extraction was then performed by adding 200 uL of HCl (6N) and 1000 uL of Hexane:EtAc (95:5). After vortexing and centrifugation at 5000 rpm for 2 min, 6 uL of the organic layer was deposited in the LazWell Plate (pre-coated with EDTA 200 ug/mL). The LDTD laser power was ramped to 45% in 3 seconds, and shut down after 1 second. Negative ionization mode was used, and API-5500 QTrap system was operated in MRM mode with MS transitions of 343->245 and 352->254 for THCC and THCC-d9 respectively.

Results: The calibration curves show excellent linearity with r2 = 0.9991 between the quantification range of 5 to 5000 ng/mL. Inter-run, intra-run accuracy, and precision ranges between 95,8 % and 103,1 % with RSD from 8,5 to 13,5%, respectively. No matrix effect or carryover was observed. The stability of THCC in and out (dry sample) of solution was evaluated for a period of 3-days. This method was cross validated with results from a traditional LC-MS/MS method for 49 real patient specimens. A good correlation between LC-MS/MS and LDTD-MS/MS data is obtained with r2 = 0.9956. All negative samples correlated accordingly.

Conclusion: LDTD technology provides unique advantages in developing an ultra-fast method for analysis of 11-Nor-9-Carboxy-THC in urine. This method has demonstrated accurate, precise and stable results with a run time of 9 seconds.

B-430

High-Throughput Quantitative Analysis of 5 Antifungal Drugs in Human Serum

M. Youssef, V. Miller, W. LaMarr. *Agilent Technologies, Wakefield, MA,*

Research laboratories traditionally rely on HPLC and more recently LC/MS/MS for quantitative analysis of antifungal drugs (AFs). The objective of this study was to develop a rapid and very robust online SPE/MS/MS method for high throughput and accurate quantitation of 5 antifungal drugs (voriconazole, ketoconazole, fluconazole, itraconazole, and posaconazole) in human serum. This method employs protein precipitation followed by dilute and shoot on the SPE/MS/MS system, enabling analysis of all 5 AFs at 14 seconds per sample producing >20x savings in analysis time and solvent consumption compared to typical analytical methods. Samples were prepared by spiking AFs into drug-free human serum followed by adding internal standard mix, a protein crash with acetonitrile and then diluting samples 10-fold with a basic dilution. Samples were then analyzed via SPE/MS/MS using a reversed-phase C18 cartridge at 14 seconds per sample. A simple protein precipitation methodology followed by (dilutes and shoot) and analysis by SPE/MS/MS allows for the accurate and precise measurement of these analytes in human serum over a linear range of (0.2 - 25 mcg/mL). Standard curves consisting of each AF spiked into serum had excellent linearity within the measured range with an R2 value greater than 0.995. This methodology is capable of throughputs greater than 240 samples per hour providing a high-throughput and very efficient mode of analysis. Carryover was assessed by analyzing the AUC of the blank calculated as % of the mean peak area of the 0.2 mcg/mL samples. No significant carryover (< 5.0%) was determined for all of the AFs. QC standards for each AF were run over a series of days to establish both intra- and interday precision and accuracy values. The accuracies determined were within 10% and coefficient of variation values were all less than 10% for concentrations within the measured range. The reproducibility of the method was evaluated by measuring >2000 sequential injections of all five AFs spiked into serum. The instrument response was stable for each of the five analytes with coefficient of variation ranging from 2.3-6.9 % showing the robustness of the system, SPE cartridge lifetime and consistency of quantitation for the analytes in the panel.A panel of five antifungal drugs including: voriconazole, ketoconazole, fluconazole, itraconazole, and posaconazole were quickly, accurately, and precisely measured in serum using a simple protein precipitation protocol and an SPE/MS/MS system. Samples were analyzed at 14 seconds per sample, providing a high-throughput method capable of analyzing more than 240 samples per hour. This methodology provides comparable results to HPLC, and LC/MS/MS, but at > 20x the speed and efficiency of typical methods.

B-431

Measurement of the antiepileptic drugs, lacosamide and lamotrigine, in human serum by liquid chromatography-tandem mass spectrometry

D. M. Garby, R. DelRosso, J. A. Edwards, L. A. Cheryk. *Mayo Medical Laboratories, Andover, MA,*

Background: Lacosamide and lamotrigine are antiepileptic drugs for whom the monitoring of drug levels allows for maximization of their seizure suppressing effects while minimizing the prevalence of adverse side effects. Lacosamide (VIMPAT®) is approved for adjunctive therapy to treat partial-onset seizures in epileptic patients 17 years of age and older. Lamotrigine (Lamictal) is approved for treatment of bipolar I disorder and a variety of seizure disorders including Lennox-Gastaut syndrome, primary generalized tonic-clonic seizures and partial-onset seizures. Both lacosamide and lamotrigine are completely absorbed after oral administration with negligible first-pass metabolism. Peak plasma concentrations occur within one to five hours after oral dosing, and the elimination half-life (in adults) is approximately 13 hours for lacosamide and 25 to 33 hours for lamotrigine. Both are believed to modulate the activity of voltage-gated sodium channels, in turn stabilizing neuronal activity.

Methods: Stable drug isotopes (lacosamide-$^{13}C,D_3$ and lamotrigine-$^{13}C,^{15}N_4$) are added to 50uL of serum as internal standards. Protein is precipitated from the mixture by the addition of acetonitrile. The supernatant is then further diluted with deionized water. Lacosamide, lamotrigine and their respective internal standards are then separated from the other serum constituents by liquid chromatography (TLX4, Thermo Fisher Scientific, Waltham, MA) using a Kinetex™ 5µm C18 50x4.6mm column (Phenomenex, Torrance, CA) followed by analysis on a tandem mass spectrometer (QTRAP 6500, AB SCIEX, Framingham, MA) equipped with an electrospray ionization source in positive mode. Ion transitions monitored in the multiple reaction monitoring (MRM) mode are m/z 255.9 → m/z 211.0 for lamotrigine, m/z 255.9 → m/z 144.9 for lamotrigine ion pair, m/z 261.0 → m/z 144.9 for lamotrigine-$^{13}C,^{15}N_4$, m/z 251.0 → m/z 108.0 for lacosamide, m/z 251.0 → m/z 90.9 for lacosamide ion pair, and m/z 255.0 → m/z 108.0 for lacosamide-$^{13}C,D_3$. Calibrators consist of six standard solutions ranging from 0 to 50ug/mL.

