SCIENTIFIC POSTER SESSION SCHEDULE

Posters of accepted abstracts were viewed in the Exhibit Hall of the Pennsylvania Convention Center on Tuesday, August 2 and Wednesday, August 3, 2016.

Below are the topics and their scheduled times.

TUESDAY, AUGUST 2, POSTER SESSIONS

9:30am – 5:00pm

WEDNESDAY, AUGUST 3, POSTER SESSIONS

9:30am – 5:00pm

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Tuesday, August 2, 2016

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

Cancer/Tumor Markers

A-002

Unusual Case of Follicular lymphoma presenting IgM Monoclonal Gammopathy overlapped with Polyclonal peak in Capillary electrophoresis

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BACKGROUND: Follicular lymphoma (FL) represents a third of non-Hodgkin lymphoma (NHL) in western countries. Although the association of monoclonal gammopathy (MG) in B-cell NHL is a well-known phenomenon, the precise incidence rate among subtypes of NHL and the prognostic significance is still unclear. Especially, the association of MG with FL has been rarely reported in asian population. We report a case of follicular lymphoma showing IgM and Kappa chain restriction accompanying with polyclonal gammopathy detected in capillary electrophoresis.

METHODS: We investigated an unusual case of a 63-year-old male patient who was diagnosed to FL presenting IgM MG overlapped with polyclonal peak in capillary electrophoresis. We performed CBC tests using Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY) and biochemical tests with Toshiba chemical analyzer (Toshiba, Nasushiobara, Japan). The monoclonal components were detected in capillary electrophoresis (CE) via capillary 2 (Sebia, Lysse, France) and reconfirmed through a conventional gel electrophoresis (EP) with high-resolution gel EP in a Hydrasys analyzer (Sebia) using Hydragel 15 HR gels (Sebia). To confirm the diagnosis of lymphoma, endoscopic biopsy and bone marrow biopsy were performed in this patient.

RESULTS: Computed tomography of neck revealed highly suggestive of lymphoma with multiple enlarged conglomerated lymph nodes along both internal jugular veins, submandibular, parotid, supraclavicular, superior mediastinal. Endoscopic incisional biopsy of both lingual tonsilar mass was done and a final diagnosis of FL was made. Peripheral blood finding was unremarkable except normocytic normochromic anemia. Bone marrow aspiration showed normocellular marrow pattern except slightly increased plasma cell portions to 5.4%. In the bone marrow clot sections, there were several nodular lesions composed of various-sized lymphocytes, suggestive of lymphoma infiltration. Immunohistochemical staining on this portion of cell aggregations on Rt and Lt clot sections were done and showed CD20, BCL2, BCL6 positive reaction. Serum electrophoresis showed a distinct M-peak in front of a broad polyclonal peak. Due to the partial overlapping between monoclonal and polyclonal peaks, gamma region of serum electrophoresis test presented discrete dual peaks. Following immunotyping electrophoresis tests with immunosubtraction method clearly revealed MG of IgM and Kappa type in spite of overlapped polyclonal gammopathy. The gel electrophoresis showed corresponding result with CE. Finally the patient was diagnosed as stage 4 FL accompanying IgM MG and was started on chemotherapy.

CONCLUSIONS: Our report shows an unusual case of IgM and Kappa type MG overlapped with polyclonal gammopathy in a patient with FL. Although in aggressive B-cell NHL, the presence of MG might be an adverse prognostic factor, the definite clinical significance of MG in B-cell NHL has not been clarified. As reported by others, FL accounts for minor portion of lymphoid neoplasms associated with serum IgM paraprotein. IgM paraproteinemia may also, however, be seen in other B-cell lymphoproliferative disorders including Waldenstrom macroglobulinemia and chronic lymphocytic leukemia. Thus, careful differential diagnosis among these diseases is critical to apply proper treatment. Further studies are also necessary to estimate the value of paraprotein profile as an early indicator for a hidden lymphoma, a tool for evaluating a prognostic outcome and disease severity in lymphoma patients.

A-004

Genomic DNA Breakpoints in *MLL* Gene in Infants with Acute Leukemia

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Background. There are several prognostic factors in infant acute leukemia (AL), including high white blood cell count, presence of 11q23/*MLL* rearrangement, CNS disease, response to initial steroid monotherapy. Although less is known about prognostic factors within group of infant AL patients carrying 11q23/*MLL* rearrangements. So the aim of this study was to evaluate the relation between genomic DNA breakpoints in *MLL* and clinical parameters of infant AL.

Methods. 87 infants (32 boys (37%) and 55 girls (63%) with median age of 4.9 months) with *MLL*-rearranged acute lymphoblastic leukemia (ALL) (n=63), acute myeloid leukemia (AML) (n=22) and mixed phenotype acute leukemia (MPAL) (n=2) were included in the current study. Genomic DNA breakpoint detection in *MLL* gene was performed by long-distance inverse PCR.

Results. Majority of ALL cases was characterized by presence of *MLL-AF4* fusion gene (FG) (n=35;55%), less frequently *MLL-MLLT1* (n=12;22%), *MLL-MLLT3* (n=8;13%) and others were found. The most common breakpoint location within *MLL* gene in ALL patients was intron 11, detected in 31 cases (49%), less frequently breakpoints in intron 10 (n=13;21%) and intron 9 (n=9;14%) were found. The highest variability of *MLL* breakpoints was found in *MLL-AF4*-positive patients: only 15 of 35(43%) had breakpoints in intron 11. The most stable pattern of *MLL* genomic DNA breakpoints was observed in *MLL-MLLT1*-positive patients: 9 of 14 (64%) had breakpoints in intron 11. In AML patients the most prevalent FG was *MLL-MLLT3* (n=8;36%). The most frequent breakpoint location was intron 9 (n=10;45%), less often they were found in intron 10 (n=5;23%) and 11 (n=4;18%). The most stable pattern was revealed for *MLL-MLLT10* FG: *MLL* breakpoints in 4 of 5 (80%) cases were found in intron 9. Distribution of DNA breakpoints in *MLL* gene was similar in boys and girls and did not depend on type of translocation partner gene. ALL patients who had breakpoints in intron 11 were significantly younger (median 3.0 mo, range 0.03-11.6) than all others (median 5.6 mo, range 0.7-11.9) (p=0.025) and than patients with *MLL* breakpoints in intron 9 (median 6.6 mo, range 3.1-11.9)(p=0.017). For AML cases we did not find any relation between age and breakpoint locations. We estimated prognostic significance of *MLL* breakpoint locations in 46 cases of infant ALL uniformly treated by multicenter MLL-Baby protocol. 5-year even-free survival was significantly lower in patients with breakpoints in intron 11 (n=29) in comparison to patients with breakpoint localized from intron 7 to exon 11 (n=17) (0.16±0.07 vs 0.38±0.14 p=0.039). While cumulative incidence of relapse was remarkably higher in the first group of patients (0.74±0.09 vs 0.52±0.17 p=0.045). Median follow-up time was 42 months. Although in Cox regression model including breakpoint location in intron 11 together with age, immunophenotype, initial white blood cell count, initial CNS involvement, type of *MLL* rearrangements, absolute blast number at day 8 of dexamethasone profase, minimal residual disease (MRD) at time point 4 (TP4) of MLL-Baby protocol, the only significant covariate was the presence of MRD at TP4 (HR 5.994, 95% CI 2.209-16.263, p<0.001).

Conclusions. Our data provide new information of molecular genetic features of *MLL*-rearranged infant AL.

A-007

Urinary cell-free microRNA expression signatures serve as novel noninvasive biomarkers for diagnosis and recurrence prediction of bladder cancer

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Background: Cell-free microRNA (miRNA) open up a new field for noninvasive diagnosis and prognosis of bladder cancer (BC) based on their stability in urine supernatant. The aim of the present study was to investigate the role of cell-free miRNA in diagnosing and predicting recurrence of BC.

Methods: Miseq sequencing was performed to identify candidate miRNAs for diagnosing cancer in a screening cohort of 12 participants (BC patients and controls). Real-time polymerase chain reaction was employed to evaluate the expressions of candidate miRNAs which were then analyzed by logistic regression in 540 participants. Meanwhile, urine cytology was conducted for comparison with the miRNA panel and correlation between miRNAs and tumor recurrence was further assessed in the validation cohort. **Results:** We identified a seven-miRNA panel (miR-22-3p, miR-29a-3p, miR-375, miR-7-5p, miR-126-5p, miR-423-5p and miR-200a-3p) that provided high diagnostic accuracy of BC with an AUC of 0.923 and 0.916 for training and validation set, respectively. The corresponding AUCs of this panel for Ta, T1 and T2-T4 were 0.864, 0.930 and 0.978, significantly higher than those of urine cytology, which were 0.531, 0.628 and 0.724, respectively (all at $p < 0.05$). Moreover, nonmuscle-invasive BC (NMIBC) patients with high miR-22-3p level and low miR-200a-3p level had worse recurrence-free survival (RFS) ($p = 0.002$ and $p = 0.040$, respectively). MiR-22-3p and miR-200a-3p were independently associated with recurrence of NMIBC ($p = 0.024$ and $p = 0.008$, respectively).

Conclusion: MiRNA expression signatures from urine supernatant may have considerable clinical value in diagnosis and recurrence prediction of BC.

A-008

Combining prostate cancer antigen 3 (PCA3) and prostate-specific antigen (PSA) improves diagnostic accuracy in men at risk of prostate cancer

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Background: Prostate cancer (PCa) is the most common cancer and the second leading cause of cancer-related death in men of the United States. Currently, serum prostate-specific antigen (PSA) is widely used as an aid for screening, diagnosis, and patient management of PCa. However, the low specificity of PSA results in unnecessary invasive biopsy and high negative biopsy rate. Prostate cancer antigen 3 (PCA3) is a non-coding prostate-specific mRNA that is highly overexpressed in PCa tissue and excreted in urine in PCa patients. The objective of this study is to assess the clinical utility of urinary PCA3, and to compare the performance characteristics of urinary PCA3 and serum PSA test in men at risk of prostate cancer.

Methods: A cohort of 142 men (mean age 64 years; range 43-79 years) with elevated PSA, and/or strong family history, and/or abnormal digital rectal examination (DRE) were investigated. Urinary PCA3 mRNA level and score were assessed using the Progensa assay and serum PSA was tested on TOSOH automated enzyme immunoassay analyzer AIA-2000. Diagnosis of PCa was confirmed by biopsy using a 12- or 18-core biopsy scheme. The performance characteristics including diagnostic sensitivity, specificity, positive and negative predictive values (PPV, NPV), and test efficiency were evaluated. Area under the receiver-operating characteristic curve (AUC) was quantified using R software to compare the performance of PCA3, PSA, and combination of the two biomarkers. A multivariable logistic regression analysis was conducted to incorporate the two biomarkers and other risk factors such as age, race, family history, DRE, and prostate volume.

Results: Of the 142 patients, 10 (7.0%) were excluded with no biopsy analysis. Among the 132 patients underwent biopsy, 40 (30.3%) were detected with prostate adenocarcinoma. Urinary PCA3 score at the cutoff value of 25 had a diagnostic sensitivity of 77.5%, specificity of 51.1%, PPV of 40.8%, NPV of 83.9%, and test efficiency of 59.1%. Serum PSA had a sensitivity of 87.5% and 25.0%, specificity of 22.8% and 84.8%, PPV of 33.0% and 41.7%, NPV of 80.8% and 72.2%, and test efficiency of 42.4% and 66.7%, at the cut-off of 4 ng/mL and 10 ng/mL, respectively. The AUCs for PCA3 and PSA were 0.697 and 0.577 respectively (P=0.14). A logistic regression algorithm combining PCA3 with PSA increased the AUC from 0.577 for PSA-alone to 0.708 (P= 0.02). Combination of urinary PCA3 score and serum PSA also improved the performance characteristics with a diagnostic sensitivity of 67.5%, specificity of 63.0%, PPV of 37.5%, NPV of 77.3%, and test efficiency of 64.4%. Incorporating patients' demographic and clinical characteristics did not significantly improve the performance of the combined biomarkers.

Conclusion: Our data suggest that PCA3 improves the diagnostic sensitivity and specificity and the combination of PCA3 with PSA gives a better overall performance characteristics in identification of PCa compared with serum PSA alone in high risk population. Implementing the urinary biomarker PCA3 together with serum PSA measurement into clinical practice would guide effective biopsy and lead to a considerable reduction of the number of unnecessary prostate biopsies.

A-009

Circulating plasma microRNAs as potential biomarkers for HCV related hepatocellular carcinoma in Egyptian patients

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Background: Circulating microRNAs are aberrant in cancer patients so the potential use of microRNAs (miRNAs) as ideal tumor markers has been the focus of recent research**.**

Objective: Our hypothesis was that circulating miRNAs are differentially expressed in pretherapeutic sera of HCV related hepatic cancer patients compared to controls and to HCV induced chronic liver disease to find out their ability in differentiate among them.

Materials and Methods: Two stages procedure, the first one aimed to determine which microRNAs are aberrant in HCV related hepatic cancer patients' pool (ten patients) compared to 10 normal donors' pool using Human Liver miFinder miRNA PCR Array. The second stage was done by using real-time quantitative polymerase chain reaction (qPCR) analysis, levels of six candidate miRNAs (miR-122-p5, miR-192-p5, miR-106b-p5, miR-34a-p5, miR-195-p5 and miR-199a-p5), which were chosen based on the previous miRNAs array step, were quantified in sera of 70 HCV related hepatic cancer patients, 50 HCV induced chronic liver disease and 50 healthy controls.

Results: Generally, increased expression levels of some microRNAs (miR-122-p5, miR-192-p5, miR-106b-p5 and miR-34a-p5) were noticed in HCC patients' pool as compared to healthy controls' pool while others (miR-195-p5 and miR-199a-p5) showed decreased expression levels. A diagnostic accuracy of a panel made of combination of 6-serum miRNAs which included in this study was evaluated , ROC curve showed that AUC was 0.990 (95% CI: 0.943 - 1.000, P <

0.001). In discrimination between study groups, this panel showed an excellent diagnostic performance with higher AUC as compared to each studied miRNA separately. When comparing between HCC patients and healthy controls the AUC of 6-serum miRNAs panel was 1.000 (95% CI: 0.951 - 1.000, P < 0.0001). The AUC was 0.977 when comparing between HCC and CLD patients (0.912 - 0.998) and was 0.924 when comparing between CLD patients and healthy controls. logistic regression was made to determine the best predictor miRNA considering P value < 0.05 a probability of entry. MiR-195 and miR-192 were the best predictors (p = 0.0155 and 0.0275 respectively). A second 2-miRNAs (miR-195/miR-192) panel was made and its diagnostic performance is evaluated. The ROC curve of the 2-miRNAs panel showed that AUC was 0.978 (995% CI: 0.925 - 0.997, P< 0.001). The 2-miRNAs panel was also excellent in discriminating between HCC patients and healthy controls (AUC=0.996, 95% CI: 0.942 - 1.000, P < 0.0001) and also between HCC and CLD patients (AUC=0.961, 95% CI: 0.886 - 0.992, P < 0.0001).

Conclusion: These findings suggest that systemic circulating miRNAs have potential use as novel biomarkers for diagnosis of HCV related hepatic cancer patients and at least five of them can be used as early diagnostic marker to differentiate between HCV related hepatic cancer patients and HCV induced chronic liver disease . However, future larger studies are needed to confirm our findings.

A-010

novel flowcytometry-based approach to detect tumor cells in body fluid using sysmex automated hematology analyzer

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Background: Nucleated cells differential analysis of body fluid (BF) samples is important diagnostic tool for several diseases including cancer metastasis. Detection

of tumor cells in BF requires the manual microscopic scanning of slides by the cytopathologist to visually identify cells with suspicious features, which is time-consuming and labor-intensive. Furthermore, the cytological examination of BF for detection of malignancy is not always reliable, because of a relatively low overall sensitivity rates (ranging 40-90%) with the higher false-negative rates for lymphomas and mesotheliomas. This study aimed to develop the scattergram gating analysis for detection of tumor cells in BF using the BF mode on the Sysmex automated hematology analyzer XN-1000 (XN BF mode; Sysmex, Kobe, Japan).

Methods: We used a total of 220 BF samples (53 cerebrospinal fluids, 73 pleural or ascitic fluids, and 94 peritoneal dialysis effluent) obtained from patients with cytological diagnoses (papanicolaou stain) including negative and positive for malignancy, and chronic inflammation with an elevated lymphocyte and histiocyte fractions. As a reference method, morphological manual differential (200 cells counts) was performed by two experienced technologists using cytospin slides stained with the May-Grunwald Giemsa. The gating criteria were based on the WDF scatter plots; #1: detect the cells with larger and clumped cell signal in comparison with general leukocytes, which mainly derived from clustered tumor cells, #2: to detect the middle sized mononuclear cells with less granules rather than neutrophils and similar fluorescence signal to monocytes, which targeting hematological malignant cells and solid tumor cells. BF samples that meet at least one criterion were interpreted as positive for tumor cells.

Results: The malignant BF samples containing tumor cells showed the different scattergram patterns from the benign ones with chronic inflammation. Our scattergram gating analysis achieved an overall sensitivity of 78.6% and specificity of 97.1% in detecting tumor cells positive samples when screening against all samples outcomes. The positive predictive value was 64.7% and the negative predictive value was 98.5%. For the samples of positive for malignancy and/or chronic inflammation (n=125) by morphological manual differential, the sensitivity and the specificity were 78.6% and 94.6%, respectively, with 64.7% of the positive predictive value and 97.2% of the negative predictive value. For the samples with absence of tumor cells and inflammatory observations (=95), no false positive was detected.