Results: Method performance was assessed using accuracy, precision, linearity and specimen stability. Accuracy of the method was assessed by comparison to external reference laboratories performing analysis of these drugs by tandem mass spectrometry. Comparison to the external laboratories demonstrated excellent agreement between the methods with overall differences of less than 3%. Precision studies were performed using commercially available quality control material (AEDII, UTAK Laboratories Inc., Valencia, CA) and an in-house prepared serum pool fortified with lacosamide and lamotrigine standard solutions. Intra-run precision coefficients of variation (CVs) ranged from 1.9% to 2.1% for lacosamide and 1.4% to 2.8% for lamotrigine. Inter-run precision CVs ranged from 3.3% to 4.7% for lacosamide, and 3.1% to 7.1% for lamotrigine. Linearity studies were performed using serum fortified with standard drug solutions. Linearity was demonstrated over the assay range (0.2 to 50ug/mL) for each analyte, yielding the following equations: observed lacosamide value = 1.0331*(expected value) - 0.1118, $R^2 = 0.9999$; observed lamotrigine value = 1.0194*(expected value) - 0.0590, $R^2 = 1.0000$. Specimens were stable when stored at ambient, refrigerate and frozen temperatures for up to 32 days.

Conclusion: This method provides for the simultaneous and reliable analysis of lacosamide and lamotrigine in human serum.

B-432

Development of a comprehensive drug screen in urine using liquid-chromatography quadrupole time of flight mass spectrometry.

K. L. Thoren, J. M. Colby, S. B. Shugarts, A. H. B. Wu, K. L. Lynch. *UC-San Francisco/San Francisco General Hospital, San Francisco, CA,*

Background: Recently, there has been much interest in using high resolution mass spectrometry (HRMS) for drug screening applications as it offers several advantages over tandem-based methods (LC-MS/MS). Importantly, HRMS instruments are often used in an untargeted manner. This is especially attractive for broad spectrum drug screening because it allows for potential identification of unknown or unexpected compounds. Despite the interest in these techniques, few studies have investigated the performance of high resolution instruments in comprehensive drug screening. The objective of this study was to develop a broad spectrum drug screen for urine using LC-HRMS and to determine its performance in identifying drugs and metabolites in 100 routine clinical samples.

Methods: All chromatographic separations were performed on a Phenomenex Kinetex 2.6-µm C18 column (3 x 50 mm) thermostatted at 30 degrees C. A binary mobile phase consisting of (A) 0.05% formic acid in 5 mM ammonium formate (aq.) and (B) 0.05% formic acid in acetonitrile/methanol (50:50 v/v) was ramped linearly from 2% to 100% B over 10 minutes. To determine the performance of our assay, 100 patient comparison urine samples were diluted 1:5 with 12.5% acetonitrile/methanol (50:50 v/v) in water and spiked with fentanyl-d5 internal standard. Data were collected on an ABSciex TripleTOF® 5600 System operating in full-scan positive ion mode with IDA-triggered acquisition of product ion spectra. Data were analyzed using the MasterView function of PeakView software (AB Sciex) and Analyst TF (AB Sciex, Version 1.5.1). Criteria for positive identification of a drug included chromatographic retention time, accurate mass, isotope pattern, and library match. Results from the HRMS analysis were compared to several other methods (LC-MS/MS, LC-Orbitrap, gas chromatography-mass spectrometry, immunoassay and patient prescription history) to determine whether a compound was a true positive or false positive.

Results: Retention times were established for 210 compounds with coefficient of variations that ranged from 0 to 9%. The lower limit of detection was 25 ng/mL or less for 83% of the compounds. Using a targeted approach for data analysis and an in-house spectral library, our HRMS method found 523 positive hits in the patient comparison samples. Of these candidate hits, 509 (97%) were verified by another method meaning that only 14 were deemed to be false positives. The HRMS missed 52 compounds that were identified by one or more of the reference methods.

Conclusion: Overall, the HRMS method identified compounds with high confidence; 97% of the compounds found by HRMS were verified by another method. The majority of the 52 missed compounds could be attributed to differences in lower limits of detection. In general, the targeted, LC-MS/MS method had slightly better sensitivity than the HRMS method. However, the ability to detect unknown or unexpected compounds still makes HRMS very appealing. Therefore, a combination of LC-MS/MS and HRMS may be the most effective approach for broad spectrum drug screening.

Wednesday, July 30, 2014

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

Technology/Design Development

B-433

Determination of Aminothiol Adsorption Properties of Nano-Sized Titanium(IV) Oxide by HPLC-FLD

A. Stella[1], S. Hsieh[2], M. Garelnabi[1], J. Horta[1], E. J. Rogers[1]. *[1]University of Massachusetts Lowell, Lowell, MA, [2]National Taiwan University, Taipei, Taiwan,*

Metal oxide nanoparticles are known for their optical, electrochemical, electrical, gravimetric, acoustic, and magnetic properties which make them appropriate for laboratory diagnostics. Among these, titanium(IV) oxide (a.k.a. titanium dioxide, TiO_2) nanoparticles display superior photocatalytic properties. In the present study, the adsorptive properties of eight TiO_2 polymorphs were evaluated when exposed to the toxic thiol-containing amino acid homocysteine. Homocysteine is an amino acid known to cause cardiovascular toxicity and neurodegenerative disorders and can be adsorbed to TiO_2 polymorphs to different degrees depending on the physicochemical characteristics of the polymorph. This adsorption quality may provide an alternative/point of care biosensing approach to homocysteine measurement.

A homocysteine standard solution was combined with dispersions of each TiO_2 polymorph under physiological conditions. After exposure, an HPLC fluorescence detection (HPLC-FLD) method optimized for quantification of total, unbound homocysteine was utilized and showed a range of results for the polymorphs studied. An initial centrifugation (1,280 RCF) step, followed by two microcentrifugation (11,152 RCF) steps were performed to ensure nanoparticle removal. The supernatants produced were used for homocysteine analysis. A thiol-specific derivatization agent, 7-fluorobenzofurazan-4-sulfonic acid (SBD-F) was employed to derivatize non-adsorbed homocysteine. The excitation wavelength was set to 385 nm and the emission wavelength was set to 515 nm. The peaks produced by the fluorescence detector were non-adsorbed homocysteine in the reduced form. It is possible that the homocysteine may have become oxidized by the nano-sized polymorphs upon contact, however tris-(2-carboxyethyl)phosphine (TCEP) was used to convert all homocysteine back to the reduced form for derivatization.