Conclusion: A simple measurement of BF by automated hematology analyzer in which cells are minimally handled has a potential to reduce costs and allow routine cell screening in clinical applications. For BF malignancy diagnostics, a scattergram gating analysis is promising to (i) augment diagnostic routines without requiring additional sample preparation procedure, (ii) limit operator bias, and (iii) provide a standardized measurement.

A-011

Development of a New Biochip Array for the Simultaneous Detection of Pepsinogen I, Pepsinogen II and Gastrin 17

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Background: Atrophic gastritis is a condition that is associated with a significantly higher risk of developing gastric cancer; the fifth most common cancer worldwide. Atrophic gastritis involves a loss in the gastric glands, affecting the secretion of pepsinogen II (PGII) from all areas of the stomach and pepsinogen I (PGI) and gastrin 17 (G17) more specifically from the corpus and antrum. During atrophic corpus gastritis, the levels of PGI in circulation are decreased. The ratio of PGI to PGII (which is produced by chief cells in the gastric mucosa) is also lowered. G17 is a crucial peptide hormone of the gastrointestinal tract and is secreted by the gastrin cells in the antrum. During antral atrophy the levels of G17 are ultimately decreased. These serum biomarkers therefore, are valuable in the screening of atrophic gastritis and can provide a comprehensive diagnosis on the condition of the stomach mucosa.

Enzyme-linked immunosorbent assays (ELISAs) have been developed for the single detection of PGI, PGII and G17 in serum and plasma (Biohit Oyj, Helsinki, Finland). Applying ELISA principles, Biochip Array Technology (BAT) allows the multiplex determination of analytes from a single sample. Therefore this collaborative study aimed to develop a biochip array for the simultaneous detection of PGI, PGII and G17 in serum/plasma in order to provide a patient profile to facilitate the non-invasive screening and diagnosis on the condition of stomach mucosa.

Methods: Simultaneous chemiluminescent sandwich immunoassays were employed, the anti-human capture antibodies were immobilised on the biochip surface defining discrete test sites. The immunoassays were applied to the Evidence Investigator analyser.

The multi-analyte calibrators were developed using native human antigen. A correlation study was carried out on a cohort of 76 serum/plasma samples using this biochip array and individual ELISAs (Biohit Oyj, Helsinki, Finland).

Results: Nine-point calibration curves for each individual analyte were simultaneously generated. The assay ranges were 0-200ng/mL for PGI, 0-50ng/mL for PGII and 0-40pmol/L for G17. Cross-reactivity testing demonstrated that each individual assay was specific for its target analyte (<1% cross-reactivity with the other analytes). When 76 serum/plasma samples were tested using BAT and individual ELISAS, the regression analysis showed the following values for the coefficient of

determination (r^2) and slope: PGI assay r^2=0.826, slope 0.7267; PGII assay r^2=0.9439, slope 0.929 and G17 assay r^2=0.9816, slope 1.068.

Conclusions: The results of this collaborative study indicate applicability of BAT to the simultaneous measurement of PGI, PGII and G17 from a single serum/plasma sample. Good agreement was found between this technology and individual ELISAs. The use of this biochip array facilitates the screening and diagnosis of patients at risk of developing gastric cancer and offers advantages over current diagnostic methods such as gastroscopy, which can be highly invasive and costly. This newly developed array uses low sample volume and will offer a cost effective and efficient method of testing for patients.

A-012

LncRNAs expression can distinguish B and T lineage acute lymphoblastic leukemias

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Background: In the late 1990s, gene expression profiling (GEP) based on microarray studies showed that different leukemia subtypes had distinct gene signatures. Microarray technology is not currently applied to leukemia diagnosis because of its high cost and need of deep technical expertise, but it provides a tool for biomarker discover and improvement of the knowledge of the cellular pathways deregulated in the disease. In a previous GEP study, we identified genes differently expressed in acute lymphoid and myeloid leukemias, some of which are long non-coding RNAs (lncRNAs). Recently, lncRNAs have been associated to cancer, including hematological malignancies, although evidences of their role in acute lymphoblastic leukemias (ALL) are still scarce. Objective: The aim of the present study was to investigate lncRNAs that can distinguish B lineage ALL from T lineage.Patients and Methods: 97 Brazilian patients diagnosed with ALL were included in this analysis. RNA was extracted from bone marrow samples obtained at diagnosis using PAXgene Bone Marrow RNA (QIAGEN/BD, Valencia, CA, USA). RNA integrity was determined on Bioanalyzer™ 2100 (Agilent TechnologiesPalo Alto, CA, USA). Gene expression was carried out using the Low Input Quick Amp Labeling kit - One Color and the Sure Print G3 Human GE 8 x 60K array (Agilent Technologies). Data were extracted with the Feature Extraction Software v7.5 and normalized using the Gene Spring software v12.5 (Agilent Technologies). From a set of 32,640 probes, 60 were selected in a supervised analysis using K-Nearest Neighbor (K-NM) algorithm, in order to build an effective classifier based on the differential gene expression signatures of B-ALL and T-ALL patients. We searched those probe´s sequences in genomic databanks, identified the ones that corresponded to lncRNAs and performed an unsupervised Hierarchical Clustering analysis with them to group ALL individuals with close gene expression patterns. Programs were run within a local installation of the GenePattern suite (Broad Institute). Differential expression was validated with TaqMan quantitative reverse transcription real time PCR assays (ThermoScientifc) probes. Results: We identified nine lncRNAs differentially expressed between B-ALL and T-ALL. Six lncRNAs were upregulated in T-ALL (NCBI Genbank sequences XR_248137, NR_034143, NR036502, NR036476, NR_104614, NR_015410), while three were down-regulated (NR_027406, NR_026779, NR_040662). None of them has been previously related to ALL, however, two were reported as associated to cancer: the NBAT1 (CASC14) that suppresses neuroblastoma growth and the CASC15 that is involved in melanoma progression. qRT-PCR confirmed microarray data in four out of the five markers tested ($p<0.05$). B-ALL and T-ALL patients could be clustered separately based solely on the expression data of these lncRNAs. Conclusion: lncRNAs have emerged as an important class of molecules that regulate cell processes. Our findings corroborate the hypothesis that these transcripts are relevant to the biology of acute lymphoblastic B and T leukemias. Besides their role as lineage biomarkers, lncRNAs should be investigated as potential targets for drug and therapy development.

A-013

EGFR analysis in cfDNA reflects tumor heterogeneity and has prognostic value in non-small cell lung cancer

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Background: Mutation analysis of epidermal growth factor receptor (*EGFR*) gene is essential for treatment selection in non-small cell lung cancer (NSCLC). Treatment with EGFR inhibitors is indicated for patients with EGFR activating mutations. Cell-free DNA (cfDNA) has been proposed as a less invasive and more informative alternative to tissue biopsy. We evaluated the clinical utility of *EGFR* mutation analysis in cfDNA from NSCLC patients by droplet digital PCR (ddPCR). Evaluated mutations were the two most prevalent EGFR activating mutations (L858R and delE746-A750) and T790M mutation, associated with resistance to treatment with EGFR inhibitors.**Methods:** We selected 36 NSCLC patients with *EGFR* activating mutations detected in cytological samples obtained by fine-needle aspiration and negative for T790M mutation, including 29 patients with advanced disease (63 ± 22 years, 69% female and 55% never smokers) and 7 patients with early stage NSCLC (64± 24 years, 43% female and 43% never smokers). Wild-type *EGFR* copies and mutated copies for L858R, delE746-A750 and T790M mutations were analyzed by ddPCR in a QX100 system (Bio-Rad) in cfDNA isolated from plasma at baseline and during treatment at best response, pre-progression and progression.

Results: First, we evaluated ddPCR sensitivity and found that for the three *EGFR* mutations, we could quantify maintaining linearity, mutated copies diluted as much as 0.005% in wild-type DNA. *EGFR* mutations were detected in basal cfDNA from 71% of advanced stage patients with positive cytological samples. Concordance between cytological samples and plasma results for EGFR activating mutations was 62%, being only significant for L858R mutation (87%, p=0.001). We detected in cfDNA mutations in patients with negative cytological samples: L858R in 12% of patients, delE746-A750 in 31% and T790M in 13%. We even detected *EGFR* double mutations in 17% of patients, which only presented one of them in cytological sample. Total *EGFR* copy levels in cfDNA in stage I patients were lower than in stage IV (1003 copies/mL versus 3523 copies/mL; $p<0.01$). Patients with basal concentration of *EGFR* activating mutations higher than 94 copies/mL had lower overall (317 versus 805 days; $p<0.05$) and progression free survival (195 versus 724 days; $p<0.05$) than those with lower levels. Similar findings were observed for total *EGFR* copy levels for a cut-off of 3462 copies/mL. Although we observed a decrease in EGFR activating mutations levels for between baseline and best response, this decrease did not reach significance. During follow-up, T790M was detected in 53% of patients. **Conclusion:***EGFR* cfDNA analysis by ddPCR seems a relevant tool for clinical management of NSCLC patients

A-014

A Laboratory Validation of the Dual Measurement of hCG and AFP in CSF

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Background: Primary central nervous system (CNS) germ cell tumors are a rare, heterogeneous and a diagnostically challenging group of neoplasms. The primary diagnosis of intracranial germ cell tumors can be aided by the analysis of cerebrospinal fluid (CSF) for increased concentrations of human chorionic gonadotropin (hCG) and α-fetoprotein (AFP). Following successful treatment new-onset elevations of hCG and AFP in CSF can precede radiologic or symptomatic tumor detection; however, the matrix effect on alternate sample types such as CSF should be taken into consideration prior to analysis as it can impact test results. Assays need to be validated before use in the clinical laboratory. Prior studies have validated measurements of total hCG and AFP in CSF on the Siemens Centaur; here we report on the validation of hCG and AFP assays in CSF on the Roche COBAS 6000 (Roche Diagnostics, Indianapolis, IN, USA). The objective of our study was to perform an in-house validation of total hCG and AFP concentrations in CSF on the Roche COBAS 6000.

Methods: Institutional Review Board approval was obtained prior to beginning the study. Validation testing was performed on remnant CSF sampled from physician-ordered clinical testing at Dartmouth-Hitchcock Medical Center. Serum samples with high AFP or hCG concentrations were used to spike aliquots of pooled CSF. Precision studies, linear range, limit of quantitation and carryover studies of the hCG and AFP assays on the COBAS 6000 analyzer were performed and data analyzed using available templates in EP Evaluator.

Results: Within day precision studies demonstrated acceptable imprecision of 2.4% and 4.7% for AFP concentrations of 12.0ng/mL and 602.8ng/mL and acceptable imprecision of 1.8% and 3.6% for hCG concentrations of 10.5mIU/mL and 118.8mIU/mL. Day-to-Day precision studies demonstrated acceptable imprecision of 5.6% and 3.7% for AFP concentrations of 11.6ng/mL and 538.6ng/mL and acceptable imprecision of 3.4% and 2.1% for hCG concentrations of 9.9mIU/mL and 110.5mIU/mL. The LOB, LOD and LOQ (10% CV) of hCG were 0.4 mIU/mL, 0.8 mIU/mL and 0.8 mIU/mL, respectively. The Linear Range for the hCG assay was established as 1.0 to 10,000 mIU/mL. The LOB, LOD and LOQ (20% CV) of AFP were 1.1 ng/ml, 1.6 ng/ml and 1.6 ng/ml, respectively. The Linear Range for the AFP assay was established to be 1.6 to 1100 ng/mL. The recovery experiment demonstrated no appreciable matrix effect with AFP and hCG recovery differing less than 10% of the target concentration, with the exception of the level one AFP sample. This sample demonstrated over-recovery of 18%; however this over-recovery was deemed analytically acceptable and likely due to a combination of clearer CSF matrix and assay imprecision.

Conclusion: The Roche COBAS 6000 total hCG and AFP assay can accurately quantify hCG and AFP in CSF facilitating the rapid and accurate diagnosis and monitoring of germ cell tumors.

A-015

Detection and characterization of serum free light chains by MALDI-TOF MS in immunofixation electrophoresis-negative specimens with abnormal free light chain ratios

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Background: Monoclonal free light chains (FLCs) play an important supportive role in diagnosis, prognosis, and monitoring of monoclonal gammopathies. FLCs are secreted in larger quantities by abnormal plasma cells undergoing clonal expansion. Quantitative immuno-nephelometric serum FLC (sFLC) assays are used to measure concentrations of circulating kappa (K) and lambda (L) chains unbound to their heavy chains. An abnormal K/L FLC ratio can indicate a low abundance monoclonal clone which is typically undetected by serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Patients with nonsecretory multiple myeloma, light chain multiple myeloma, primary systemic amyloidosis and light chain deposition disease often are only detected by the FLC ratio. On the other hand, hypergammaglobulinemia patients can also present with abnormal FLC ratios. There is a need to directly detect monoclonal FLCs independent of the K/L ratio. Recently, we have developed a sensitive technique that uses nanobody enrichment-coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for detection of monoclonal proteins in serum.

Objective: The objective of this study was to evaluate the ability of a MALDI-TOF MS method to detect monoclonal FLCs in IFE-negative patient sera with abnormal K/L FLC ratios.

Methods: Residual patient serum specimens (n=48) that were negative by IFE and displayed an abnormal FLC ratio (K/L <0.26 or >1.65) were collected for analysis. Nanobody enrichment was performed with CaptureSelect™ affinity resins to purify IgG, IgM, IgA, K light chains and L light chains. Specimens were reduced to dissociate heavy and light chains. Additionally, FLCs were affinity purified with sepharose beads conjugated with polyclonal antibodies used for the sFLC assay (n=31), which have been shown to have high specificity towards FLCs and low cross-reactivity with light chains bound to heavy chains. Purified specimens were subjected to MALDI-TOF MS in automated acquisition mode (Bruker Microflex). FlexAnalysis software was used to interrogate spectra for isotypes and the molecular masses of monoclonal proteins.

Results: Monoclonal abnormalities were detected in 14 (29%) of nanobody-purified serum samples analyzed by MALDI-TOF MS, thereby supporting positivity by the sFLC assay. These included monoclonal GK isotypes (n=7), free K (n=3), AK (n=2), GK and K FLC (n=1), and a GL (n=1) of varying molecular masses. Seven of these specimens had evidence of monoclonal FLC proteins based on sFLC antibody purification spectra. Interestingly, all three specimens of free K isotype had FLC ratios greater than 100. FLCs were detected in nine additional specimens purified with sFLC assay antibodies. FLC ratios of specimens with detectable FLC were significantly higher than those without (p-value=0.0002, Mann Whitney U test). The remaining specimens (46%) did not harbor any detectable monoclonal protein abnormalities according to MALDI-TOF MS, thereby, corroborating the negative IFE.

Conclusion: MALDI-TOF MS is a rapid monoclonal immunoglobulin isotyping method that generates information rich spectra. This study suggests that IFE-negative serum with abnormal K/L FLC ratios may harbor monoclonal abnormalities undetectable by current routine laboratory methods. Further work is underway to corroborate these findings.

A-016

Two missense mutations in a female patient with a strong familial history of breast cancer: A case report

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Background: Breast Cancer is the most common cancer in women and is the first leading cause of women cancer death in developing countries. Death rates have been declining and it is believed that this decrease is result of earlier detection through increased awareness, screening and improved treatment. Around 5-10% of these tumors present a strong hereditary component due to mutations in highly penetrant genes. *BRCA1* and *BRCA2* are the two most frequently mutated genes and account for up to 45% hereditary breast cancers. Recent advances in sequencing technologies allowed the discovery of several novel genes related to breast cancer increased risk, such as *PALB2*. **Case report:** This report describes a 59-year-old female who was diagnosed with invasive breast cancer at age 38. She presented an important familial history of different types of cancers on both sides of the family. On her mother's side, five cases of breast cancer (three female cousins, one aunt and one great-aunt), one case of ovarian cancer (the same great-aunt with breast cancer), one grandmother with uterine cancer and one male cousin with stomach cancer. On her father's side, only one uncle with bone cancer. Since she was *BRCA* mutation negative, breast cancer screening was done by next generation sequencing in 15 related genes: *ATM, BARD1, BRIP1, CHEK2, CDH1, MRE11A, NBN, PTEN, PALB2, RAD50, RAD51C, RAD51D, STK11, TP53,*and *XRC22*. Two heterozygous mutations were identified: a known benign mutation in exon nine of the *PALB2* gene (rs45551636: c.2993G>A; p.G998E) and a previously undescribed mutation in exon nine of *MRE11A* gene (c.908C>T; p.T303I). Prediction programs SIFT and PolyPhen-2 classified this mutation as possibly damaging. Onlineprediction program Mutation Taster suggested that this variant is a disease causing mutation with a probability value of 0.999 since the protein structure might be affected due to splicing changes. In addition, this is a variant of unknown clinical significance (VUS) and need to be further investigated. We considered as VUS undescribed missense mutations or described variants with minor allele frequency (MAF) <0.02.**Conclusions:** Breast cancer increased risk is linked to genetic factors and shared lifestyle factors. Genetic screening of these novel susceptibility genes in families with a strong history of the disease is of utmost importance for clinical diagnosis, appropriate treatment, prophylactic interventions and genetic counseling, since it significantly impact patient's and family member'swell-being and survival.

A-017

Evaluation of the Immunoassay Reagent Kit for PIVKA-II (ARCHITECT® PIVKA-II) for the Fully-Automated Chemiluminescent ARCHITECT Analyzer

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Background: Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is used as an aid in the diagnosis of hepatocellular cancer (HCC), in monitoring of high risk patients for development of HCC, and in the management of HCC. The goal of this study was to evaluate the analytical performance of the ARCHITECT PIVKA-II assay.