The nano-sized anatase polymorph adsorbed 2.92 µg of homocysteine per mg of polymorph, whereas amorphous TiO_2 only adsorbed 3.65 $x10^{-3}$ µg of homocysteine per mg of polymorph; both values were corrected for primary particle size. Results were determined by peak area comparison to the 5 µmol blank standard. Other polymorphs produced values between the anatase and amorphous polymorphs. Surface chemistries are distinguishing characteristics of these polymorphs and are responsible for their individual physicochemical properties. Variations in the observations were attributed to the unique combinations of size, surface area and polarity of each polymorph. The aminothiol adsorptive property of titanium dioxide polymorphs has potential applications in nanomedicine, biosensing, diagnostics, and amino acid tagging. This HPLC-FLD evaluation of homocysteine adsorption effectively determined which polymorphs may be best suited for applications in the clinical laboratory for diagnostic purposes.

B-434

Versatile Electrical Platform for Accelerated Development and Commercialization of In Vitro Diagnostic Assays

C. Cheng[1], B. Reddy[2], F. Lai[1], P. Yen[3], C. Duarte[2], E. Salm[2], F. Tsui[1], C. Wen[1], T. Chen[1], J. Huang[1], Y. Hsieh[1], C. Mauracher[4], G. Bauer[4], M. Yamamoto[4], R. Bashir[2], Y. Liu[1]. *[1]Taiwan Semiconductor Manufacturing Company, Hsinchu, Taiwan, [2]University of Illinois at Urbana-Champaign, Champaign, IL, [3]National Taiwan University, Taipei, Taiwan, [4]SONY DADC Bioscience, Salzburg, Austria,*

Background: Diagnostic techniques have become critical to modern health care not only for patient diagnosis and optimization of treatment, but also for providing vital information regarding pathways for complex diseases. Ideal biosensors provide the maximum desirable data with the least amount of complexity. However, to date, most biosensors either yield very specialized data or are very complex and expensive. Researchers have attempted to address this need with the development of point-of-care sensors that can provide accurate data without the need for expensive equipment. Semiconductor manufacturing techniques offer a particularly attractive opportunity to design a biosensor that is inexpensive, scalable, easy to integrate with portable electronics, and highly sensitive to target analytes due to device size. Such fabrication techniques have been honed to perfection with optimization and standardization. Prototyping of new device designs in a semiconductor manufacturing manner can critically enable rapid development and commercialization of new in vitro diagnostic assays.

Methods: We present here a versatile electrical biosensor platform consisting of tens of thousands of devices fabricated with a 0.18 µm silicon-on-insulator technology. The platform consists of a unit cell transistor integrated seamlessly with control and read-out circuitry that is amenable for immediate commercialization. Each cell consists of a sub-micron FET Sensor with a near-Nernst pH sensitivity of around 56-59 mV/pH and a resolution of <0.01 pH. The gate oxide is directly exposed to the target analytes, instead of to the commonly employed floating gate architecture. This enables many critical advantages, including increased sensitivity due to the elimination of parasitic coupling capacitances and reduced vulnerability to crippling factors such as electrostatic discharge. The unit cell can be coupled to a variety of different surface chemistries for different target analytes and applications.

Results: We verified the device performance and potential applications by detection of urea level via an enzyme (urease)-catalyzed reaction. A high level of urea in blood plasma can indicate both potential kidney failure and the onset of kidney diseases. Compared to reported literature (Lai et al, 2001) and commercially available kits (SIGMA ALDRICH®), our devices exhibited a linear detection response in the hundreds of picomoles to nanomoles range with a sample volume of 0.1 µl, an improvement of one order in detection limit and three orders in required sample volume. In addition, we verified the device performance by the detection of DNA hybridization. As low as picomoles of DNA molecules were easily detectable, a limit that could be pushed down to femtomoles with integrated polymer microfluidics.

Conclusion: In summary, this work introduces a fully electronic biosensor platform produced using a semiconductor manufacturing foundry. As proofs of concept of the functionality of the unit cell, we demonstrated the detection of enzymatic reactions and DNA hybridization. Foundry fabricated FET sensors with integrated polymer microfluidics have the potential to enable highly cost effective, mass fabricated POC devices with better sensitivity and resolution than currently commercially available solutions. The finely tuned and high throughput nature of a semiconductor foundry can translate to immediate commercialization of electronic biomedical assays.

B-435

An easy, portable and rapid way for performing PCR reactions

M. Gramegna[1], L. J. Turner[1], L. Ventura[1], M. L. Incandela[1], M. Bianchessi[2], M. Cereda[2], A. Cocci[2], A. Moiana[1]. *[1]Sentinel CH, Milano, Italy, [2]STMicroelectronics, Agrate Brianza, Italy,*

Background. Limits to the wide diffusion of molecular diagnostics are the elevated cost of thermalcyclers and the need of trained technicians that perform PCR reaction and analysis. Another limit is that PCR mixes, enzymes and components are typically stored at -20° or +2-8°C. Repeated freeze-thaw cycles could negatively impact on the assay's performance. We developed innovative technology to produce a freeze-dried mix for the amplification of nucleic acids, which is ready-to-use, pre-dispensed, flexible and room-temperature storable. This technology is successfully applicable to PCR or RT-PCR, End-Point PCR or Real-Time PCR, in singleplex or multiplex reactions. **Objective.** The aim of the present work was to couple two technologies, real-time chip technology and a technology that stabilize PCR reagents at room-temperature, in order to obtain a rapid, user-friendly and easy-to-store PCR system. **Methods.** A PCR mix was prepared as follows: reaction buffer, dNTPs, $MgCl_2$, DNA polymerase, primers, probe, preservatives and stabilizers. Around 5 µL of this mix was spotted in each chamber of a chip and freeze-dried using an Epsilon 2-12D freeze-dryer (Martin Christ). Performances were evaluated on a portable PCR system (STMicroelectronics). **Results and conclusions.** Real-time PCR performances evaluated on chip with the portable PCR system matched those obtained with a classical 7500 Real-time PCR System (Applied Biosystems). This suggests that it is possible to perform real-time PCR on a small, portable instrument with reduced test turnaround time, low-volume needs and with a user-friendly software; with freeze-dried reagents that are easy-to-store. This PCR system can be used at the point of impact in point-of-care molecular diagnostics.

B-437

Antioxidant profiles of human serum and biological fluids

A. Sharma[1], K. Kline[2], A. M. Sawaya[1], R. L. Leveille[1]. *[1]Central Michigan University, Mt. Pleasant, MI, [2]McLaren Central Hospital, Mt. Pleasant, MI,*

Background: Free radicals are strongly associated with numerous human diseases such as cancer, cardiovascular and neurodegenerative disorders.

Objective: The objective of the present study was to develop a test to assess the antioxidant profile of human serum and other biological fluids.

Methods: Human serum samples were subjected to serum protein electrophoresis in agarose gels using conditions similar for separation of lipoproteins or proteins (migration towards anode). The gels were then stained with an activity stain based on the ability of antioxidants in serum to reduce ferricyanide to ferrocyanide. In the presence of ferric ions, strong antioxidant components in serum yielded a dark blue band.