Methods: The ARCHITECT PIVKA-II assay is a quantitative two-step, double monoclonal antibody sandwich assay (3C10 and MCA1-8), for the fully automated chemiluminescent ARCHITECT *i* Systems analyzer. This assay has an assay time of approximately 29 minutes and an analytical range of 0.00 to 30,000.00 mAU/mL. Precision was performed based on guidance from National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-A2. The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) was performed based on guidance from CLSI Document EP17-A2. Linearity was performed based on guidance from NCCLS Document EP6-A. Potential interferents were performed based on guidance from CLSI Document EP7-A2. Correlations to Picolumi and Lumipulse PIVKA-II assays were performed with samples sourced from European and USA sites.

Results: The within-run and total imprecision showed %CVs of 1.9-5.0 and 2.0-8.6 over the range of 38.55 to 26880.54 mAU/mL. The LoB, LoD and LoQ ranged from 0.45 to 0.64, from 1.05 to 1.45 and from 4.93 to 5.06 mAU/mL. The assay is linear up to 30,000.00 mAU/mL. There were no differences between sample types and no interference of common drugs and endogenous substances was observed. The correlation between the Picolumi PIVKA-II and the ARCHITECT PIVKA-II was 1.03 for the regression slope and 1.00 for the Spearman's correlation coefficient. The correlation between the Lumipulse PIVKA-II and the ARCHITECT PIVKA-II was 1.07 for the regression slope and 0.98 for the Spearman's correlation coefficient.

Conclusion: The ARCHITECT PIVKA-II assay demonstrated good analytical performance and compared well with other on-market assays. The ARCHITECT PIVKA-II assay is a convenient fully automated assay with high throughput (200 tests/hour) without the pretreatment of specimens.

A-018

Comparison of Freelite™, N Latex and Luminex serum free light chain assays in subjects with end stage renal disease on haemodialysis

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BACKGROUND: Quantification of serum free light chains (FLC) is important in the diagnosis of plasma cell diseases where abnormal kappa:lambda FLC ratio infers a population of monoclonal plasma cells. Whereas the Freelite™ assay uses a separate renal range for the kappa:lambda ratio compared to the normal population range, N Latex does not require a different range. A third FLC assay based on a multiplex bead array assay (Luminex) and using anti-FLC monoclonal antibodies was compared to Freelite™ and N Latex assay in an end stage renal disease population on haemodialysis.

METHODS: We completed a cross-sectional study comparing the performance of three FLC assays on 104 haemodialysis patients without paraproteinaemia. We quantified FLC pre- and post-dialysis using both the Freelite™, N Latex and Luminex assays.

RESULTS: FLC concentrations were elevated by all assays for pre-dialysis samples. Median kappa FLC was lower by Luminex (77 mg/L) compared to Freelite™ (155 mg/L) and N Latex (130 mg/L). Median lambda FLC was more than 2-fold higher by N Latex (250 mg/L) compared to Freelite™ (110 mg/L) and Luminex (95 mg/L). Of the 104 samples tested pre-dialysis, kappa:lambda FLC ratio was elevated in 29 by Freelite™ (diagnostic range 0.26-1.65), in 2 by Luminex (0.40-1.59) and none by N Latex (0.31-1.56). Only one ratio was above the Freelite™ renal range (0.37-3.1).

Correlation between assays for both pre- and post-dialysis samples was better for kappa FLC (R value 0.869 to 0.939) compared to lambda FLC (R value 0.750 to 0.864). Mean difference for lambda FLC post-dialysis decreased from 140 mg/L (95% C.I. 128-152) to 21 mg/L (95% C.I. 14.8-27.3) for Freelite™ versus N Latex, from 156 mg/L (95% C.I. 142-170) to 59.5 mg/L (95% C.I. 51.6-67.4) for Luminex versus N Latex, and increased from 16.1 mg/L (95% C.I. 8.0-24.2) to 38.5 mg/L (95% C.I. 31.7-45.2) for Luminex versus Freelite™ assay comparisons. Mean differences between assays were minimal for kappa FLC post-dialysis.

Post-dialysis median FLC concentrations decreased for all assays but remained elevated above the reference limit for a normal population. Median kappa FLC decreased 58% for Luminex, 68% for Freelite™, and 66% for N Latex. Median lambda FLC decreased 63% for Luminex, 36% for Freelite™, and 63% for N Latex.

CONCLUSIONS: Significant differences in FLC concentration existed between the three assays in an end stage renal disease population, pre-dialysis. This affected the kappa:lambda ratio, which was lowest by N Latex and highest by Freelite™. Markedly elevated lambda FLC contributed to the low N Latex ratios. Clearance of FLC by dialysis reduced kappa FLC by two-thirds in all assays and lambda FLC by a similar amount in N Latex and Luminex assays compared to just over a third reduction of lambda FLC by Freelite™. This difference in clearance of lambda FLC by Freelite™ possibly reflects a difference in antibody reactivity of various molecular forms of lambda FLC that may be present in renal disease.

A-019

circulating free DNA assessment of prognostic biomarkers in prostate cancer.

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Background: Cell-free DNA (cfDNA) is of crucial interest in oncology. Several studies have shown the potential role of cfDNA levels in the prognostic assessment of different solid malignancies. However, the quantification of pure cfDNA is a prerequisite for a reliable genotype analysis focused on the detection of cancer-specific DNA mutations signatures and/or epigenetic modifications. In the present study, the quality and quantity of the cfDNA were assessed by two different quantification procedures, Qubit 2.0 and Nanodrop fluorometer measurements, in order to identify the best and most cost-effective procedure in relation to the tumour stage. Further aim of this study was to evaluate the total cfDNA level and the cancer-specific DNA mutations as prognostic biomarkers in prostate cancer patients. **Methods:** We collected blood samples of patients affected by prostate cancer and healthy individuals. Blood samples were collected at the diagnosis of advanced cancer confirmed by biopsy, and at 6 and 12 months following the diagnosis. Blood collected was processed within one hour and frozen at - 80°C; cfDNA was extracted from plasma through Qiagen kit and Promega automatic extractor. Qubit 2.0 and Nanodrop were applied for measurements of total amount cfDNA before qPCR quantification performed targeting of the single copy gene *APP*. Methylated *GSTP1* and *RASSF1A* tumour specific cfDNA markers were determined in patients with prostate cancer. **Results:** A total of 25 prostate cancer patients and 30 aged matched healthy controls were evaluated. Automated DNA extractions resulted to be more accurate and cost-effective than the manual procedures. The pre-PCR quantification by Qubit and Nanodrop measurements revealed differences between the two procedures, highlighting the highest sensitivity of Qubit in the detection of small amounts of pure double strand cfDNA. On the other hand, Nanodrop spectrophotometric measurements showed to be more apt to perform quality and purity assessment of extracted DNA. Concerning the cfDNA levels in our cancer patients, preliminary data showed that patients with high cfDNA concentration at baseline had worse disease free time and overall survival, in comparison to those with a lower concentration. **Conclusions:** The automated cfDNA extraction associated to the quantification by Qubit 2.0 seems to be the best approach to quantify the patient's cancer-specific DNA mutations by qPCR assay. The spectrophotometric Nanodrop approach could be used for the evaluation of plasma samples with potentially higher cfDNA quantity in advanced cancer patients. The combination of multiple mutational/methylation and distinctive antigenic cancer biomarkers including prostatic markers is suitable to determine the total amount of cfDNA in prostate cancer patients. Cancer progression correlates with the changes in the level of cfDNA in plasma. Therefore cfDNA detection can be used as a prognostic and predictive tool for the stratification, the clinical management and the follow-up of patients with malignant melanoma and prostate cancer.

A-020

Combination of hK2, CCL11 and PSA in prognosis of prostate cancer patients

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Backgrounds: Human glandular kallikrein 2 (hK2) and prostate specific antigen (PSA or hk3) are members of kallikrein family produced by prostatic epithelium. While the production of hK2 is often increased in early stages and/or less well differentiated prostate cancer (PCa), PSA showed a decreased production. CCL11, a member of chemokines, plays an important role in regulation of tumor growth, progression and metastasis. The screening test need to be high sensitive to detect patients with early-stage disease with a sufficient specificity to prevent false-positive patients from undergoing invasive and unwarranted diagnostic evaluations. Although PSA is the best and most sensitive available screening test for PCa, there is a large overlap between PCa and benign prostatic hyperplasia (BPH) especially with PSA range 4-10 ng/ml which provoke the necessity to reveal, validate and advocate potential adjunct markers to improve PCa specificity with respect to sensitive detection. **Objective:** This study aimed to explore the diagnostic and prognostic value of hK2, CCL11 and PSA combination to improve the overall value of sensitivity, specificity and diagnostic accuracy of PCa patients. **Patients and Methods:** This study included 64 newly diagnosed PCa patients, 72 BPH and 65 apparently healthy men with matched age. Digital rectal examination (DRE) and transrectal ultrasound (TRUS) guided biopsy with volume measurements of the prostate were performed for all PCa patients. Clinical and pathologic distribution of stages were found 70.3% T2 (n=45), 21.9% T3 (n=14) and 7.8% T4 (n=5). Stages T3 and T4 were combined as advanced PCa group and compared with T2 (early PCa) to validate each biomarker in identifying early stage disease. Serum samples were collected from all patients after at least one-week gap following DRE and prior to any prostate biopsy. Serum levels of hK2, CCL11, tPSA and f/tPSA were measured. Validity (sensitivity and specificity) of each biomarker and their combination as well as possible association between parameters were assessed by analysis of ROC curve. **Results:** PSA had a sensitivity of 82% at 77% specificity with a diagnostic accuracy of 81% while f/tPSA ratio attained a sensitivity, specificity and accuracy of 81%, 82% and 85% respectively. Serum hK2 and CCL11 levels differentiated significantly among PCa, BPH and control groups ($p<0.05$). Although hk2 and CCL11 had no statistical differences among PCa stages ($p>0.05$), their ratios with tPSA significantly differentiated between early and advanced PCa stages ($p<0.05$) with a sensitivity, specificity and accuracy of (69%, 71%, 80%) and (88%, 82%, 85%) respectively. Combination of hK2, CCL11 and f/tPSA ratio seems to improve the overall value of sensitivity, specificity and diagnostic accuracy to 93%, 84% and 88% respectively. **Conclusion:** hK2 and CCL11 may provide a useful diagnostic information helping distinguish between BPH and PCa. Combined use of these biomarkers with the standard ones, PSA, can improve the overall value of sensitivity, specificity as well as accuracy of PCa especially in PSA overlap zone eventually sparing unnecessary prostate biopsies. However, larger prospective studies are warranted to validate the diagnostic value of combining these markers.

A-021

Quantification of Death Receptors as Tumor Markers for the Prediction of TRAIL Sensitivity by Flow Cytometry

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Background: The clinical evaluation and classification of biopsied tumors for genotypic and phenotypic characteristics allows for the application of appropriate anti-cancer treatments for specific tumor types. Personalized cancer treatments are critical to tailor therapies to individuals resulting in more efficacious therapies while minimizing negative side effects. Individualized cancer plans can be applied to the utilization of death ligand Tumor necrosis factor-Related Apoptosis Inducing Ligand (TRAIL) as an anti-cancer drug. Administration of TRAIL has been shown to be highly effective in selectively killing cancer cells. The selectivity of TRAIL is through its interaction with extracellular death receptors (DR), DR4 and DR5, the binding to which ultimately leads to apoptosis or programmed cell death in cancer cells. In Phase I studies, TRAIL was found to be safe and well-tolerated, however, Phase II studies only show a small cohort of patients responded to TRAIL therapy while others displayed resistance. Consequently, we propose the pre-treatment analysis of biopsied tumors for expression of death receptors to determine patient's suitability for TRAIL treatment.

Methods: An analytical flow cytometry method is described to determine tumor expression of death receptors and predict an individual's sensitivity to TRAIL. We employed various established malignant melanoma cell lines that have been reported to show varied sensitivity to TRAIL. Cells were stained with saturating amounts of anti-human CD261 (DR4) and anti-human CD262 (DR5) conjugated to phycoerythrin (PE) along with an IgG1κ isotype antibody and analyzed on the BDFACS Canto II using FACS Diva software. Additionally, we measured the degree of apoptosis experienced by the malignant melanomas in response to TRAIL treatment. Post treatment cells were collected and stained with FITC-AnnexinV and Propidium Iodide (PI) and analyzed by FACS.

Results: Three malignant melanoma cell lines, A375, MeWo and WM164 were analyzed for membrane expression of DR4 and DR5. Cell line A375 had the highest expression of both DR4 and DR5 with a mean fluorescence intensity (MFI) of 42.0±2.7 (n=9) and 1958.0±25.9 (n=9), respectively. Compared to A375, MeWo and WM164 had significantly less expression of DR4 with a MFI of 15.7±3.6 (n=9) and 18.0±1.4 (n=9), respectively, and DR5 with a MFI of 928.4±47.0 (n=9) and 552.7±10.8 (n=9), respectively. Cell line A375 was the most sensitive to TRAIL-induced apoptosis. Treatment with 50 ng/ml TRAIL resulted in the apoptosis in 30.7%±0.6 (n=9) of the cells, whereas, MeWo and WM164 were highly resistant to TRAIL and there was no significant induction of apoptosis.

Conclusion: A flow cytometry technique is proposed to measure DR expression of biopsied tumors to predict their sensitivity to TRAIL-induced apoptosis. Preliminary results show a direct correlation between DR expression and sensitivity to TRAIL-induced apoptosis as TRAIL-resistant cells lines, MeWo and WM164 had 59.9%±4.5 (n=18) less DR4 and 62.2%±2.6 (n=18) less DR5 membrane expression compared to the TRAIL-sensitive cell line, A375. These data provide the rationale for personalized cancer treatments and the analysis of biopsied tumor pre-treatment to determine the best anti-cancer therapy that will be most effective and possess minimal side effects.

A-025

LINE_1 Open Reading Frame 1 (ORF1) Protein Concentrations in Men with Prostate Cancer

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Background: Retrotransposones are repetitive DNA sequences capable of copying and moving themselves and other sequences to new location throughout the genome. Long interspersed nuclear element 1 (LINE1) is the most abundant and active group of these retrotransposons. Methylation of the LINE1 promoter regulates its activation and the generation of ORF1 and ORF2 proteins. LINE1 hyper-activation has been demonstrated in many types of cancer including, colon, prostate, lung and breast. We investigated whether L1 ORF1 concentrations in serum from men with borderline PSA concentrations were different based on the clinical decision of performing a biopsy or not. We further compared L1 ORF1 values in men who had undergone biopsy and had confirmed cancer versus those who did not have malignancy.

Methods: Remnant serum samples from 63 men >50-year-old with clinically measured PSA values of 4 -14 ng/mL (borderline) were included in this study. The clinical decision of having biopsy (n=20) or not (n=43) had been made during their routine clinical workup. Of the 20 biopsied subjects, 9 had confirmed prostate cancer, one had suspected atypia, 7 had no abnormality, and 3 had prostatitis. In order to measure ORF1 protein concentration in serum, we used an in-house competitive ELISA using a custom-made antibody against the select amino acid sequence of ORF1 protein. The same peptide sequence was coupled with biotin and used as anchor in streptavidin coated 96-well plates. Then, a secondary antibody (GAR-HRP) and colorimetric substrate were used to generate a blue color. Absorbance values at 450 nm were measured and patient sample concentrations were calculated based on a logistic 4-parameter standard curve generated from calibrators of known ORF1 concentrations.

Results: The mean ORF-1 protein concentration in biopsied and non-biopsy group were 26.60 (SD=14.9) and 16.40 (SD=9.2) ng/ml respectively. The difference between these groups was significant (P <0.003). The mean ORF-1 protein concentration in biopsy-confirmed prostate cancer subjects (n = 8) was 33.7 (SD=15) ng/ml, and 23.7 (SD=10) in biopsy-confirmed normal subjects (n=7). ORF1 protein concentration in biopsy-confirmed cancer subjects was significantly higher than non-biopsy group (P <0.001). **Conclusion:** ORF1 is a novel biomarker of cancer that can potentially serve as an aid in making the decision of whether a man with borderline PSA values should undergo biopsy or not. More data is required to confirm this finding as well as to the effect of smoking status of the subject at the time of sample collection.

A-026

Mutation spectrum and frequencies of *BRCA1* and *BRCA2* genes among 1,011 Brazilian patients

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Background: Breast cancer is the most common cause of mortality among women worldwide and this rate has increased rapidly in all countries. Around 10% of women who are diagnosed with breast cancer report a family history of the condition and a significant proportion of individuals with hereditary breast cancer have mutations in *BRCA1* and *BRCA2* genes. Several studies of *BRCA* mutations spectrum and frequencies have been done in populations from distinctive geographic regions. However, most are restricted to North America and Europe and studies in Brazil are scarce and restricted to a few reports with limited sample sizes. **Objective:** In this context, the aim of this study was to survey the spectrum and prevalence of *BRCA* mutations in Brazilian patients with breast/ovarian cancers or with family history of the condition, based on mutation analysis of DNA. **Methods:** Coding and flanking regions of the *BRCA1/2* genes from 1,011 patients were sequenced by next generation sequencing at Progenética Laboratory/ Hermes Pardini Institute. Patients come from different regions of the country and have anaverageage of 45.74 (range 17-84 years).**Results and Discussion:** We identified a total of 259 mutations: 115 in *BRCA1* and 144 in *BRCA2* gene. In *BRCA1*, 80 mutations were classified as pathogenic mutation, five as probably pathogenic and 29 as variants of unknown clinical significance (VUS).For *BRCA2*, 52 were classified as pathogenic, 11 as probably pathogenic and 76 as VUS. Overall, 15% of patients (148/1011) harbored a pathogenic/ probably pathogenic mutation in these genes, 57% of them in BRCA1 and 43% in BRCA2. This data is consistent with previously observed frequency mutation in BRCA genes for the Brazilian population, since *BRCA1* presents a higher mutation rate than *BRCA2*. Among 115 mutations in *BRCA1*, we observed 63 distinct mutations and 16 were identified more than once, including 27 women that carried the mutation 5382insC (the most common mutation identified worldwide and found at Ashkenazi Jews),nine with the 3450del4 mutation and five with 917delTT mutation, strengthening the relevance of these mutations at Brazilian population. For *BRCA2*, 92 mutations were identified once and 52 were identified more than once, which the more frequent one, rs80358547 (c.2T>G), was identified in nine patients.We were able to identify 61 previously undescribed mutations, 17 at *BRCA1* (threepathogenic and threeprobably pathogenic) and 44 at *BRCA2* (11 pathogenic and nine probably pathogenic). **Conclusion:** These results reveal the importance of this kind of survey to discovery new pathologic variants at countrieswith high rate of miscegenation and with restricted mutational information of *BRCA1/2*. Furthermore, knowledge of the proportion of normal individuals and cancer patients carrying mutations and the frequency of these mutations are needed for determining relevant mutations for the prevalence of hereditary breast cancer in the population studied, as well as defining public health and screening strategies for these patients. With these data,we will be able to offer a better genetic screening and counseling of patients with breast cancer and/or a family history for the disease.

A-027

Brazilian women with double heterozygosity for *BRCA1* and *BRCA2* mutations: two rare case reports

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Background: Breast Cancer is the most common cancer in women worldwide. Around 5-10% of these tumors are a result of mutations in *BRCA1* (MIN #113705) and *BRCA2* (MIN #600185) genes. Since *BRCA1/2* are associated with a large number of DNA repair pathways, mutations in these genes increase the probability to develop genetic alterations that can lead to malignant transformation and cancer. Among general population, the mutation frequency of these genes is very low (0.2%). However, it is higher in high-risk families, reaching until 20%. In recent years, several families have been described with more than one *BRCA* mutation. Nevertheless, the identification of individuals with two independent mutations in both genes is still rare. Only few individuals or families have been reported to have more than one non-Ashkenazi *BRCA* mutation. **Objective:** This report describes two independent cases of two non-Ashkenazi mutations in each BRCA1 and BRCA2 genes by next-generation sequencing (NGS) analysis. **Methods:** Genomic DNA was extracted from blood samples of the patients, using DNA micro kit (Qiagen). Mutation screening in the entire coding regions of BRCA1/2 genes was carried out on the Ion Torrent PGM™ sequencer. All procedures for library preparation, emulsion PCR and next-generation sequencing were performed with Ion Torrent equipment and Ion Torrent kits following the manufacturer's instructions. The sequence data were processed using standard Ion Torrent Suite™ Software running on the Torrent Server. Reads were aligned to the human genome reference (hg19/GRCh37) and variant calling were performed by Ion Reporter™ Software. Mutations were also validated by conventional Sanger sequencing. **Results and Discussion:** In a 49-year-old woman, we detected two independent deletions resulting in frame shifts: a *BRCA1* mutation in exon 11 (c.798_799delTT/p.V266fs/ rs80357724) and a *BRCA2* mutation in exon 11 (c.2808_2811delACAA/ p.K936fs/ rs80359352).In a 48-year-old woman, we identified a *BRCA1* mutation in intron 13 (c.4357+1G>A/IVS13+1G>A/ rs80358027) and a *BRCA2* mutation in exon 11 (c.6402_6406delTAACT/ p.N2134fs/rs80359584). All these mutations have been reported previously and classified as pathogenic. Since these mutations have a very low mutation frequency, the identification of two of these mutations in the same individual has never been described before. **Conclusion:** These findings reinforce the recommendation for mutation screening in both BRCA genes into clinical practice, avoiding misleading caused when only a familial mutation or a single gene is tested. In these cases, relatives of these patients could be falsely reassured if only one mutation or gene is excluded while an unrecognized one could still be present.

A-028

Prevalence of *EGFR* mutation in non-small cell lung cancer patients from Brazil: A personalized medicine for EGFR-TKI treatment

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Background: Lung cancer is the leading cause of both new cancer diagnoses and cancer-related deaths all over the world and approximately 85% of this kind of cancers are diagnosed as non-small cell lung cancer (NSCLC). Clinically, most of NSCLC patients are diagnosed at the advanced stages of disease, leading to a short survival. However, target therapy recently has achieved promising successes in NSCLC patients harboring Epidermal Growth Factor Receptor (EGFR) active mutations. Tyrosine kinase inhibitors (TKIs) can inhibit the EGFR TK domain reversibly through competitive binding with ATP. In this way, TKI shave been used to treat cancers harboring *EGFR* mutations or aberrant activation of EGFR, significantly prolonging patients's survival. For EGFR-TKI therapy, EGFR mutations need to be first detected in patients to allow oncologists to decide which first-line treatment should be offered to improve the efficacy of the treatment. **Objective:** In this context, the objective of this study was to survey the spectrum and prevalence of *EGFR* mutations identified in genomic DNA samples obtained from tumor tissues, using Cobas® EGFR Mutation Test real time PCR. This study was conducted in the Progenética Laboratory/ Hermes Pardini Institute. **Results and Discussion:** This study involved a total of 2,009 patients, with mean age of 64.8 years (range 52 until 77), with53% women and 47% men. Ten percent of the patients showed invalid results and these samples were associated with poor DNA quality and/or quantity (p>0,01).From the remaining patients, we identified mutations at 432(24%).A total of 416 patients presented only one mutation in *EFGR* gene and 16 were double mutant. Mutations in exon 19 (19Del) and exon 21 (L858R) were the most frequent, accounting for 53.7% and 32.9%, respectively. According to the literature, these mutations account for up to 90% of all *EGFR* mutations. Other concordant result was the prevalence of *EGFR* mutations in women, representing 64% of the mutated patients (p>0.0001). The mutations associated with TKIs resistance (S768I e T790M) were identified in 4% of the patients and in most cases (75%) they were associated with a sensitivity mutation (19Del or L858R). **Conclusion:** In conclusion, this work revealed similar results for mutation prevalence and spectrum with other previously analyzed European and American populations. Additionally, our results regarding the influence of DNA quantity and quality in obtaining conclusive test results reinforce the need of proper pre-analytical sample handling for paraffin-embedded tissue, especially when a limited tissue size is available. A recent alternative standing emerging is the utilization of circulating free DNA (cf-DNA) in the blood originating from tumor lesions, as surrogate sample for detecting *EGFR* mutations. This alternative is a less invasive source for obtaining genomic samples than surgical biopsy.

A-029

Light Chain Escape: A infrequent relapse in Multiple Myeloma

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Background: Free light chain escape (FLC escape) is defined as an increase in monoclonal serum free light chain (sFLC) without a corresponding increase in monoclonal intact immunoglobulins in a patient diagnosed with intact inmunoglobulin Multiple Myeloma (IIMM). The frequency at which IIMM patients relapse with FLC escape may be as high as 20%. We reported the clinical case of a patient with an IIMM where the serial measurement of sFLC after autologous stem cell transplantation (ASCT) allow us to detect a relapse as a FLC escape.

Case presentation: A 64 years old man was diagnosed with IgA Kappa MM in ISS stage 3. At diagnosis, he presented a monoclonal protein of 1,90 g/dL detected by serum protein electrophoresis (SPE) which was typed by serum immunofixation (IFE) as IgA Kappa. The sFLC levels were 510.5 mg/L for kappa and 13.8 mg/L for lambda with a ratio of 36.9. The Bence Jones proteinuria (BJP) was positive for kappa. A bone marrow biopsy showed a 20% of plasma cells and multiple lesions in axial skeleton and skull were found by PET/CT. After induction therapy with 6 cycles of VD (bortezomib and dexamethasone) he received an ASCT and achieved a status of complete response with negative IFE, 0.1% of plasma cells in bone marrow, an abnormal sFLC ratio of 9.5 (kappa=11.5 mg/L and lambda 1.2 mg/L) and the presence of a selective IgA deficiency (<10 mg/dL). Two months after ASCT, sFLC kappa began to increase with levels of 33 mg/L (ratio of 14.7) and normal SPE and BJP. Five months after ASCT, sFLC kappa levels were 150 mg/L (ratio of 57) with negative SPE and BJP. The sFLC kappa rapidly increased to a maximum of 21.282 mg/L (ratio 41.7) with negative SPE at seventh month after ASCT. Only sFLC kappa was identified by IFE with absence of IgA and the selective IgA deficiency was persistent since ASCT. A biological relapse was documented consistent with FLC escape. At this time, the bone marrow plasma cells were 42% and a PET/CT was performed showing a hypermetabolic focus confirming a clinical relapse. The patient began treatment with lenalidomide, dexamethasone and clarithromycin. After first and second cycles, sFLC kappa levels decreased to 1121 mg/L (ratio of 124.6 mg/L) and 379.9 mg/L (ratio of 61.8), respectively but the patient presented a severe thrombocytopenia during this period with adverse outcome.

Conclusion: In patients with IIMM that achieved a status of remission is very important the periodic assessment of sFLC to detect early if a FLC happens. Without the quantification of sFLC levels after remission or ASCT, the FLC escape couldn´t be detected and therefore the relapse of the disease. Furthermore, this case is an example of the clonal heterogeneity in MM with different clones at diagnosis (IgA Kappa) and relapse (Kappa) due to the different sensitivity of clones to the treatments, remaining the more resistant clones.

A-030

Improving quantification of M-protein Using Capillary Electrophoresis Immunosubtraction

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Background:

M-protein quantification is routinely performed by demarcating serum protein electrophoresis (SPE) regions. However, quantification of beta-migrating M-proteins is difficult due to overlapping non-immunoglobulin proteins. Therefore, some groups using techniques that do not separate beta-1 and beta-2 regions only quantify symmetric beta-migrating M-proteins > 2 g/dL. For this reason, recent guidelines have recommended following beta-migrating IgA M-proteins with total IgA levels. Immunosubtraction on capillary zone electrophoresis is a method currently used qualitatively to subtract out (and therefore highlight) immunoglobulins in serum, thus reducing the masking effect of normal serum proteins. This study expands on traditional immunosubtraction by developing a quantitative immmunosubtraction (qIS) suitable for measuring beta-migrating M-proteins as low as 0.1 g/dL.

Methods:

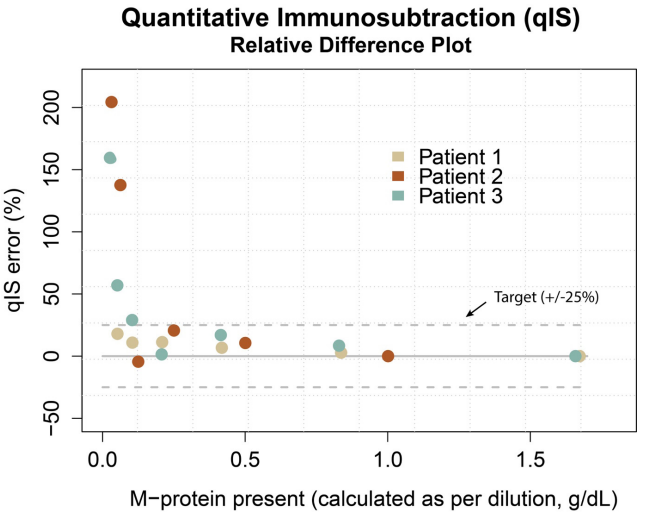
qIS is achieved by quantifying the subclass-specific immunoglobulin contribution to the SPE region containing the M-protein. We performed a comparison study with serial dilutions from three patients with beta-region M-proteins > 1 g/dL (n=20) as measured by SPE. We performed SPE and immunosubtraction on each dilution with the Sebia Capillarys 2™. Capillarys 2 immunosubtraction produces only qualitative traces. To quantify, we used traditional SPE analysis to calculate protein concentration in a region including both M-protein and normal protein. We then imported immunosubtraction images into Image J™ and performed region of interest analysis to calculate the involved immunoglobulin subclass contribution to the SPE region. In this way, we quantified pure immunoglobulin concentrations within a band of restriction without contamination by non-immunoglobulin proteins.

Results:

Passing-Bablok regression between qIS and the expected M-protein recovery produced a slope of 0.98 (95% CI 0.96 1.03), r = 0.999. Using a quality target of 25% error, our analytical measurable range spanned the maximum concentration tested (1.6 g/dL) to 0.10 g/dL (Figure).

Conclusion:

qIS achieves quantification of beta-migrating M-proteins at concentrations an order of magnitude lower than traditional SPE methodology, thus allowing earlier detection of M-protein recurrence or reduction.



A-031

Serum heavy/light chain analysis and specific isotype pair suppression in the monitoring of multiple myeloma

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Background: The immunoglobulin heavy/light chain (HLC) analysis has been recently proposed for monitoring the monoclonal protein (MP) in multiple myeloma (MM). The HLC ratio (rHLC) has been suggested as a new marker of monoclonality and early indicator of biological progression of the disease. **The aim** of this study is to compare HLC and its ratio in the follow-up of MM patients with standard laboratory techniques **Methods:** 24 diagnostic and 239 post-treatment serum samples from 26 MM patients (15 IgG, 10 IgA, 1 IgAK-GK) were included, with a median follow-up time of 37.5 months (range 21-67) and 9.5 samples per patient on average (range 6-19). Samples were analyzed for HLC and standard tests. Serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) were performed on the Capillarys and Hydrasys (Sebia) respectively, total immunoglobulins were measured on the Immage 800 (Beckman Coulter), HLC and serum free light chain (Hevylite®, Freelite®, Binding Site) on the BN proSpec (Siemens). **Results:** At **diagnosis**, all the samples presented an abnormal rHLC, in agreement with the IFE, elevated involved HLC (iHLC) and suppressed uninvolved HLC (uHLC) levels. During **follow-up**, 97.4% of the samples with MP detected by SPE had an abnormal rHLC, 83,5 % presented increased iHLC and 80.7 % decreased uHLC. Moreover, iHLC levels correlated well with the MP measured by SPE (y=0.46+1.06x, r=0.943). In 96 samples with normalized SPE, there was a moderate agreement between the IFE and the rHLC and uHLC suppression (kappa coefficient, 0.510 and 0.439, respectively). 25/42 IFE positive and 5/54 IFE negative samples had an abnormal rHLC. 5 IgG MM samples presented oligoclonal bands (OCB), impacting the IFE agreement with the HLC parameters (kappa coefficient excluding OCB samples, 0.591-0.593). After **treatment**, 11 patients achieved Complete Response (CR), of which 3 relapsed. rHLC was normal and in agreement with SPE and IFE in 6 patients. In 4 patients (1 IgG and 3 IgA) the rHLC normalized 2-5 months before the IFE became negative, and in 1 IgA patient the rHLC indicated relapse 3 months before IFE. In 10 patients who achieved Partial Response (PR) and 5 Very Good Partial Response (MP detected only by IFE), the rHLC was in agreement with the conventional tests, except for 9 samples from 3 IgG patients with normal rHLC that presented OCB and low levels of MP (< 3 g/L).

In PR patients, an increase in the iHLC was usually observed at progression.

In the 5 VGPR patients, the rHLC abnormality was mainly attributable to the suppression of the uHLC, while the iHLC is within the reference interval in most of the samples. The 5 patients subsequently progressed. **Conclusion:** These results confirm rHLC as a monoclonality marker, which may be an early indicator of degree of response to treatment and relapse. Despite the effect of oligoclonal bands on the IFE vs HLC agreement, HLC correlated with the clinical outcome. HLC is useful and complementary to other techniques for monitoring response, and adds information about the suppression of the uninvolved immunoglobulins

A-036

Clinical value of ANNA-1 antibodies in patients with paraneoplastic syndromes

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Background: The ANNA-1 or anti-Hu antibodies are directed against an antigen localized in the nucleus of all neurons. They are directed against a family of RNA binding proteins with a molecular size of 35-40 kDa. They are expressed in the nuclei of neurons of the central and peripheral nervous system. Paraneoplastic syndromes associated with this antibody are sensory neuropathy, encephalomyelitis, cerebellar degeneration with autonomic dysfunction and limbic encephalitis. The tumours associated with the presence of this antibody are small cell lung cancer, prostate cancer, breast cancer, neuroblastoma and sarcoma. The aim of this study is to show the clinic value of this antibody in the study of paraneoplastic syndromes.

Methods: We report four patients with paraneoplastic syndromes and the presence of anti-Hu antibodies were detected. Onconeural antibodies were identified in serum sample by indirect immunofluorescence (Euroimmun AG) based on primate tissues (cerebellum, nerves and intestine). The positive results were confirmed on recombinant immunoblot assay (Ravo-Diagnostika) that detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies.

Results: The results obtained are shown in the table.