Results: Evaluation of the activity staining methodology gave positive bands only with strong antioxidants such as vitamin C, quercetin and Trolox (a water-soluble derivative of vitamin E). Uric acid and certain amino acids gave very weak bands. Twenty random serum samples were evaluated for their antioxidant profiles after serum electrophoresis. Different profiles were observed for various samples suggesting that this test may be potentially used for diagnosis and management of human diseases that are strongly associated with free radicals. Some of the antioxidant bands corresponded to LDL, VLDL and HDL. However, there was no direct correlation between the intensity of the antioxidant and lipoprotein bands. For example, some samples showed weak lipoprotein bands but strong antioxidant activities. All samples also showed a strong reducing activity at the point of origin (gamma globulin region).

Conclusion: This is the first report of visualizing antioxidant activities of serum components. Profiles of other human biological fluids will also be presented. Studies are currently underway to assess the serum antioxidant profiles of patients with various diseases compared to a healthy population.

B-438

Bioenergetic health index: a predictive biomarker of human health that combines the effects of systemic stress and disease susceptibility

B. K. Chacko, P. Kramer, T. Mitchell, S. Ravi, M. Johnson, G. Benavides, R. Hardy, V. Darley-Usmar. *University of Alabama at Birmingham, Birmingham, AL,*

Background: The increasing incidence of bioenergetics-related diseases and the personalized nature of these disease progression is one of the major health concerns worldwide. Emerging literature suggest the existence of substantial diversity in individual susceptibility to diseases associated with energetic dysfunction such as diabetes, alcoholoc liver disease and cancer. This opens up a valuable avenue to develop a personalized predicitive biomarker in the diagnosis and management of diseases involving bioenergetic alterations. However, no clinical test capable of determining "bioenergetic health" exists to stratify these patients. Several studies studies have demonstrated the influence of genetic, environmental and lifestyle factors and age in disease susceptibility and progression and also in the individual bioenergetic function. Our recent findings support an emerging concept that circulating leukocytes and platelets can act as sensors or biomarkers of bioenergetic dysfunction that occurs in chronic diseases. It is proposed that chronic disease-induced systemic stress (inflammation, oxidative stress etc) will cause alterations in leukocyte bioenergetics, which is the resultant of the intensity of stress and individual health. This suggests that oxidative stress induced typical changes in leukocyte mitochondrial function can be used as an indicator of bioenergetic health of individuals. Hence it is hypothesized that systemic stress alters the bioenergetic capability in leukocytes and platelets and these alterations can be used to assess individual health and disease susceptibility.

Methods: In this study, by inducing oxidative stress, the impact of stress on bioenergetic capability of human leukocytes and platelets isolated from different individuals is demonstrated. The bioenergetic capability of cells is determined using the extracellular flux analyzer (Seahorse Biosciences). Oxidative stress was induced in isolated leukocytes (monocytes, lymphocytes and neutrophils) and platelets using well characterized oxidants, such as dimethylnaphthoquinone and lipid peroxidation product 4-hydroxynonenal. Using the individual parameters of bioenergetic assay, 'the bioenergetic index(BHI)' is calculated for each cell type.

Results: We demonstrate that oxidative stress induces different degrees of mitochondrial dysfunction in different individuals. Our results also show that these oxidant-induced changes in the profiles of bioenergetic parameters is characterized by increase in basal respiration, proton leak and non-mitochondrial respiration and a decrease in ATP-linked and maximal respiration in freshly isolated monocytes, lymphocytes, neutrophils and platelets. These alterations also show typical profiles for different individuals. In addition, different degrees of oxidative stress is required for bioenergetic dysfunction in different individuals suggesting the personalized nature of this parameter. Using the bioenergetic parameters of oxidant-treated leukocytes and platelets we also demonstrate the development of the bioenergetic health index, a functional measure that can define the health of individuals.

Conclusion: These novel findings suggest that peripheral blood leukocytes from different individuals significantly differ in their response to oxidative stress which is reflected in the bioenergetic health index in monocytes, lymphocytes, neutrophils and platelets. Taken together, bioenergetic health index is a novel parameter that is derived from the mitochondrial function of cells and demonstrates the interplay between innate health and acquired risk factors. It is also suggested that the 'bioenergetic health index' has the potential to be used as a clinical test in developing personalized management/therapeutic strategies in patients.

B-439

Development of an HCV Ag-Ab COMB ELISA Kit and Evaluation in Taiwan Population

Y. Wu, I. Huang, W. Lu, J. Lin. *General Biologicals corporation, Hsinchu, Taiwan,*

Background: Worldwide infection statistics showed 184 million people with antibodies to Hepatitis C (HCV). HCV infection is important risk factor of hepatocellular carcinoma, which was ranked in the second place among worldwide cancer incidences. There is still no vaccine or immune globulin products specific to HCV infection, so the prevention is relied on screening. To provide a screening method that reduces window period and testing cost, we developed an enzyme-linked immunosorbent assay (ELISA) kit that can detect HCV antigen and antibody simultaneously.

Methods and results: We used 218 positive and 204 negative serosamples of Taiwanese population to compare the sensitivity and specificity of our HCV Ag-Ab COMB kit and Monolisa HCV Ag/Ab kit. The result shows as table; the sensitivity and specificity of our kit were 100% and 100%, which were better than Monolisa kit. We also used seroconvertion panels and British Working Standard to evaluate and compare the performance of our kit and Monolisa kit. The test HCV seroconversion panels included BBI: PHV901, PHV906, PHV914, PHV917, PHV919, PHV920 and BCP: 6214, 6215, 6227, total 9 panels. When our kit was compared to the Monolisa kit, 6 of the 9 seroconversion panels tested gave the same results on both kits. For 2 of the 9 panels tested, our kit detected HCV infection earlier than Monolisa kit. The analytical sensitivity of our kit (1:32 dilution) was higher than Monolisa kit (1:2 dilution) by using serial dilutions of the NIBSC British Working Standard for Anti-HCV.

Conclusion: Although previous data shown the antigen detection limit of our kit was still higher than compared one, our kit shown a lower antibody detection limit, a better clinical sensitivity and a better specificity. Besides, the total reaction time only takes 105 minutes. We therefore provide a useful HCV infection screening choice.