Gender	Age (years)	Paraneoplastic syndromes	Antibody	Diagnosis of the patient after study	Survival
Male	79	Limbic encephalitis	Anti-Hu 1/100	Squamous cell lung cancer	Deceased (2 months)
Female	46	Sensory neuropathy	Anti-Hu 1/100	Multiple Sclerosis	Alive (25 months)
Male	67	Paraneoplastic encephalitis	Anti-Hu 1/1000	Lung adenocarcinoma	Deceased (19 months)
Female	50	Paraneoplastic encephalitis	Anti-Hu 1/1000	Small cell lung cancer	Deceased (7 months)

Conclusion: The presence of anti-Hu antibodies was associated to lung cancer in three patients while in the remaining patient was not found a tumour. In these three patient, the presence of anti-Hu antibodies was associated with a poor prognosis with short survival time. In the patient with multiple sclerosis and positive anti-Hu was not found a tumour despite the presence of continuous positive anti-Hu. In this patient, the possible interference due to anti-nuclear and anti-mitochondrial antibodies was studied with negative results. In summary, the presence of this antibody should help the clinician towards finding a hidden tumour, foremost among them, small cell lung cancer presents in 80% of cases of positivity for this antibody.

A-038

Lumipulse *G* HE4 Assay for Monitoring of Patients with Epithelial Ovarian Cancer

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Background: Human epididymis protein 4 (HE4) demonstrated to be a useful biomarker for ovarian cancer (Hellström I, 2003; Drapkin R, 2005; Moore RG, 2007). This study was to analytically verify the Lumipulse ***G*** HE4 assay and clinically validate the assay for monitoring recurrence and progression of epithelial ovarian cancer (EOC).

Methods: Lumipulse ***G*** HE4 assay is a Chemiluminescent Enzyme Immunoassay for the quantitative determination of HE4 in human serum and plasma on the Lumipulse ***G***1200 System via a two-step sandwich immunoassay method using two monoclonal antibodies against HE4. The amount of HE4 in the specimen is obtained from the luminescence signals derived from the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1,2-dioxetane disodium salt).

Results: The Lumipulse ***G*** HE4 assay demonstrated linearity from 20 to 1,500 pM, and an analytical sensitivity with the LoQ (Limit of Quantitation) ≤ 20.0 pM. The precision study of 2 controls and 8 sera (n = 120 for each sample) revealed a total %CV ≤ 6.1% at 3 testing sites using 3 lots of reagents. There was no High Dose Hook effect with up to 300,000 pM of HE4 antigen in samples. Interference studies showed an average percent difference ≤ 10% between test and control samples for potential interferents, including 9 endogenous substances (human anti-mouse antibody, rheumatoid factor, conjugated bilirubin, unconjugated bilirubin, human immunoglobulin G, biotin, triglycerides, hemoglobin, and human serum albumin) and 23 drugs, which were spiked individually into sera (test samples). In the monitoring study, changes in HE4 levels in serial serum samples collected in SST tubes from 72 subjects with EOC were compared to changes in disease status, that is, progression or no progression. A total of 330 observations were undertaken with an average number of 5.6 observations per subject. A positive change in the HE4 value was defined as an increase of HE4 in the observation value that was at least 18% greater than the previous observation value. Of the 61 samples with a positive change, 49% of them correlated with the progression of EOC while 80% of the 269 subject serial samples with no significant change in the HE4 value correlated with no progression. The total concordance with diagnosis was 74%, PPV (positive predictive value) 35%, and NPV (negative predictive value) 87%. A comparison of Lumipulse ***G*** HE4 with the predicate device, HE4 EIA, was analyzed using weighted Deming regression. The slope and correlation coefficient (r) obtained were 1.03 and 0.9891, respectively, for the tested specimens (n = 143) which ranged from 33.4 - 969.5 pM, and slope and r of 1.03 and 0.9917, respectively, for the tested specimens (n = 168) ranged from 33.4 - 4602.0 pM.

Conclusion: The Lumipulse ***G*** HE4 assay has demonstrated to be accurate, precise, and sensitive for the quantitative determination of HE4 antigen in human serum and plasma, and is useful in monitoring the course of disease in women with epithelial ovarian cancer.

A-039

A Risk of Ovarian Malignancy Algorithm (ROMA) Derived from Lumipulse *G* HE4 and Lumipulse CA125II Assays

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Background: The FDA-cleared ROMA, a combination of HE4 EIA and ARCHITECT CA 125 II, is intended to assess the likelihood of finding malignancy on surgery of a woman with an ovarian adnexal mass (Chudecka-Glaz, 2015). The current study was to evaluate the clinical utility of Lumipulse ***G*** ROMA, a new ROMA assay under development.

Methods: Lumipulse ***G*** ROMA is a qualitative serum and plasma test that combines the measurements of Lumipulse ***G*** HE4 and Lumipulse ***G*** CA125II assays as a ROMA score = exp(PI) / [1 + exp(PI)]*10, where PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125] for a premenopausal woman, and PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125] for a postmenopausal woman. The cut-points for defining a high likelihood of finding malignancy from a low likelihood was set as ≥ 1.31 and ≥ 2.77 for a pre and postmenopausal woman, respectively. Lumipulse ***G*** ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. Lumipulse ***G*** ROMA is for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. Lumipulse ***G*** ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay. PRECAUTION: Lumipulse ***G*** ROMA should not be used without an independent clinical /radiological evaluation and is **not** intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of Lumipulse ***G*** ROMA carries the risk of unnecessary testing, surgery, and/or delayed diagnosis.

Results: A precision study of 5 panels spanning the range of Lumipulse ***G*** ROMA scores revealed a total %CV ≤ 8.1 (n = 120, 3 sites). The method comparison between the Lumipulse ***G*** ROMA and the FDA-cleared ROMA showed a Deming regression slopes of 1.0, and a correlation coefficient (r) of 1.0 (n = 130). To assess the likelihood of finding malignancy on surgery of a woman with an ovarian adnexal mass, samples from patients presenting to a generalist with an ovarian adnexal mass were tested. For diagnosis of EOC only, a sensitivity of 93.6% (n = 47), specificity of 76.0% (n = 366), PPV of 33.3% (n = 132), NPV of 98.9% (n = 281), PLR of 3.894 and NLR of 0.084 was achieved at an EOC prevalence of 11.4% (n = 413). For diagnosis of EOC + LMP, a sensitivity of 87.7% (n = 65), specificity of 76.0% (n = 366), PPV of 39.3% (n = 145), NPV of 97.2% (n = 286), PLR of 3.647 and NLR of 0.162 was achieved at an EOC + LMP prevalence of 15.1% (n = 431).

Conclusion: The Lumipulse ***G*** ROMA under development appeared to be precise and sensitive for assessing whether a woman with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery.

A-040

Development of RT-qPCR Gene Expression Assays for Multiple New Cancer Therapies

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Background: Gene expression profiling to identify candidates for targeted cancer therapies is a growing need. Once molecular biomarkers are identified using massive parallel processing technologies such as microarrays or NGS, development of a robust quantitative assay for biomarker expression, such as RT-PCR, is the logical next step. In addition to their established and reliable use in in-vitro diagnostics, quantitative PCR (qPCR) assays have the advantage of being cost-effective. Here we present biomarker A and biomarker B RT-qPCR gene expression assays for targeted antibody-drug conjugate therapies that accept FFPE tissue as input and demonstrate, with initial research, high performance characteristics for measuring gene expression (i.e., RQ).

Methods: RT-qPCR was performed using the LifeTech QuantStudio 7 employing low-density (384-well) array cards, allowing for evaluation of multiple target and reference genes (2 target and 12 reference genes in this study). Stable normalization genes for three different tissue types (breast, ovary, lung) were selected from 12 candidate reference genes across 4 normal and 17 clinical tumor FFPE samples. Reference gene normalization was achieved using the geNorm algorithm. RNA extraction from 10 µm sections of FFPE tissue was performed using the automated Tissue Preparation System (TPS) from Siemens Healthcare. PCR amplicon specificity for the different assays was assessed using electrophoresis and NGS of end-point PCR product. Cell line controls were selected for low and high expression of target genes. Analytical reproducibility was assessed using both cell line control and clinical samples. Analytical sensitivity was assessed using mixtures of normal and high-level expressors of each biomarker. Differential expression of biomarkers A and B was assessed across 4 normal and 17 clinical tumor FFPE samples for each tissue type.

Results: The Siemens TPS System showed highest yield (>700 ng/sample), reproducibility (<0.25 SD between runs and operators), and scalability (up to 48 samples/run) over other manual RNA extraction methods. PCR efficiency for all primer/probe sets was found to be 100 ±6%. Electrophoresis of amplicons showed >95% on-target products based on size, while results from NGS showed mapping to the gene of interest >90% for the majority of assays, including selected target and references assays. Of 132 data points per indicated target or reference gene, normalized Cq values were below 0.2 SD for triplicates, and Cq values were <31 for control and clinical FFPE samples. The minimum detectable level of elevated biomarker expression, differentiated from normal, also known as lower limit of quantification (LLoQ), was determined to be 1.22 for biomarker A and 1.34 for biomarker B. Fold difference for mean expression values (i.e. RQ) between normal and tumor samples was highly significant in lung (fold difference = 3.7; p-value of 4.4E-4) for biomarker A.

Conclusion: Together, these RT-qPCR assays demonstrate the potential for robust performance and ease of use for companion diagnostics applications, such as identification or screening of tumor patients for personalized treatment.

A-041

Disease spectra and clinical characteristics of serum PIVKA-II-producing cancers other than hepatocellular carcinoma

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Background: The serum des-gamma-carboxyprothrombin (protein induced by vitamin K absence or antagonist-II, PIVKA-II) is a putative specific marker of hepatocellular carcinoma (HCC), but may also be produced by various tumors in the stomach, lung, colon, and pancreas. Although case reports of PIVKA-II-producing cancers other than HCC have been gradually increasing in number, their disease spectra and clinical characteristics remain unclear. In particular, there is no systematic study about clinicopathological features in PIVKA-II-producing cancers except HCC. The aim of this study was to identify clinical characteristics and diagnostic value of serum PIVKA-II in PIVKA-II-producing cancers excluding HCC.

Methods: We evaluated the serum PIVKA-II levels in 172 patients with various cancers (primary tumor sites: 59 stomach, 24 colon, 13 bile duct, 11 lung, 9 esophagus, 6 prostate gland, 4 rectum, 4 gall bladder, 20 etc.) excluding HCC in Chonnam National University Hwasun Hospital (Hwasun, Korea). The serum PIVKA-II level was determined using a chemiluminescent enzyme immunoassay system and an automated immunoassay analyzer (Lumipulse G1200; Fujirebio, Japan). In patients with more than 40 mAU/mL (cutoff value) of serum PIVKA-II, we investigated the clinicopathological characteristics of enrolled patients.

Results: Serum PIVKA-II levels in 172 patients with non-HCC cancers ranged from 10~110,179 mAU/mL (median, 24 mAU/mL). Of these patients, 22 patients (12.8%) showed PIVKA-II levels above 40 mAU/mL (median 102 mAU/mL). The most common type of cancers was gastric cancer (8 cases), followed by pancreatic cancer (4), cholangiocarcinoma (3), colon cancer (3), and renal cell carcinoma (2). Among 22 patients with PIVKA-II producing cancer, 8 patients (36%) had metastases to multiple organs, including 4 liver metastasis (50%). However, serum AFP levels (cutoff value, < 5.8 IU/mL) were abnormally high in 3 of all 22 patients, and in 1 of 4 patients with liver metastasis.

Conclusions: About 13% of various cancers excluding HCC showed elevated serum PIVKA-II level. Our results disclosed that serum PIVKA-II was not restricted in HCC and elevated serum PIVKA-II value was observed in mainly, gastrointestinal tract cancer. Additionally, when the serum PIVKA-II level is abnormally high in patient with gastric cancer, the possibility of liver metastasis should be considered.

A-042

Antibody-free microfluidics-based circulating tumor cell enrichment by Angle PLC Parsortix and downstream molecular characterization by Affymetrix branched DNA technology

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Background Enumeration of circulating tumor cells (CTC) in blood is a prognostic and predictive marker in metastatic breast cancer. However, enumeration of CTC by current approved methodology is of limited clinical utility and could be enhanced by molecular characterization. The unique feature of the Angle PLC Parsortix system that sets it apart from many other existing and nascent technologies is that it captures CTC without antibodies. It relies on the size and deformability of CTC with the advantage of harvesting them for subsequent downstream molecular characterization. The prime objective of this study is to validate the isolation of spiked breast cancer cell lines in healthy donor blood (HDB) with Parsortix followed by molecular characterization using Affymetrix QuantiGene Plex, a sensitive assay exploiting branch DNA technology.

Methods Four breast cancer cell lines (SUM190, MCF-7, MDA-MB-453 and MDA-MB-231) were separately spiked into 7.5 ml of HDB with EDTA anticoagulant and processed through Parsortix 10µm microfluidic cassettes for tumor cell enrichment. The harvested tumor cells were then suspended in 300µl of lysis buffer and analyzed by QuantiGene to detect the transcripts of 5 epithelial genes (*CDH1, EGFR, ERBB2, KRT18,* and *MUC1*) and 20 additional CTC and/or breast cancer-related genes. A gene was considered detectable if the transcript level was 2.5 standard deviations above the average transcript level of the gene in four unspiked HDB samples. Individual cell lines were similarly analyzed to determine the linearity and sensitivity of QuantiGene. Human Universal RNA was included as technical control for QuantiGene.

Results Tumor cells harvested by Parsortix were assayed for five epithelial genes customarily expressed by these cell lines. In terms of *specificity* of epithelial genes known to be expressed by these cells, 4 of 5 were detected in SUM190 and MCF-7 cells; 2 of 4 were detected in MDA-MB-453 cells; and 3 of 3 were detected by MDA-MB-231. In *linearity* studies, expression levels correlated well with the number of cells spiked into HDB and such a correlation was maintained ($R^2 > 0.9$) for most of the 25 genes tested. The *precision* of QuantiGene bead array assay for the molecular characterization of CTCs harvested by Parsortix was excellent with most CVs under 10%. In terms of *sensitivity*, dilution of the harvested cell extracts suggested that highly expressed genes such as KRT18 could be detected in as few as 20 SUM190 cells. KRT18 gene expression was detected when as few as 50 SUM190 (basal) cells or MCF-7 (luminal) cells were spiked into HDB; several genes were expressed when >50 cells were spiked. None of the 25 genes were detected in MDA-MB-453 (Her2-positive) when <500 cells were spiked. Gene expression was detected in the highly mesenchymal cell line MDA-MB-231 only when >500 cells were spiked.

Conclusion Molecular characterization of cells harvested by Parsortix is more informative and clinically useful than the enumeration of CTC alone. As liquid biopsy provides repeated blood sampling, such characterization may provide a novel avenue for personalized noninvasive assessment of therapy response and warrants further exploration in controlled cohort studies.

A-045

Development and Initial Evaluation of a Multi-Protein Biomarker Blood Test for Organ Confined Prostate Cancer Diagnosis (OCProDx)

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Background: About one in six men will get a diagnosis of prostate cancer during their lives. Generally, prostate cancer is treated effectively, but for many men the disease is not life threatening and they will die with prostate cancer rather than because of it. Too many men are treated unnecessarily. For them active surveillance of the disease would be a better option. Unfortunately, the existing readily available tools for disease diagnosis (PSA test, digital rectal examination and trans-rectal ultrasound guided biopsy), do not adequately guide this key decision of whether to pursue active surveillance or invasive treatment. Through analysis of the key decisions in prostate cancer patient management we highlighted that establishing whether the disease is organ confined (localized, OC) or has spread beyond the extracellular capsule of the organ (non-organ confined, NOC) would provide important information to guide this decision [Oon SF, Pennington SR, Fitzpatrick JM, Watson RW. Nature Reviews Urololgy (2011) 8:131-8.]. Our objective was to identify serum protein biomarkers to determine disease status in terms of organ confinement.

Methods: We undertook unbiased protein discovery experiments using gel and LC-MS based proteomics. Discovery was undertaken with affinity depleted (MARS14) serum samples (n>50 for gel and n=30 for LC-MS) taken from patients at time of diagnosis and for whom OC or NOC status was determined following radical prostatectomy. Statistical analysis of differentially expressed proteins was undertaken at univariate (Student t-test) and multivariate levels to assemble a panel of 59 candidate proteins. We supplemented this panel of 59 proteins with 5 proteins identified from the literature and developed a multiplexed MRM assay to support the simultaneous measurement of 63 of the proteins. The protein panel was evaluated its by undertaking two initial validation studies in which first 31/63 and then 63/64 of the candidate proteins were measured using patient samples distinct from those used for the discovery experiments. Serum samples were from the Irish Prostate Cancer Research Consortium.

Results: Initially, the relative abundance of the highest MRM transition from 50 peptides was used to measure 31 proteins in 63 clinical samples. The data, extracted using Skyline, were fitted into a PLS-DA model and the predicted performance was assessed through 200 times bootstrapping. The predictions in the out-of-bag samples were compared with the true group information and ROC curves were generated. The AUC for differentiating between OC and NOC was 0.824. Subsequently, 63 candidate proteins were evaluated with total of 116 patient samples and data analysed using a range of different statistical approaches. The AUC values for distinguishing organ confined from non-organ confined disease were >0.8. It was notable that proteins within the second phase of MRM development (n=32) made a contribution to these AUC values.

Conclusions: This initial evaluation data clearly demonstrates the potential of the 63 protein multiplexed MRM assay to discriminate OC from NOC prostate cancer. With incorporation of appropriate QC methods we suggest the OCProDx MRM assay may be capable of translation to diagnostic use to support the discrimination between OC and NOC prostate cancer.

A-046

USING HEVYLITE OVERCOMES PROBLEMS WITH THE MONITORING OF MONOCLONAL PROTEINS DIFFICULT TO MEASURE BY CONVENTIONAL TECHNIQUES

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Multiple Myeloma (MM) monitoring is most frequently done by quantifying serum monoclonal immunoglobulins (MP) in agarose electrophoresis gels (SPE). This procedure is often complicated when the MP migration pattern overlaps with normal serum proteins, appear as broad band, multiple peaks or small peaks, which may occur in up to 40% of IgA type MM. Thus, the follow-up of these MP may result less accurate, require additional techniques and ultimately result in equivocal evaluation of patients' response to treatment. The Hevylite® immunoassay for the determination of immunoglobulins' specific heavy/light chains pair has been developed, which allows the exact quantification of the MP without the over- or underestimation that may occur when monitoring with SPE, mainly in IgA MM patients.

Objective: utility of Hevylite® versus SPE quantification in the follow-up of IgA MM patients.

Methods: Hevylite® measured by turbidimetry on a SPA_{PLUS} (Binding Site); SPE on a Capillarys Hydrasys Focusing device (Sebia). Population: 335 samples from 36 IgA MM patients followed at our center between 2012-2015.

Results: A high correlation was found between the MP quantification by SPE and Hevylite (iHLC=-0.203+1.15 SPE; r=0.928; p<0.0001) and between total IgA and the sum of Hevylite IgAk+IgAlambda (∑HLC=-1,63+1,12 totIgA; r=0.912).

Analyzing 21 patients with a medium of 13 (range:5-26) follow-up samples we found that when the MP is clearly distinguishable by SPE the evolution of the MP during follow-up by either SPE or HLC is virtually superimposable, validating the role of HLC as monitoring tool. In turn, some patients with complicated MP migrating patterns benefited from the use of HLC (see table).

Patient	MM Isotype	SPE M-spike migration pattern	Hevylite contribution
1	AL	Alpha 2	HLC allows to continue monitoring MP even after SPE becomes negative due to total overlap with normal serum proteins
2	AL	Beta	HLC identifies relapse earlier than to SPE. Also, HLC never normalizes in contrast to SPE which remains non-quantifiable for 11 months
3	AK	Small Beta	Very small M-spike by SPE, hard to identify and quantify. HLC allows an easy and accurate follow-up of the MP
4	AL	Split peak in Beta	SPE probably underestimating the amount of MP due to gross interference from other serum proteins
5	AK	Triplet spanning Beta and gamma	Hevylite confirms disappearance of MP while SPE shows a peak later identified by IFx as oligoclonal IgGL band
6	AK	Broad beta	Allows follow-up up to 4 months after the last positive SPE and IFx. Identifies relapse 3 months before SPE

Conclusion:

-Hevylite is an alternative method for MP quantification , adding value to the follow-up of MM patients particularly when SPE shows limitations.

- Additional Hevylite value might come from early indication of relapse. However this observation lacks confirmation from larger studies.

A-047

Precision profile of a second-generation multivariate index assay for malignancy risk assessment of adnexal masses

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Objective: A second generation multivariate index assay (MIA2G) has been developed for improved detection of ovarian cancer among women undergoing removal of adnexal masses. This test combines the levels of serum proteins apolipoprotein A-1, CA 125, HE4, FSH and transferrin using ensembles of classification models integrated into a 0-10 risk score. The analytical precision can be estimated empirically using specimens spanning the range of risk scores, but there are currently no methods that account for imprecision from the possibly large combination of biomarker values that can result in any one score. The objective of this study was to determine precision across the range of risk scores resulting from all possible combinations of biomarker concentrations.

Relevance: Multivariate index assays have multiple sources of imprecision. A complete precision profile could help determine the allowable error that would permit reliable assessment of risk of malignancy.

Methodology: Single-site precision and multi-site reproducibility studies were performed on pooled patient serum samples that spanned the range of risk scores. Biomarkers levels were determined using the Roche cobas® 6000 clinical analyzer. Variance components were determined from analysis of variance using a restricted maximum likelihood method. Monte Carlo (MC) simulations of all possible combinations of biomarker concentrations from the studies -- resampled from their empirical standard deviations (SD) -- were used to generate MIA2G scores, assuming biomarker values varied independently. A second MC simulation used data from intended use clinical studies, which retained the true correlation structure of biomarkers in the benign and malignant conditions. The precision profile is a graph of the coefficient of variation of the sampled MIA2G scores as a function of the median of the score

Validation: Repeatability (within run) of MIA2G ranged from a SD of 0.000 to 0.130 risk score units (CV of 0.00% – 2.57%) and reproducibility (total of all components) ranged from a SD of 0.000 to 0.175 risk score units (CV of 0.00% - 3.43%), depending on the pool tested. The CV of individual biomarkers never exceeded 2.69% for any component. MC simulations assuming independence of biomarkers showed decreasing CVs with increasing risk scores, as well as higher variabilities of CVs at lower scores. The highest CVs were never found at the cut off value. None of these simulations exceeded a 5% CV. Simulation of within run precision using data from clinical samples resulted in about 1% of the simulations showing a CV of ≥5%. MC simulations of repeatability using data from clinical samples resulted in <2% of the cases where the 2.5%-97.5% quantiles crossed the cut-off. Estimates of the sensitivity and specificity of MIA2G within the 2.5% - 97.5% quantiles of values obtained from the simulation resulted in no significance change in test performance.

Conclusions: MIA2G was implemented on high-quality clinical instrumentation using well-controlled assays. The MIA2G risk score imprecision was generally lower than the component assays, resulting in reliable and robust outputs. The novel application of MC simulations demonstrated that the algorithms were robust to random individual biomarker perturbations over the range of risk scores.

A-048

Hyperhomocysteinemia results from and promotes hepatocellular carcinoma via CYP450 metabolism

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Background & Aims: Hyperhomocysteinemia (HHcy) can result from liver cancer or dysfunction and further alters intracellular lipid metabolism. Cytochrome P450 (CYP) arachidonic acid epoxygenases are expressed in human cancers and promote human cancer metastasis. This study explored the cross-talk of homocysteine (Hcy) and CYP450 metabolism in hepatocellular carcinoma (HCC). Methods: We first screened arachidonic acid and Hcy metabolism by liquid chromatography-mass spectrometry, Meta-analysis, and ELISA. Hcy regulation of CYP450 enzymes was verified by ELISA, immunostaining, and quantitative PCR in 42 tissue samples of human HCC and their adjacent non-tumor tissue, as well as in an HepG2-cell orthotopic-injected model of HCC in BALB/C nude mice. The bioluminescence imaging system was used for sensitive detection of tumor growth in the mice. Results: Arachidonic acid was the most abundant in tumor tissue, about 721.04±358.32 ng/mg. Importantly, the accumulation of metabolites in the CYP450 pathway (5,6-EET, 8,9-EET, 11,12-

EET, 14,15-EET and their corresponding DHETs) but not COX or LOX pathways was higher in tumor versus adjacent non-tumor tissue. Among three enzymes of EET synthesis: CYP2J2, but not CYP2C8 and CYP2C9, expression at mRNA and protein levels were higher in tumor versus non-tumor tissues. CYP2J2 protein levels were positively associated with poorly differentiated tumors, tumor sizes, and levels of alpha-fetoprotein. Meta-analysis of 13 eligible studies of 1,144 cases and 1,147 controls were performed and revealed that HCC risk associated with high serum Hcy levels (odds ratio [OR] 12.0; 95% confidence interval [CI] 9.13~14.88) and low folate levels (OR -8.3; 95% CI -12.9~ -3.71). Furthermore, intracellular Hcy levels were higher but folate levels were lower in HCC tumor versus non-tumor tissue. Importantly, high intracellular Hcy level was frequently found associated with high 11,12-EET and CYP2J2 protein levels, and positively correlated with CYP2J2 mRNA in HCC. A mouse model of HCC was generated orthotopically using HepG2-GFP cells and mediated by Hcy, with 2%L(wt/wt) L-methionine in a chow diet or not. The significant increase of tumor growth rate, size, and weight in the mice with methionine diet compared with the control diet, and the similar increase in CYP2J2 protein and mRNA level, as well as, poorly differentiated HCCs were observed. Moreover, the tumors became smaller in mice with CYP2J2 knockdown in both control and methionine diet groups. Conclusion: HHcy may result from but also promotes hepatocarcinogenesis via CYP450-EET metabolism through CYP2J2. The Hcy-CYP2J2-EETs pathway might be a target for the diagnosis and treatment of HCC.

Keywords: hepatocellular carcinoma, homocysteine, CYP2J2

A-049

Evaluation of a newly developed lateral flow system for kappa and lambda free light chains in urine

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Introduction: We describe the evaluation of a newly developed lateral flow device for the detection of kappa and lambda free light chains in human urine. This dual analyte, lateral flow immunoassay uses highly specific and characterised monoclonal antibodies (Campbell et al., 2013 JIM) in a competitive/inhibition format. This arrangement does not suffer from antigen excess (high does hook effect), making it suitable for the detection of elevated urinary free light chains in the clinical investigation of plasma cell dyscrasias. The system comprises of a lateral flow device, application buffer and a small portable reader, which, coupled with short incubations to provide rapid near patient results in the physicians office laboratory. **Method:** The monoclonal antibodies to either kappa or lambda free light chain were conjugated to colloidal gold and dried in a pad within the lateral flow device. The nitrocellulose strip has two test zones; comprising of either immobilised kappa or immobilised lambda light chain. Urine is prepared in an application buffer and added to the device where the gold labelled monoclonal antibodies rehydrate and travel by capillary action along the nitrocellulose. In the presence of the specific light chain, inhibition of binding will occur during the incubation period. The device also has a third zone on the nitrocellulose that acts an independent immunoassay control. The reader performs the 10-minute incubation, interprets the line intensity and converts these into a concentration via a predetermined calibration (10 to 100 mg/L for each light chain) contained within a barcode. **Results:** Each assay demonstrated acceptable inter-assay precision; repeated analysis of a patient urine sample across 30 devices over several days yielded mean values of 35.2 mg/L (CV, 10.4%) and 22.4 mg/L (CV, 10.6%) for kappa and lambda free light chains respectively. A normal urine sample was analysed at the same time; all kappa and lambda values for this read less than 10mg/L. There was no influence of pH: Negative synthetic urine was tested at pH 5, 6, 7 and 8 and remained negative. Aliquots at each pH were fortified with kappa (concentration 28 mg/L) and lambda (concentration 14 mg/L) and tested and were also unaffected at these pH levels. No significant interference was seen from glucose tested at 2000 mg/dL, human albumin tested at 1000 mg/dL or hemoglobin tested at 0.67mg/dL (equivalent to 200 RBC/microlitre).

Clinical testing has started with the retrospective analysis of 71 pathological urine samples submitted for immunofixation electrophoresis. The limit of detection for this method is 10 mg/L). This urine population comprised of 31 samples that were negative for monoclonal free light chains, 18 samples where kappa light chains were present and 22 samples that had lambda light chains. The new lateral flow device correctly identified each sample versus its immunofixation status. Kappa results for the lateral flow device ranged from 22 to 3995 mg/L whilst lambda results ranged from 27 to 2484 mg/L. **Conclusion:** Further work is planned to expand the patient cohorts and to assess potential interference from other paraproteins.

A-050

EFFECT OF REAGENTLOT-TO-LOTVARIABILITY ON CARCINOEMBRYONICANTIGEN PATIENT´S RESULTS

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Background Carcinoembryonic Antigen (CEA)is widely recommended in the follow up of some types of epithelial cancers. There is evidence that the reagent lot-to-lot variability has influence on patient's result. CLSI recommends verifying this circumstance, especially valuable in immunoassay methods, having an impact in their clinical applicability and affecting the decision-making process. **Aim** To evaluate the effect of reagent lot-to-lot variability on CEA patient's results. **Patients and Methods** Patient results were retrospectively obtained from the Laboratory Information System over a 2 years period (2014-2015). Serum CEA was measured by chemiluminescence(Centaur XP®, Siemens HCD). Manufacturer traceability statement:Internal standard (highly purified material). We performed the Kolmogorov-Smirnoff test and calculated the median and interquartile ranges (IQR) for every group of results related to each reagent lot. Kruskall-Wallis and median test were used to evaluate if differences were statistically significant. To assess the clinical significance we applied two criteria: desirable biological variation (BV) specification for systematic error (14.3%) and NACB guideline criteria of clinical significance (30%). **Results** 45,987CEA results from 20,871 patients were recruited. Kruskall-Wallis and median test showed statistical significant differences between serum CEA patient results grouped by reagent lot ($p<0.01$). Table shows median and IQR, relative difference related to the previous lot (RelDif), maximum difference between each lot and the rest (MaxDif),expressed as percentage and the number of results obtained with each lot.

Lot number	Median (ng/mL) (IQR)	RelDif(%) (CI 95%)	MaxDif(%) (CI 95%)	N
152	1.14 (0.54-2.20)	(First Lot)	22.8 (21.56-24.04)*	4,561
154	1.36 (0.78-2.29)	19.3 (18.13-20.47)*	19.1 (17.93-20.27)*	4,479
155	1.40 (0.78-2.40)	2.9(2.40-3.40)	21.4 (20.19-22.61)*	4,564
156	1.20 (0.6-2.3)	-14.3(-15.34- -15.34)*	16.7 (15.14-18.26)*	2,291
157	1.10 (0.5-2.1)	- 8.3(-9.45- -7.15)	27.3 (26.30-28.30)*	7,995
158	1.30 (0.6-2.3)	18.2(17.34-19.06)*	15.4 (14.38-16.42)*	5,041
159	1.30 (0.7-2.3)	No Difference	15.4 (14.44-16.36)*	5,601
160	1.10 (0.5-2.20)	- 15.4(-16.36 - -14.44)*	27.3 (26.08-28.52)*	5,377
161	1.10 (0.5-2.1)	No Difference	27.3 (25.62-28.98)*	2,813
162	1.40 (0.7-2.4)	27.3(25.62-28.98)*	21.4(19.96-22.84)*	3,265
Total	1.25 (0.6-2.28)	-	-	45,987
*Desirable SE -BV (%) No one exceeded NACB criteria (30%)				

Conclusions Differences observed in CEA results in this study based on BV, although do not exceed the NACB criteria, reinforce the need of performing an evaluation of new reagent lots prior to analyze patient samples. This could be a source of analytical error so laboratories should apply protocols for detecting them in order to avoid erroneous clinical decisions.

A-051

Biomarker Discovery by Proteomic Analysis of Ubiquitin Modification in Ovarian Cancer Cells

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Background: Cancer biomarkers are essential for screening, diagnosis, treatment assessment and monitoring disease recurrence in patients. Preliminary identification of biomarkers is challenging and initial screening is often conducted in cell models where the protein profile is examined with respect to expression, mutations and abnormal post-translational modification. In this study, ubiquitin post-translational modification is examined in an ovarian carcinoma cell line with the aim of establishing a ubiquitination profile and identifying alterations in ubiquitin signaling that can be translated to disease presentation. Ubiquitination is primarily associated with degradation by the 26S proteasome. However, ubiquitination is also involved in other signaling mechanisms that impact protein function in a degradation-independent manner. The objective of this project is to utilize stable isotope labeling by amino acids in cell culture (SILAC) and LC-MS/MS as a preliminary biomarker screen through the identification of ubiquitinated proteins in SKOV3 ovarian cancer cells. This approach assesses ubiquitin-dependent changes in protein levels, while differentiating between proteins targeted for degradation and those ubiquitinated for degradation-independent signaling.

Methods: SKOV3 cells are cultured in RPMI media with 10% FBS (Light) and SILAC RPMI media containing $^{13}C_6$-L-lysine and $^{13}C_6{}^{15}N_4$-L-arginine (Heavy) media supplemented with 10% FBS. Light cells are treated with MG132 proteasome inhibitor, a combination of MG132 and tunicamycin (N-glycosylation inhibitor), as well as DMSO (control). Both Light and Heavy cells are harvested 48 hours post-treatment and lysed in urea buffer. Protein concentration is determined for each sample and the Heavy and Light lysates are combined at a 1:1 ratio for each treatment. The samples then undergo reduction and alkylation, followed by trypsin digestion, and offline basic reversed phase (bRP) fractionation (Global samples). A sub-faction of the peptides undergoes further K-ε-GG ubiquitin remnant motif peptide enrichment, following the initial bRP fractionation step (Ubiquitin-enriched samples). Samples are subjected to LC-MS/MS using an Orbitrap mass spectrometer, and protein identification and quantification are conducted using MaxQuant. To validate the reproducibility and precision of this preliminary screen, the experiment is repeated and varying MG132 concentrations and treatment times are tested for optimization.

Results: Proteasome inhibition by MG132, shows overall enhanced protein up-regulation in Global and Ubiquitin-enriched samples compared to DMSO and MG132/tunicamycin treated cells. Ubiquitin-enriched samples represent the ubiquitinated portion of proteins, where MG132 treatment results in up-regulation of 46% of proteins compared to 0% in the DMSO control. Only 12% of proteins are up-regulated in the MG132/tunicamycin treated Ubiquitin-enriched sample, suggesting an effect of glycosylation on ubiquitin modification. Proteins identified in the Ubiquitin-enriched samples, especially ones up-regulated with MG132, are assessed in the Global samples to determine if increased protein levels are due to stabilization by proteasome inhibition or degradation-independent ubiquitination. This approach enables differentiation between protein targets modified by ubiquitin for turnover and those ubiquitinated for trafficking or other non-proteasome signaling.

Conclusion: Protein ubiquitination in SKOV3 ovarian carcinoma cells can be classified into degradation and non-degradation signaling functions. The ability to distinguish these proteins by SILAC-based quantitative proteomics allows screening for cancer biomarkers whose functions are altered in ovarian cells due to abnormal non-degradation mediating ubiquitination.