		Our HCV Ag-Ab COMB kit		
		HCV+	HCV-	Total
Monolisa HCV Ag/Ab kit	HCV+	215	0	215
	HCV-	3	204	207
	Total	218	204	422

B-441

Integration of Microarray and qPCR system for quantitative and multi-qualitative analysis of MTB, NTM and MDR

D. Lee, J. Kim, S. Park, S. Ku, M. Peak. *K-MAC, Daejeon, Korea, Republic of,*

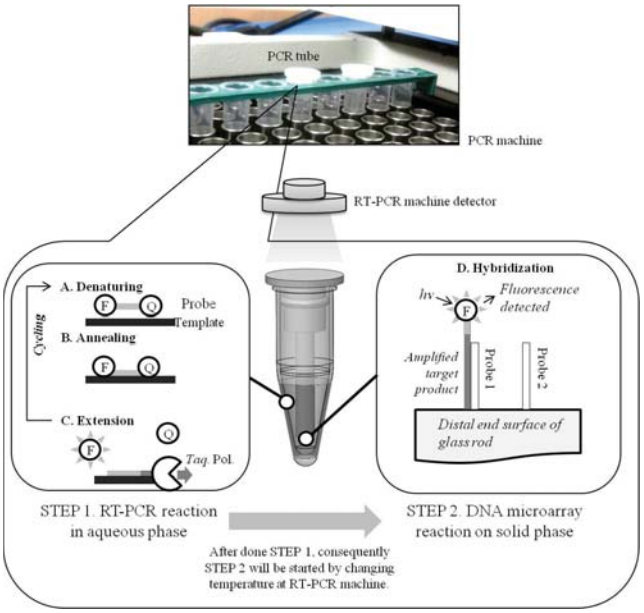
Background: qPCR and DNA Chip analysis have been widely used for molecular diagnosis. We developed a novel platform for molecular diagnosis, called Ampli & Array system, which integrates the qPCR and hybridization in a DNA microarray. In this system, entire reaction of qPCR and microarray are carried out in same container. So, quantitative and multi-qualitative analysis can be achieved at the same time. In this study, the Ampli & Array system was applied to the detection of *Mycobacterium tuberculosis* (MTB) to verify the usefulness in diagnosis.

Methods: Probes identifying mutation genes and genotyping of MTB/NTM are immobilized on the surface of Ampli & Array platform. We can recognize clinically important target genes, rpoB, katG and inhA that are related with rifampin and

isoniazid by using these probes. Amplification of the target DNA was carried out in a normal qPCR machine. After qPCR step, hybridization was performed sequentially by simply changing temperature at the qPCR machine.

Results: We measured 10 copies of MTB in qPCR process, and detected NTM genotypes and mutation genes of rpoB, katG and inhA specifically. In hybridization, we screened 94% of NTM by detecting 5 types of NTM. And, we also classified the mutation related to MDR. In rifampin related genes, we have screened L511P, D516V, D516Y, H526Y, H526D and S531L. Also, we have detected 2 types of mutation in isoniazid related genes.

Conclusion: With the Ampli & Array system, we could quantitatively analyze various genotypes in samples of large number at once. Also, more accurate results could be provided with more economical manner. The system encouraged us to overcome the limitations of current molecular diagnosis/DNA analysis in revolutionary way. For this reason, we expect that the Ampli &Array system will replace the existing qPCR and microarray in molecular diagnosis market.



B-442

A Novel and Robust Real-time PCR System for DNA Polymorphism Analysis Directly from Crude Clinical Samples

T. Kobayashi, H. Matsumoto, T. Kuroita. *Toyobo Co., Ltd., Osaka, Japan,*

Background: Real-time quantitative PCR (qPCR), a powerful technology utilized in many scientific disciplines, is also used for analysis of clinical samples. However, conventional qPCR systems using Taq DNA polymerase suffer from the following problems: (1) target size limitations (<200 bp), (2) poor amplification of GC-rich targets, and (3) PCR inhibition from the biogenic substances present in clinical samples. To circumvent these problems, we developed a novel qPCR system (KOD-SYBR) that uses KOD exo(-) DNA polymerase and SYBR Green I dye. Thus far, KOD-SYBR has been used to amplify large targets (<2 kb), GC-rich targets, and targets from crude clinical samples (whole blood, tissue lysates, hair roots, Gram-negative and Gram-positive microorganisms).

Objective: To determine the effectiveness of the KOD-SYBR system for DNA polymorphism analysis of crude clinical samples.

Methods: First, we evaluated the KOD-SYBR system using amplified fragment length polymorphism (AFLP) PCR with melting curve analysis at the end point. We used three primers to generate fragments of two different sizes [wild type (WT) allele: 100 bp; Inserted allele (IN): 341 bp]. Mouse tissue lysates prepared by a rapid method were used as templates. Next, single nucleotide polymorphisms (SNPs) in the alcohol dehydrogenase gene were detected using allele-specific primers containing 3' end mismatched bases followed by melting curve analysis. Diluted whole blood and oral mucosa specimens were used. A G-specific primer-bearing tail sequence at the 5' end was used to obtain a larger fragment (57 bp) than that of the other primer set (an A-specific primer and a common primer), which had no tail sequence (45 bp). The G-specific and common primer set detects an A>G SNP that leads to a missense mutation (E487K).

Results: On melting curve analysis, the 100- and 341-bp-amplicons obtained from crude mouse tissue lysates gave distinct peaks at 81°C and 84°C, respectively. Additionally, all of the variants tested (WT/WT, WT/IN and IN/IN) were distinguishable from each other. In the SNP assay, A/A, A/G, and G/G variants were detected in the crude specimens. Peaks from 45- and 57-bp-amplicons were detected by melting curve analysis at 73°C and 80°C, respectively. However, when tested, the conventional qPCR system failed to amplify any of the target DNA molecules. We found that to obtain separation of distinct peaks on melting curve analysis required melting temperature differences of at least 3°C between the amplicons, and amplicon sizes of 45 bp to 1 kb.

Conclusions: KOD-SYBR is a robust system for polymorphism analysis of crude clinical samples. The flexibility of being able to use relatively large amplicon sizes (<2 kb) for melting curve analysis is a distinct advantage of this system. KOD-SYBR has potential to be a powerful tool for high-throughput genotyping of clinical samples.

B-444

Highly sensitive quantification of oxalate in plasma and dialysate fluid by ion chromatography.

N. Voskoboev, T. S. Larson, J. C. Lieske. *Mayo Clinic, Rochester, MN,*

Background: Oxalate is an end product of glyoxalate and glycerate metabolism that is excreted in the urine. This dicarboxylic acid is a common component of up to 75% of kidney stones. In addition to kidney stones, more extreme hyperoxaluria due to either genetic (primary hyperoxaluria) or acquired/secondary causes (enteric hyperoxaluria) can cause nephrocalcinosis and/or renal failure. If patients with primary (or sometimes enteric) hyperoxaluria develop renal failure, plasma oxalate levels and removal rates in dialysate need to be monitored closely to prevent oxalosis and/or rapid loss of a transplanted kidney. Previously we have validated enzymatic assays of oxalate in plasma and dialysate. Use of ion chromatography to quantify oxalate has the potential advantages of improved precision, automation, and integration with the laboratory information system.

Methods: A Dionex ion chromatography system and IonPac column were used to measure oxalate. Dionex ion chromatography system was modified to accommodate a Boric Acid eluent. Waste dialysate and plasma samples from patients with and without hyperoxaluric diseases were obtained for clinical and analytic validation. All samples were acidified to a pH 2.5 - 3.0 within 1 hour of collection, previously shown necessary for oxalate stability. Accuracy was assessed via oxalate spike recovery and comparison to our laboratory's current enzymatic oxalate oxidase method that is based on the Trinity Biotech oxalate kit (Trinity Biotech plc, Bray, Co. Wicklow, Ireland).

Results: During initial development it was demonstrated that oxalate levels increased in plasma and dialysate samples, even after acidification. This is consistent with previous observations that oxalate levels can increase in samples exposed to basic conditions, perhaps due to conversion from ascorbic acid. Thus the Dionex ion chromatography system and IonPac column were modified to accommodate a Boric Acid eluent. Using this set-up, plasma and dialysate oxalate measurements were linear over the range of 1- 50 mcmol/L. Intrassay precision was acceptable (10%) at 1 mcmol/L, and improved to <2 % for values above 10 mcmol/L. Average recovery with serial dilutions was 102%. Oxalate was stable refrigerated or frozen (-20°C or -80°C) when plasma or dialysate was acidified (pH 2.5), but oxalate levels variably increased in samples that were stored refrigerated or frozen but unacidified. Results for 48 plasma samples across the normal and abnormal range compared well with the current assay with a mean difference of only 2% between the two methods.

Conclusion: Ion chromatography using borate buffer can be used to reproducibly quantitate oxalate in plasma and hemodialysate fluid. Use of common methods based on aqueous and carbonate eluents are not acceptable for highly sensitive quantification of oxalate. Results of a new chromatographic method compare well with a previous enzymatic oxalate oxidase method.

B-445

Evaluation of the Sebia Capillarys 2 Flex Piercing hemoglobin A_{1c} (HbA_{1c}) assay

Z. Zhao[1], J. Basilio[1], S. Hanson[2], A. Tennill[2], R. Little[2], D. Sacks[1]. *[1]National Institutes of Health, Bethesda, MD, [2]University of Missouri, Kansas City, MO,*

Background: Hemoglobin A_{1c} (HbA_{1c}) is used to monitor long-term glycemic control in patients with diabetes, guide therapy, predict the risk of microvascular complications, and diagnose diabetes. It is vital that methods to measure HbA_{1c} be accurate, precise, reliable and subject to minimal interference. Recently, Sebia (Lisse, France) developed an automated liquid-flow capillary electrophoresis method to

measure HbA_{1c} on the CAPILLARYS 2 Flex Piercing instrument. We evaluated the performance of the Capillarys for the measurement of HbA_{1c}.

Methods: Technical evaluation was performed in two clinical centers (center 1, a NGSP Secondary Reference Laboratory (SRL9) and center 2, a clinical laboratory). The instruments and reagents were provided by Sebia and used according to the manufacturer's procedures. Linearity, carryover, and interference studies were performed on fresh or fresh frozen samples collected in each center. Blood samples were shared between the centers for precision and accuracy studies. Precision evaluation was performed following CLSI EP-5 using pooled whole blood daily-use aliquots frozen at -70ºC at four HbA_{1c} levels (4.7%, 6.3%, 7.4% and 11.1%) and compared to National Academy of Clinical Biochemistry (NACB) recommendations. Accuracy was assessed using 100 single-donor patient samples with ≤ 16 samples analyzed in duplicate each day. HbA_{1c} values obtained with the Capillarys were compared to those obtained with two other analyzers used routinely at the two centers: NGSP SRL9, G8 (Tosoh Bioscience, South San Francisco, CA) in center 1 and D-10 (Bio-Rad Laboratories, Hercules, CA) in center 2. For interference studies, a difference of ±0.2% HbA_{1c} was used as an acceptable limit.

Results: The Capillarys was linear for HbA_{1c} results from 4.2 to 17.6%. There was no carryover when samples with HbA_{1c} of 4.7 and 14% were analyzed alternately. There was no interference from labile HbA_{1c} as high as 11%, carbamylated hemoglobin (blood samples incubated with 0.15 to 1 mmol/L of KCNO at 37ºC for 3 hours), or triglycerides (93 to 4666 mg/dL). No additional peaks (i.e. for labile, carbamylated, etc) were observed on the Capillarys electropherograms. Total CVs were < 2.0% in both centers. HbA_{1c} values obtained with the Capillarys and comparison methods were well correlated, with minimal bias. Linear regression analysis yield the following: y (HbA_{1c} Capillarys center 1)= 1.033 × (HbA_{1c} Tosoh G8)-0.34, r=0.997, S_{yx}=0.16; y (HbA_{1c} Capillarys center 2)= 1.084 × (HbA_{1c} Bio-Rad D10)-0.67, r=0.995, S_{yx}=0.22. Results of the Capillarys were comparable between centers with mean bias ≤ 0.1% HbA_{1c} (y (HbA_{1c} Capillarys center 1) = 0.982(HbA_{1c} Capillarys center 2) + 0.05, r=0.996, Syx=0.18). Ninety-five % (center 1) and 97 % (center 2) of single Capillarys results were within ±6% of the SRL9 mean (NGSP manufacturer certification criteria: ≥ 92.5% of results within ±6%).

Conclusion: The analytical performance of Capillarys HbA_{1c} is within NACB and NGSP recommendations, has minimal interference, and is suitable for clinical application.

B-446

Homogeneous high-sensitivity CRP assay on MagArray biosensors

H. Yu[1], L. Carbonell[1], M. Momiyama[2], M. Murakami[2]. *[1]MagArray Inc, Sunnyvale, CA, [2]IMRA America, Inc, Ann Arbor, MI,*

Background: MagArray platform is based on the detection of magnetic particles as labels in bioassays. In contrast to systems based on optical signals, magnetic signals are not affected by the common optical interference in complex matrices. In addition, since the biosensors are designed to detect magnetic particles only when particles are bound or captured to the sensor surface, this proximity detection mechanism allows the possibility of homogeneous immunoassays. We report here that we have developed a one-pot homogeneous assay for CRP with high sensitivity. We wish to demonstrate MagArray platform is well suited for homogeneous assays that require both simplicity and sensitivity.