A-055

Frequency of Somatic TP53 Mutations in Combination with Known Pathogenic Mutations In Non-Small Cell Lung Carcinoma as Identified by Next-Generation Sequencing

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Background: The tumor suppressor gene TP53 is the most frequently mutated gene in human cancer and encodes p53, a DNA-binding transcription factor that regulates multiple genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis and senescence. TP53 is associated with human cancer by either frameshift or nonsense mutations that lead to a loss of wild-type p53 function or by missense mutations that confer alternate oncogenic functions (gain of function) that enable them to promote invasion, metastasis, proliferation and cell survival. Identifying TP53 mutations in tumor cells may help direct more effective therapies for treating cancer; gene therapies to restore the function of TP53 are currently being evaluated. In this study, we identified which TP53 somatic mutations predominated in non-small cell lung carcinoma (NSCLC) using Next Generation Sequencing (NGS) technology. We also identified somatic mutations in numerous actionable genes including *BRAF*, *EGFR*, *KRAS*, *and PIK3CA* that occurred concurrently with these TP53 variants.

Methods: DNA was extracted from 592 NSCLC tumors from formalin-fixed-paraffin-embedded sections and used to prepare barcoded libraries using the Ion Torrent Cancer 50 gene Hotspot Panel v2. Samples were multiplexed and sequenced using Ion Torrent 318v2 chips on the PGM Sequencing Platform. Variants were identified using the Variant Caller Plugin (v4.0.2) available in the Torrent Suite and Golden Helix SVS (v7.7.8) was used to assess quality and functional predictions.

Results: 215 of 592 (36.3%) of NSCLC cases were found to have one or more mutations in the TP53 gene. The two most common P53 mutations in our patient population were V157F and R158L; however, both mutations were present in only 6/215 patients (2.8%). Pathogenic variants were observed in 100 (46.5%) of tumors, including *KRAS* (59/215, 27.5%) *EGFR* (25/215, 11.6%), *PIK3CA* (9/215, 4.2%) and *BRAF* (7/215, 3.3%).

Conclusion: Intense efforts to develop drugs that could activate or restore the original p53 pathway have reached clinical trials. For this reason, the identification of both the particular TP53 mutation and concurrent known actionable genes present in the tumor could lead to appropriate treatment and improved clinical outcomes for the patient.

A-056

Comparison of two methods for determination of squamous cell carcinoma antigen in serum: electrochemiluminescence immunoassay (ECLIA) and chemiluminescent microparticle immunoassay (CMIA).

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Background: Squamous cell carcinoma antigen (SCC) is a serum tumor marker for the diagnosis and management of squamous cell carcinoma. Squamous epithelial cells are the main part of the epidermis, but are also present in the lining of the digestive tract, lungs, and other areas of the body. SCC occurs as a form of cancer in diverse tissues, mainly the lung, uterine cervix, vagina as well as lips, mouth and esophagus. The aim of this study was the comparison of two methods for determination of serum SCC: ECLIA and CMIA. **Methods:** We studied samples of patients who were required to determine serum SCC. Serum SCC were analyzed by two methods: 1. ECLIA: electrochemiluminescence immunoassay in Modular E-170 (Roche diagnostic®), with reference range < 2.3 ng/mL. 2. CMIA: chemiluminescent microparticle immunoassay in Architect i 2000SR (Abbott®), with reference range < 1.5 ng/mL. Statistical analysis was performed by Bland and Altman test and Passing and Bablock regression using the software MedCalc®. **Results:** We analyzed 56 samples. Descriptive statistics are showed in following table (CI: confidence interval; IR: interquartile range):

	Lowest	Highest	Median (95% CI)	IR
ECLIA (ng/mL)	0.66	7.13	1.55 (1.24-1.87)	0.9
CMIA (ng/mL)	0.7	8.1	1.30 (1.00-1.80)	0.8

Spearman's coefficient of rank correlation (rho) was 0.911 ($p<0.0001$). The mean of differences between ECLIA and CMI using Bland and Altman test was 0.15 ng/mL. The Passing and Bablock regression was: CMIA = 0.0254 + 0.8972 ECLIA. **Conclusions:** The serum SCC values were highers using ECLIA than CMIA. The serum SCC determined on samples by different assay methods cannot be used interchangeably.

A-057

Elevation of serum human chorionic gonadotropin level in a patient with giant cell tumor of the bone

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Background: Human chorionic gonadotropin (hCG) is a glycoprotein that is predominately secreted by the trophoblastic cells of the placenta after implantation of the fertilized ovum. hCG test on urine and serum is the most popular and reliable approach for early detection or serial monitoring of a pregnancy. Moreover, serum hCG elevation has been well documented as a tumor marker in a number of gestational trophoblastic and non-trophoblastic neoplasms. However, the alteration of hCG level in patients with giant cell tumor of bone (GCTB) is still largely unknown. The objective of this study is to report a case of GCTB with a concomitant elevated serum hCG, highlighting the variability based on the specific hCG isoforms.

Method: Serum samples were collected from a patient with GCTB and a healthy pregnant woman as a positive control, and hCG level was tested by three immunoassays (Beckman, Abbott, and Roche)

Results: We presented a case of an 18 year old woman with a slow growing painful mass located at the proximal head of her left humerus. Radiologic imaging revealed a large lytic bone lesion which on core needle biopsy contained spindled and osteoclast type giant cells. Surgical excision was planned. On the day of her surgery, her urine and serum hCG were positive with the serum level at 38 IU/L (Beckman, reference interval: <5 IU/L). Since patient was not sexually active, a potential false positive result was further investigated. The elevated level of HCG was confirmed by our lab and other two local clinical labs (using immunoassays of Abbott or Roche). However, a significant inter-assay variation (ranging from 19 to 40 IU/L) was noticed among the three assays. Meanwhile, a serum sample of a known healthy pregnant patient also was tested concurrently in the three labs as the positive control, which showed the consistent hCG levels of 48 IU/L. Twenty four hours post tumor resection, the patient's serum hCG level dropped to 2 IU/L and remained at this level during her

hospital stay. The identical low serum hCG levels detected by the three labs after tumor resection, suggested that the variable results of tumor-derived hCG might be due to different isoforms. Moreover, post-surgical histopathology diagnosis of the lesion indicated GCTB with hCG expression in mononuclear cells, atypical giant cells, and S100-positive dendritic cells. Our results indicated that an elevated serum hCG level in this patient was primarily produced by GCTB. The different isoforms other than the intact form of hCG derived from GCTB contributed to the inter-assay variation of hCG results by the three immunoassays.

Conclusion: Since GCTB often occurs in younger females, it is important to be aware of the possibility of hCG expression by GCTB leading to a false positive pregnancy result. hCG may serve as an ancillary marker in diagnosis, post-treatment follow-up and monitoring for recurrence. Finally, our results also indicate a standardized assay targeting specific hCG isoforms is needed for the diagnostic test of GCTB.

A-060

Detection of non-innoculated fecal immunochemical test kits

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Introduction: Fecal immunochemical testing (FIT) is now considered a "gold standard" for colorectal bleeding and cancer screening programs. As with any patient collected specimen, FIT runs the risk of improper sample collection, including failure to collect a sample before the kit is returned to the lab. The spectrophotometers currently available for analysis of FIT kits cannot detect whether the kit is inoculated or not. Thus, a low absorbance can be due to lack of blood in the specimen or no specimen. This could pose a patient safety risk if an uninnoculated specimen was not caught and was incorrectly reported as a negative, especially for a large lab than runs several hundred specimens daily. We found that the problem also exists at other labs using the same kit.

Methods: FIT is performed at our lab using the OC-Sensor Diana system from Polymedco. The screening cutoff is 75 ng/mL that has been set by the provincial colorectal cancer screening program. Specimens are collected into FIT kits also from Polymedco. Kits are distributed through laboratory services to patients along with provincially standardized instructions on how to properly collect the test. The collection kits are often prelabelled with a laboratory information system patient identifier label to ease identification and sample return. Twenty non-innoculated FIT kits were run on the instrument to determine the absorbance for empty tubes.

Data pulls were made from the OC-Diana sensor instrument as well as from our laboratory information system (Cerner Millennium). All data was analyzed in Microsoft Excel.

Results: Results showed that non-innoculated FIT kits gave an average result of 15 ng/mL. We then reviewed over 140,000 patient results obtained over 16 months and found that over 70% of results had a reading of 15 ng/mL or lower indicating that using a cutoff was not a viable option. In addition, we investigated the use of administrative controls to prevent acceptance of non-innoculated specimens. Prelabelling of FIT kits was discontinued and patients returning a kit were required to log their specimen in with accession staff. This has been successful in reducing the number of non-innoculated FIT kits arriving at the laboratory from 1-2 a week to only 2 detected since the implementation of the administrative block. The manufacturers of the kits were also contacted for solutions, which has resulted in a short term solution of redesigning the FIT kit labels with a break-away seal to indicate that the kit has been opened.

Conclusion: Labs running FIT should evaluate their local process to ensure that non-innoculated FIT kits are detected prior to analysis.

A-061

BRCA1 and BRCA2 NGS Sequencing and Pathogenic Variants Prevalence in Female Patients in Brazil

P. Y. Nishimura, L. T. Galindo, M. P. Migliavacca, O. Fernandes, L. C. Scarpelli. *DASA- DIAGNOSTICOS DA AMERICA, BARUERI, Brazil*

Background: Breast cancer is the leading cause of death from cancer in women in Brazil. About 10-15% of breast cancer cases present a heritability pattern and pathogenic mutations in BRCA1 (Breast Cancer 1) gene , located on chromosome 17 and in BRCA2 gene (Breast Cancer 2), located on chromosome 13, are responsible for half of this type of cancer and both are associated with predisposition to Hereditary Breast and Ovary Cancer Syndrome (HBOC). The *BRCA1* and *BRCA2* genes encode tumor suppressor proteins that act in DNA repair pathways and are important to maintain the stability of genomic DNA. Mutation in one of these genes, decreases cell repair effectiveness facilitating accumulation of mutations that can lead to cancer. The cumulative risk throughout life for a female who carries a germinative deleterious mutation in either of these genes is around 85% whereas it is about 12,5% in total population. Therefore, molecular testing to identify these mutations becomes a powerful tool that enables the identification of individuals at risk and initiate a surveillance and early prevention. Sanger sequencing was the established technique used to identify these mutations but with the advent of Next Generation Sequencing (NGS) we are able to sequence a larger amount of samples in a faster and cheaper way, increasing the availability of molecular tests to those eligible for screening. **Objective:** The aim of this study was to evaluate the prevalence of BRCA1 and BRCA2 genes mutation with NGS in female patients in a large Brazilian private laboratory.

Methods: 104 DNA samples obtained from a female group of patients with breast and/or ovarian cancer were sequenced in the Ion PGM platform (Thermo Fisher). The region of interest was amplified using Ion AmpliSeq BRCA1 e BRCA2 Panel (Thermo Fisher) and the sequencing analysis was obtained using the Ion Torrent Browser. The medium coverage was 200X. After identification of candidate variants IGV (Integrative Genome Viewer) analysis was performed and then additional biological annotation for each candidate variant was made consulting the following data bases: ClinVar, Breast Cancer Information Core (BIC), Leiden Open variation Database (LOVD), ARUP and EVS (Exome Variant Server). Sanger sequencing then confirmed the variants classified as pathogenic. To identify intragenic deletion or duplication, MLPA (Multiplex Ligation Dependent Probe Amplification) was performed using the SALSA MLPA KIT P002 BRCA1 and the SALSA MLPA KIT P045 BRCA2/CHEK2 (MRC Holland Amsterdam, The Netherlands).

Results: From the 104 samples analyzed, 26 (25%) presented pathogenic variants, of which 12 were present in BRCA1 gene and 14 in BRCA2 gene. All 26 pathogenic variants were confirmed with Sanger Sequencing. MLPA was performed in all samples and no deletions or duplications were identified.

Conclusion: In conclusion, the results obtained with Sanger Sequencing were in accordance with NGS results, suggesting a prevalence of 25% of pathogenic variants in BRCA1 and BRCA2 genes among the patients analysed.

A-062

Genomic DNA Purification From Human Whole Blood and Buffy Coat

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Background: Whole blood and buffy coat are commonly used sample types for genomic analysis in applications such as qPCR, microarrays, and sequencing among others. The Maxwell® RSC Whole Blood DNA and Maxwell® RSC Buffy Coat DNA kits have been developed to purify genomic DNA from these sample types on the Maxwell® RSC instrument, a bench top personal magnetic particle handler. A user adds sample directly to the first well of the pre-dispensed cartridge and starts the purification protocol on the instrument. Whole blood collection tubes containing common anticoagulants (EDTA, heparin, and citrate) are compatible with these purification chemistries. The 45 minute purification protocol results in large molecular weight genomic DNA of high purity and concentration that is compatible with downstream amplification. The Maxwell® RSC Whole Blood DNA kit can process between 50-500µl of blood while the Maxwell® RSC Buffy Coat DNA kit can process between 50-250µl of buffy coat sample. **Method:** To demonstrate performance of the Maxwell® RSC Whole Blood DNA kit, whole blood was collected from six Individuals and used for purification. For each Individual, four replicate blood samples of 500µl volume were purified and analyzed using a Nanodrop spectrophotometer and agarose gel electrophoresis. Quadruplicate whole blood samples from two additional individuals were purified. Eluates from these samples were analyzed using a Taqman-based qPCR assay to assess quantitation and inhibition. For the Maxwell® RSC Buffy Coat DNA kit, whole blood samples were collected from six Individuals and the blood tubes were centrifuged to separate the blood into plasma, white blood cell, and red blood cell layers. Buffy coats were drawn from the white blood cell layers and quadruplicate 250µl buffy coat samples from each individual were used for purification. Samples were analyzed for using a Nanodrop spectrophotometer and agarose gel electrophoresis. An additional sample of buffy coat was purified in quadruplicate for analysis using a Taqman-based qPCR assay to assess quantitation and inhibition. **Results:** Using the Maxwell® RSC Whole Blood DNA kit, average DNA concentrations ranged from 70 to 370 ng/µl while average yields ranged from 4µg to 16µg depending on white blood cell count of the initial blood sample. The purity ratios for A_{260}/A_{280} ranged from 1.85 to 1.91 while the purity ratios for A_{260}/A_{230} ranged from 1.92 to 2.44. The resulting DNA performed well in qPCR amplification.

Using the Maxwell® RSC Buffy Coat DNA kit, average DNA concentrations ranged from 260 to 860 ng/µl while average yields ranged from 27µg to 53µg depending on the white blood cell count. The purity ratios for A_{260}/A_{280} ranged from 1.87 to 1.93 while the purity ratios for A_{260}/A_{230} ranged from 2.19 to 2.46. The resulting DNA performed well in qPCR amplification.**Conclusions:** The data generated from the Maxwell® RSC Whole Blood DNA kit produced highly intact, amplifiable DNA with excellent purity ratios from up to 500µl of human whole blood. The Maxwell® RSC Buffy Coat DNA kit can process up to 250µl of buffy coat from human whole blood producing highly intact, amplifiable DNA with excellent purity ratios.

Tuesday, August 2, 2016

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

Cardiac Markers

A-064

Rule out myocardial infarction by admission limit of detection measurement using contemporary and high sensitivity troponin assays.

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Objective: To compare admission measurement of troponin for the rule out myocardial infarction (MI) 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. All patients were followed up to 30 days for major adverse cardiac events (MACE). Samples were analysed for cardiac troponin I (cTnI) by

the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 30 to 50,000 ng/L 10% CV 60ng/L 99th percentile 70 ng/L; the Beckman AccuTnI enhanced (B) (Access 2 , Beckman-Coulter) range 1 to 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 to 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 to 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. The use of limit of detection of the assay was used to classify patients using the admission sample only. Values below the limit of detection were classified as no MI.

Results: Samples were available from 813 /1132 patients enrolled in the study, 487 male age 23.7-92.8 years median 53.8 years. Limit of detection measured on admission allowed accurate exclusion of MI in 98.5-99.2% of patients with a final diagnosis that excluded MI corresponding to 70.6-80.8% of all patients presenting. In those who ruled out based on a single admission measurement major adverse events occurred in 0.2-0.6% and comprised readmission with suspected acute coronary syndrome and 1 myocardial infarction on follow up.

Conclusion: In low risk chest pain patients, troponin below the limit of detection measured with a sensitive or contemporary sensitive assay identified a very low risk group who can be considered for immediate further investigation or discharge.

A-065

Evaluation of the Specificity and Concordance of a Cardiac Risk Prediction Array for the Detection of Multiple SNPs from One sample of Different Biological Matrices

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Background: Coronary Heart Disease (CHD) is the most common cause of death in the Western world. Research indicates a strong genetic predisposition to CHD. Genetic studies have identified CHD-associated single nucleotide polymorphisms (SNPs). Current risk prediction methods, *e.g.* QRISK / Framingham are suboptimal at predicting CHD. The development of a predictive risk tool, incorporating personalised genetic profiling for CHD is therefore warranted. The objective of this study was to determine specificity and examine concordance rates of genotypes generated with a Cardiac Risk Prediction Array, which enables simultaneous detection of multiple CHD-associated SNPs from one sample of different biological matrices.

Methods: Biochip Array Technology (BAT) allows the development of SNP genotyping arrays, utilising rapid multiplex allelespecific PCR and product hybridisation onto a biochip. Firstly, a multiplex PCR reaction is performed, where the products amplified correspond to the genotype of the patient sample. The PCR products are then hybridised onto the Cardiac Risk Prediction biochip array and imaged using the Evidence Investigator analyser (Randox Laboratories, Crumlin, UK) to identify which alleles are present. Patient samples can be genotyped within one day.