Methods: Antibody pairs for CRPassay were screened and selected on MagArray platform, and the detection antibody is conjugated to magnetic particles for both capping CRP and generating signals. The assay consists of two simple steps of mixing the magnetic particles with serum sample and addition of the mixture to magnetic sensors. Signals can be read in as short as 2 min for a result, and more accurate results can be obtained after 5 min. The whole process requires no shaking and rinsing or other separation steps. Standard curves of CRP in both pure buffer and sera were established and compared.

Results: The detection sensitivity of CRP in serum on MagArray platform is less than 1 mg/L for a homogeneous assay. The CVs for lower concentrations are less than 10% and less than 5% for medium and high concentrations. No prozone effect was observed for CRP concentrations of up to 200 mg/L. Our preliminary tests showed no interference effects (<= 10%) from lipids, HAMA and Rheumatoid Factor.

Conclusion: MagArray platform provides a unique opportunity of detecting proteins in a simple and homogeneous fashion. Since this assay only involves mixing and adding reagents to the biosensors, the complexity of the assay format is greatly reduced. The detection of magnetic signals from magnetic particles in proximity is a key to applying MagArray platform for a homogeneous immunoassay.

B-447

Modifying tosyl-activated magnetic bead coating protocols to improve biomolecule binding efficiency

F. Sultan[1], N. Gasilova[2], H. H. Girault[2]. *[1]Merck Chimie SAS, 94126 Fontenay sous Bois Cedex, France, [2]Laboratoire d'Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland,*

Background Tosyl-activated magnetic beads are widely used as a solid phase in immunoassays and biomagnetic separations. With the right conjugation protocol any biomolecule whether it be antibody, protein, peptide or glycoprotein containing NH2- or SH- groups can be covalently coupled to the surface of tosyl-beads. Optimising the coupling protocol is key to maximizing the binding efficiency of biomolecules to beads. Using immunoaffinity capillary electrophoresis (IACE) with UV-detection, bovine β-lactoglobulin B levels bound to magnetic beads (MBs) following different antibody coupling protocols were measured. Results were further verified using a bicinchoninic acid (BCA) protein test (BCA kit, Pierce, USA) to estimate the amount of antibody bound to the MBs. A coupling protocol has been developed which shows higher binding efficiecy for the same concentration of beads when compared with a published protocol (herein referred to as Protocols 'E' and 'A' respectively). Moreover, with Protocol 'E' similar levels of antibody loading can be achieved by incubating at 37ºC for 6h rather than 12h at room temperature.

Method Protocol A: 2µl of rinsed tosyl-activated MBs were mixed with 8 µl of coating buffer (100mM sodium borate, pH9.5), 8 µl of 3M ammonium sulphate and 8 µl of antibodies (5mg/ml). The mixture was incubated for 24h at 37ºC under continuous stirring to avoid sedimentation. After incubation, beads were rinsed with 10mM PBS and stored in PBS containing 0.025% Tween-20 and 0.02% sodium azide.

Protocol E: 4µl of rinsed tosyl-activated MBs were mixed with 280µl of coating buffer, 166 µl of 3M ammonium sulphate and 54µl of antibodies (1.48mg/ml). The mixture was incubated overnight (~12h) at room temperature under continuous stirring to avoid sedimentation. Beads were subsequently incubated with a blocking buffer for 1h at room temperature under continuous stirring. Finally, beads were rinsed with washing buffer and stored in PBS containing 0.025% Tween-20 and 0.02% sodium azide.

Results Under the same conditions of IACE analysis, electropherograms showed larger peaks of antigen β-lactoglobulin B (5µg/ml) in the case of MBs coated using Protocol 'E' (area of 0.328 (±0.009) units) compared with Protocol 'A' (area of 0.155 (±0.006) units). Using the BCA test to quantify the amount of bound anti-β-lactoglobulin B antibody, it was demonstrated that 42µg of antibody was bound per 1mg of MBs using Protocol 'E' compared with 24µg for Protocol 'A'. A further enhancement of Protocol 'E' was made to decrease the antibody incubation time with the MBs. Comparative peaks were observed from the IACE electropherograms for β-lactoglobulin B levels (50µg/ml) following 12h incubation at room temperature and 6h incubation at 37ºC (areas of 2.28 (±0.06) units and 2.14 (±0.05) units respectively).

Conclusion From the IACE detection of β-lactoglobulin B levels and BCA analysis of bound anti-β-lactoglobulin B antibodies, it can be concluded that Protocol 'E' provides a higher antibody coating efficiency of MBs than Protocol 'A'. Moreover, the coating efficiency is retained at 37ºC allowing for an overall shortening of experimental time.

B-448

Performance Evaluation of a Protein-Free Reagent to Design Out Common Interferences in Diagnostic Assays

D. Pauly, T. Jentz, K. Pauly, J. Hein. *SurModics, Eden Prairie, MN,*

Background: Protein stabilizers, especially those containing bovine serum albumin (BSA), can lead to increased non-specific binding due to protein interference. Additional BSA-related issues include: anti-BSA antibodies, heterophilic false positives, cross reactivity, reliable sourcing, lot-to-lot variability. The ability to stabilize proteins in-solution, without the use of BSA or alternative proteins, would be an invaluable way to eliminate these protein-related interferences and issues. **Objectives**: To demonstrate the ability to stabilize proteins in solution using a synthetic protein stabilizing technology versus commercially available protein-containing stabilizers.

Methods: We tested 4 stabilizers: A. commercial BSA stabilizer, B. protein-free stabilizer, C. commercial alternative protein stabilizer (non-BSA), D. 1%BSA in PBS (negative control). All stabilizers were evaluated in terms of non-specific binding and retained activity of the target antibody. Data are derived from stability studies utilizing anti-rabbit antibodies from different host species. Anti-rabbit IgG-HRP conjugated antibodies (hosts of origin including: guinea pig, sheep, chicken and mouse) were diluted into the different stabilizing buffers (22 - 120 ng/mL) and equally divided for storage at 4°C and 37°C. The test solutions of each stabilizer were evaluated in an ELISA. At each time point a percent retained activity was determined by comparing the activity of the aged conjugate (37°C) to that of the control conjugate (4°C). Results: After 127 days at 37°C the A. commercial BSA stabilizer, B. protein-free stabilizer, and C. commercial alternative protein stabilizer (non-BSA) demonstrated averaged retained activities of 79, 77 and 69% respectively across all species. Based on Arrhenius projections, the stability of the conjugated antibody is equivalent to 3.4 years at 4°C. **Conclusion:**These data represent an effective protein-free approach to reduce non-specific binding and still perform equal to or better than protein-containing stabilizing reagents. When protein-related interferences arise, the protein-free stabilizer provides a valuable alternative for assay developers.

% Retained Activity after 127 days					
Stabilizer	Guinea Pig	Sheep	Chicken	Mouse	Mean
A	74.9%	77.4%	72.9%	90.9%	79.0%
B	68.7%	75.9%	73.8%	89.5%	77.0%
C	60.1%	66.3%	66.1%	85.4%	69.5%
D	0	0	0	0	0
% Retained Activity = (Abs OD of 37C sample / Abs OD of 4C Sample) x 100					
* Data points were averaged from 4 ELISA wells (n = 4)					
** %CV's for each data point were less than 10%					

B-449

A Novel Method for the Measurement of Serum Viscosity: Evaluation of the Rheosense microVISC™ Viscometer

H. Faby, L. C. Rogers. *Memorial Healthcare System, Hollywood, FL,*

Background: Measurement of serum viscosity is used to evaluate Hyperviscosity Syndrome (HVS), which is associated with plasma cell dyscrasias, myeloma, connective tissue diseases, and other inflammatory conditions. Rapid treatment of HVS is critical to ensure effective reduction of risk from serious complications. Increased viscosity, caused by the excessive intravascular paraproteins, leads to impaired transit of blood through the microcirculatory system. The vascular stasis and resultant hypoperfusion can cause severe complications which include cardiopulmonary symptoms such as shortness of breath, hypoxemia, acute respiratory failure, and hypotension; neurological effects such as confusion / mental status changes; ocular damage including dilation of the retinal veins and retinal hemorrhages; bleeding from the mucous membranes; and renal failure. Measurement of serum viscosity is essential for an accurate diagnosis, but traditional methods are labor intensive and not amenable to STAT analysis. We have evaluated a new instrument, the Rheosense microVISC™, for the rapid analysis of serum viscosity.

Methods: The microVISC™ (Rheosense, Inc.) is a small portable instrument which uses VROC® (Viscometer/Rheometer-on-a-Chip) technology. The VROC® sensor obtains a viscosity reading by measuring the pressure drop as a sample flows through a flow channel. Pressure is measured at positions of increasing distance from the inlet. The slope of the straight line in the plot of the pressure vs. sensor position is proportional to the viscosity. We evaluated this new instrument for use in the clinical laboratory. The performance evaluation in this study included within-in run and between-run precision and linearity, using standards purchased from Rheosense, Inc. Accuracy was determined by correlation of the microVISC™ (Rheosense, Inc.) to a cone and plate viscometer using patient samples. We used serum samples from patients with a normal comprehensive metabolic panel for verifying the reference range. Statistical analyses were performed by EP Evaluator®

Results: The within-run precision for normal and abnormal controls was < 1%. Mean/ standard deviation/ % CV for between-run precision was 1.54 cP/0.05 cP/3.25% and 4.12 cP/0.10 cP/2.43% for normal and abnormal controls respectively. The linear range was verified for 0-6.38 cp. The microVISC correlated well with the cone and plate method: y= 0.836x + 0.064 (r= 0.979). The small negative bias was reflected in a slightly lower reference range of 1.10-1.60 cP for serum samples.

Conclusion: We validated the microVISC instrument for the rapid, accurate, and reproducible measurement of serum viscosity in a clinical laboratory setting. The instrument is small, portable, and easy to use and maintain.

B-452

Direct Single Nucleotide Polymorphism Genotyping from Blood, Plasma or Serum

C. Yeh, J. Spurgin, J. Athanasuleas, K. Bradley, C. Willmon, J. Chimera. *Atherotech Diagnostics Lab, Birmingham, AL,*

Background and objectives: Real-time PCR is a powerful, sensitive and favorable method for large-scale single nucleotide polymorphism (SNP) genotyping. However, prior purification of genomic DNA from blood is necessary since PCR inhibitors and quenching of fluorophores from blood prevent efficient amplification and subsequent detection of PCR products. We have developed a simplified "direct-on-sample" SNP genotyping without prior DNA enrichment/isolation steps to further throughput and reduce time, cost and labor. This methodology can be applied in pharmacogenomic analysis on various platforms e.g., fluorescent-, florescence resonance energy transfer-, or electrochemical voltammetry-based detection.

Methods: Diluents and procedures designed to specifically overcome PCR inhibition and quenching of fluorescence were evaluated by genotyping on 4 SNPs from Factor II, Factor V, MTHFR genes, 3 CYP2C9, VKORC1 gene polymorphisms, and 7 SNPs of PCSK9 gene. Paired DNA and blood, plasma or serum samples are collected and analyzed for concordance using eSensor and ABI7900HT instruments.

Results: The performances of either DNA purified from blood or the same blood without DNA purification were analyzed using GenMark eSensor technology on 4 different variants prevalent in Factor II, Factor V and MTHFR genes (Thrombophilia Risk Panel), and 3 variants from CYP2C9 and VKORC1 genes (Warfarin Sensitivity Panel). Genotyping of 7 SNPs in the PCSK9 gene was conducted by TaqMan/ ABI7900 platform. Overall, genotyping from purified DNA and the corresponding blood showed concordance of 84.8% (N=66), 87.5% (N=24) and 100% (N=12) for PCSK9, Thrombophilia and Warfarin panels, respectively.

To further validate the methodology to perform large-scale high-throughput genotyping directly from blood, plasma or serum, paired DNA/blood (N=50), DNA/ plasma (N=30) or DNA/serum samples (N=20) were analyzed on 7 SNPs of PCSK9 gene using ABI TaqMan real-time PCR machine. High concordance was achieved and resulted in sensitivity/specificity of 100%/90.9% for direct blood, 97.9%/66.7% for direct plasma, and 94.5%/75.0% for direct serum genotyping.

Conclusions: The methodology described is simple and fast that allows accurate gene polymorphism test directly from a drop of blood, plasma or serum. This method can be applied to a broad range of clinical genetic tests with the advantages of immediate sample testing, improving workflow, and lowering workload, cross-contamination, costs and turnaround time.

Abbreviation: PCR, polymerase chain reaction; PCSK9, proprotein convertase subtilisin/kexin type 9; MTHFR, methylenetetrahydrofolate reductase; VKORC1, Vitamin K epoxide reductase complex subunit 1.

AUTHOR INDEX TO ABSTRACTS OF SCIENTIFIC POSTERS, AACC MEETING, 2014

(Numbers refer to Poster Numbers; see pages S2 to S263)

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

T

U

V

W

X

Y

Z

KEYWORD INDEX TO ABSTRACTS OF SCIENTIFIC POSTERS, AACC MEETING, 2014

(Numbers refer to Poster Numbers; see pages S2 to S263)

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z