To determine specificity, participants from the second UK Northwick Park Heart Study (n=3,012) were genotyped for 19 CHD-associated SNPs by Sanger sequencing. A subset of participants (n=185) were genotyped using the Cardiac Risk Prediction Array for 19 CHD-associated SNPs. To assess concordance between different biological matrices, matched blood and saliva samples were collected from a cohort of participants (n=20) and the DNA extracted was genotyped using the Cardiac Risk Prediction Array. Sanger sequencing was employed for two blood-derived DNA samples to ensure validity of results.

Results: Overall, agreement between Sanger sequencing and the Cardiac Risk Prediction Array was 99.8% (3152/3158 genotypes). Agreement for individual SNPs ranged from a minimum of 98.7% to a maximum of 100%. Fourteen out of 19 SNPs achieved values of 100%. The concordance assessment showed 100% concordance (400/400 genotypes) when comparing genotypes generated from blood and saliva. Concordance data was confirmed by Sanger sequencing of two bloodderived DNA samples.

Conclusion: Results show high specificity of the Cardiac Risk Prediction Array when comparing with goldstandard methods *i.e.* Sanger sequencing. Furthermore, with this system genetic risk profiles both from DNA derived from blood and saliva can be accurately and robustly determined. This provides evidence of its potential use in direct-to-consumer rapid genotype testing for improved cardiac risk prediction.

A-067

Impact of risperidone treatment of autistic disorders on metabolic risk factors in children and adolescents

P. Srisawasdi[1], N. Vanwong[1], Y. Hongkaew[1], S. Vanavanan[1], B. Intachak[1], N. Ngamsamut[2], P. Limsila[2], C. Sukasem[1], M. H. Kroll[3]. *[1]Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [2]Yuwaprasart Waithayopathum Child and Adolescent Psychiatric Hospital, Ministry of Public Health, Samutprakarn, Thailand, [3]Quest Diagnostics, Madison, NJ*

Background: Risperidone has been reported to affect cardiovascular and diabetic markers. Thus, we evaluated the influence of the dose and the duration of risperidone treatment on metabolic and diabetes risk markers in Thai children and adolescents with autistic spectrum disorders (ASDs).

Methods: In this cross-sectional analysis, cardiovascular and diabetic risk markers were measured in 172 ASD patients (88% male) treated with a risperidone-based regimen for ≥12 months. Based on FDA-approved dosing recommendations for pediatric patients, all patients were categorized into three groups: low dose, recommended dose, and high dose groups. Blood samples were analyzed for glucose and lipid metabolic markers, adiponectine, leptin, prolactin, cortisol, uric acid, creatinine, cystatin C and high sensitive C-reactive protein.

Results: The mean concentrations of fasting glucose, insulin, HOMA-IR, prolactin, and leptin levels significantly rose with risperidone dosage (all $P \leq 0.02$), but those of adiponectin, cortisol, uric acid, creatinine, and cystatin C did not. Dosage had minimal effect on triglycerides, total cholesterol, HDL-C, LDL-C, and lipoprotein subclasses (all $P > 0.10$). Insulin, HOMA-IR, triglyceride, and leptin levels rose with treatment duration (all $P < 0.03$). Conversely, adiponectin and HDL-C levels decreased with duration. Similar association patterns of dosage and duration of treatment with insulin, HOMA-IR and leptin were observed, all of which increased with dosage and duration.

Conclusions: Risperidone treatment disturbed glucose homeostasis and endocrine regulation (particularly leptin) in children and adolescents with ASDs, in a dose- and/or duration-dependent manner. It may directly impact glucose homeostasis by inhibiting the actions of leptin and insulin. The adverse metabolic changes associated with risperidone treatment suggest that risk for metabolic adverse effects, especially development of type 2 diabetes mellitus should be closely monitored, particularly in individuals receiving high doses and/or long-term treatment.

A-069

Standardization of Beckman Coulter Serum and Plasma Troponin-I assay in a Multi-Center Health System Using Multiple Testing Platforms

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Background: Cardiac troponin (cTn) are essential in the diagnostic evaluation for acute myocardial infarction. Our health system (WFBH, LMC, DMC) frequently assays cTnI concentration, however our hospitals use different cTnI testing platforms, sample types, and clinical cut-off values. This lack of standardization makes it difficult to monitor changes in cTnI when patients are transferred to the tertiary care hospital (WFBH). The aim of this study was to standardize cTnI testing in our care network by introducing the same cTnI method, comparing serum and plasma, and determining a clinical cut-off concentration for our patient population. This was achieved with pre- and post-study planning and evaluation between lab staff and clinicians. **Methods:** The WFBH Emergency Department collected 153 matched plasma (lithium heparin) and serum (SST) samples. Serum samples were assayed on the WFBH Siemens Centaur-XP immediately after collection and reported to physicians. Remaining samples were frozen until validation testing commenced. During validation testing, batches of samples (≈51/day) were assayed same-day on the Centaur and Beckman Coulter DxI 800 (AccuTnI+3) at WFBH and on the Access-2 at both DMC and LMC. Precision and linearity studies were conducted. Site-to-site variability was minimized by using the same lots of calibrators, QC materials, and reagents. Data were analyzed using EP Evaluator. **Results:** Linearity and precision agreed with the manufacturer's recommendations. The correlation studies are summarized below:

Comparison	Passing-Bablok Regression			Sample No.
	Slope	Y-Intercept	R^2 Coefficient	
WFBH Centaur (Serum) vs WFBH DxI (Serum)	0.609	-0.0006	0.9849	134
WFBH DxI (Serum) vs WFBH DxI (Plasma)	1.000	0.0000	0.9996	153
DMC Access-2 (Serum) vs DMC Access-2 (Plasma)	1.038	0.0000	0.9996	119
LMC Access-2 (Serum) vs LMC Access-2 (Plasma)	1.033	0.0010	0.9998	130
WFBH DxI (Plasma) vs DMC Access-2 (Plasma)	1.000	-0.0020	0.9995	131
WFBH DxI (Plasma) vs LMC Access-2 (Plasma)	1.109	0.0000	0.9984	131
DMC Access-2 (Plasma) vs LMC Access-2 (Plasma)	1.065	0.0020	0.9982	131
WFBH DxI (Serum) vs DMC Access-2 (Serum)	0.867	-0.0009	0.9997	126
WFBH DxI (Serum) vs LMC Access-2 (Serum)	1.033	-0.0002	0.9994	126
DMC Access-2 (Serum) vs LMC Access-2 (Serum)	1.083	0.0000	0.9997	126

Our observed correlation slope (m=0.609) was consistent with inherent differences between the immunoassays and supported by published CAP survey result comparisons. Qualitative method comparison between the Centaur and DxI showed a sensitivity of 93.5% and specificity of 91.0% at the manufacturer's suggested clinical cut-offs. The clinical cut-off value ≥0.025 ng/mL was appropriate for our patient population. **Conclusions:** Following this study, we will be able to standardize cTnI testing in our health system with the adoption of Beckman Coulter AccuTnI+3 reagents and instruments.

A-070

Correlation between conventional troponin and high sensitivity cardiac troponin assays in patients with chest pain

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Background: There are many methodologies available in the market, which use different targets and formats to assembly an assay, and depending on the type of assay and type of antigen used, different results and interpretations could be made. Due to the higher sensitivity and diagnostic accuracy for the detection of Acute Coronary Syndrome (ACS) at presentation, the time interval to the second cardiac troponin assessment can be shortened with the use of high-sensitivity assays

Methods: 40 consecutive samples were collected from patients of different ages admitted to the Emergency Department (ED) with chest pain and suspected ACS. The determination of Troponin I in serum samples were initially analyzed with a fluorescent immunoassay (ELFA) using the *Vidas* platform from *bio-Mérieux* ® (CT), wich was compared with a second method of high-sensitivity LOCI Cardiac Troponin I using a homogeneous sandwich chemiluminescent immunoassay in the *Dimension EXL 200* (*Siemens*) ® (HS)

Results: Excellent correlation coefficients were obtained, Pearson with r of 0.981 (expected &lt 0.95) and a concordance observed of 0.93 (expected $lt 0.90) and Kappa index of 0.85 (expected $lt 0.7).

Conclusion: There was a large correlation between the two Troponin I assays, in this particular situation both tests fulfilled their role for screening purposes using "Rule in" and "Rule out" algorithms. Further studies will answer whether there are advantages in replacing the CT methodology by HS, according to the clinical probability of ACS and delta obtained in serial samples

A-071

Identification of Predictive Proteomic and Metabolomic Biomarkers of Doxorubicin-induced Cardiotoxicity

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Doxorubicin (DOX)-based chemotherapy is commonly used for the treatment of a wide range of cancers. However, clinical use of DOX can cause cumulative dose-dependent and irreversible cardiotoxicity. Blood biomarkers such as cardiac troponin T (cTnT) and I (cTnI) have been suggested, but their ability to predict cardiotoxicity is limited. Therefore, development of new predictive biomarkers to identify potential cardiotoxicity prior to the occurrence of overt cardiac tissue damage and dysfunction would be extremely valuable for the prevention of permanent damage and/or identification of patients at risk for cardiac damage. The objective of this study was to identify potential biomarkers for presymptomatic detection of DOX-associated cardiotoxicity at the early doses of chemotherapy. Women treated for breast cancer with DOX-based chemotherapy at the University of Arkansas for Medical Sciences were enrolled in Institutional Review Board (IRB)-approved protocols of UAMS and FDA, and gave informed consent. All patients were treated following a predefined protocol which included a combination of DOX (Adriamycin, 60 mg/m^2) with cyclophosphamide (600 mg/m^2). Blood samples were collected prior to chemotherapy and after the first and/or second cycles of chemotherapy, and plasma was isolated. Cardiac function of all subjects was assessed by a multigated acquisition (MUGA) scan before the start of DOX treatment and at its completion of four cycles of chemotherapy. A decline of left ventricular ejection fraction (LVEF) by >10% or below 50% at completion of chemotherapy was considered abnormal or left ventricular dysfunction (LVD). Plasma samples of 27 patients, including 17 who maintained normal LVEF, 5 with LVEF decline by 5-10%, and 5 with LVEF decline by >10% at the completion of DOX treatment were analyzed using multiplex immunoassays for 82 proteins from 3 human cardiovascular disease biomarker panels (Millipore), one 40-plex human chemokine panel (Bio-Rad), one 9-plex human matrix metalloproteinases (MMPs) panel (Bio-Rad), and troponin T (Roche). This was to identify and verify potential utility of these proteins as predictive biomarkers of cardiotoxicity. It was found that higher abundance of CCL27, CXCL16, and GDF-15, and lower abundance of CXCL6, fibrinogen, and sICAM-1 (p<0.05) at the baseline level were associated with LVD (LVEF decline by >10%). After the second cycle of DOX treatment, increased abundance of CCL13, CXCL1, CXCL2, and MIF (p<0.05) was associated with LVD. In addition, a 1.6-fold increase of p-selectin after the second cycle of DOX treatment compared to patients' own plasma baseline levels (p<0.05) was observed in the group of patients with LVD. LC/MS-based metabolomic platform was also used to discover metabolic biomarkers in plasma. Metabolomics analysis revealed that plasma pyroglutamate and lysophosphatidylcholine (16:0) increased in the group of patients with LVD (p<0.05) while plasma docosahexaenoic acid and taurocholic acid decreased in both groups of patients with LVEF decline by 5-10% and by >10% (p<0.05) after the second cycle of chemotherapy. The differential plasma abundance of these proteins and metabolites at the baseline level or after the second cycle of DOX treatment could be potential predictive biomarkers of cardiotoxicity.

A-072

Relation of Interleukin-6 Level with Coronary Artery Disease Severity in Patients Undergoing Coronary Angiography

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Background: Atherosclerosis plays an important role in the pathogenesis of CAD. There is a closer relationship between atherosclerosis and inflammatory cytokines. Commonly used scoring systems to risk stratification in clinical practice are GENSINI and SYNTAX score for determining disease severity and method of revascularization. The aim of this study was to investigate the relationship between interleukin (IL)-6 levels and both GENSINI and SYNTAX scores in patients with CAD.

Methods: 118 patients who underwent to coronary angiography were enrolled into the study. GENSINI and SYNTAX were calculated for determining disease severity. IL-6 level was measured with immunometric assay method.

Results: There were no significant differences between the groups with respect to mean age, blood pressure, heart rate and use of alcohol. Gender and smoking status were significantly different between groups. GENSINI and SYNTAX scores of patients were significantly higher in CAD group than controls. IL-6 level was significantly higher in CAD group than controls. In correlation analysis, IL-6 level significantly correlated with GENSINI and SYNTAX scores and was an independent predictor of abnormal coronary angiography. Optimal cut-off level of IL-6 was 7.81 pg/mL to assess the ability of IL-6 to differentiate presence of CAD (Area under curve=0.78, sensitivity=78.3%, specificity=70.7%).

Conclusion: Patients with CAD have higher IL-6 level compared to control group. IL-6 level correlated with both GENSINI and SYNTAX scores. Moreover, IL-6 was an independent predictor of abnormal coronary angiography. An IL-6 value of 7.81 pg/mL

or higher predicted presence of CAD with a sensitivity of 78.3% and specificity of 70.7%.

A-073

Potential Risk Predictors for Cardiovascular Diseases in Patients with Obstructive Sleep Apnea Syndrome

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Background: An association exists between Obstructive Sleep Apnea Syndrome (OSAS) and increased incidence of cardiovascular diseases (CVDs). The high systemic oxidative stress in OSAS is considered as a major pathogenic mechanism leading to CVDs. There is limited and conflicting information in the literature investigating risk predictors for CVDs in OSAS Patients. Our aim was to investigate and define potential risk predictors for CVDs in newly diagnosed OSAS patients without manifest CVDs.

Methods: A total of 60 OSAS patients (13 moderate and 47 severe) diagnosed with polysomnography and 26 healthy volunteers were enrolled into the study. Patients were diagnosed having OSAS according to the apnea-hypopnea index (AHI) above 5. Patients were divided into subgroups according to their AHI: mild OSAS (AHI between 5 and 15); moderate OSAS (AHI between 15 and 30); and severe OSAS (AHI ≥30). There was no patient with mild OSA in the whole group. Blood samples were collected after overnight fasting, and plasma ischaemia-modified albumin (IMA), advanced oxidation protein products (AOPP), total oxidative status (TOS), total antioxidative capacity (TAC), copeptin level, myeloperoxidase (MPO) activity and soluble tumor necrosis factor receptor-1 (sTNF-R1) were measured in the patients and healthy controls. Statistical analysis was performed using SPSS Statistics Base 17.0 (SPSS Inc. Chicago, IL, USA). Receiver operator characteristic (ROC) curves were used to define sensitivity and specificity of the measured parameters for the diagnosis of OSA.

Results: Copeptin levels were significantly lower in both moderate and severe OSAS groups compared to the control group (0.42±0.18 and 0.49±0.26 ng/ml versus 0.64± 0.28; p=0.005, p=0.006, respectively). In contrast, we found no significant difference in copeptin levels between the OSAS subgroups (p=0.409). Plasma MPO activity and sTNF-R1 levels were significantly higher (43.2±21.65 vs. 30.44±8.05, p=0.0046; 2.379±1.2 vs. 1.086±0.86, p < 0.0001, respectively) in the patients compared to the control group. Plasma TOS (P<0.001) and AOPP levels (P =0.024) were significantly higher, in contrast to the TAC which was significantly lower (P=0.012) in the OSAS patients compared to the controls. There was a very strong negative correlation (r=-0.987, P<0.001) between TAC and AOPP levels. AOPP levels correlated significantly with TOS levels (P<0.001, r = 0.45). Plasma ischaemia-modified albumin levels were not statistically different between the patients and controls (P=0.74).

Conclusion: Copeptin levels were lower in the OSAS patients which might be due to low secretion of antidiuretic hormone. In contrast to our finding in the OSAS patients, copeptin levels are higher in CVDs. Thus, we conclude that measurement of copeptin is not proper to determine the risk of CVDs in OSAS patients. We found that a high systemic oxidative stress in OSAS as indicated by increased TOS and decreased TAC levels is reflected by increased AOPP without causing an increase in IMA. Elevated plasma MPO activity and sTNF-R1 levels in the OSAS patients indicate increased systemic inflammation which might contribute to the higher incidence of CVDs. Therefore, we recommend measurement of plasma MPO activity, sTNF-R1, TOS, TAC and AOPP levels in the OSAS patients as potential risk predictors for CVDs.

A-074

Alteration and association of galectin-3 levels in STEMI patients undergoing percutaneous coronary intervention

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Background: Galectin-3, secreted by activated macrophages, is both pro-inflammatory and pro-fibrotic but not cardiac specific. Most studies have focused on its prognostic utility in chronic heart failure, however few studies have investigated galectin-3 in acute coronary syndrome. Therefore, we initiated a study examining levels in adults presenting with acute ST-elevation myocardial infarction and undergoing percutaneous intervention.

Methods: Galectin-3 was measured using the Abbott chemiluminescent microparticle immunoassay on the ARCHITECT i2000 analyzer. Assay validation included precision studies and comparison with another laboratory using the Abbott assay (n=20) and one using an ELISA (n=20, BG Medicine Inc.). Results were analyzed with EP Evaluator (Data Innovations). Sample stability was studied using serum separator tubes: two patients with galectin-3 below and above the suggested cut-off (>17.8 ng/mL) were tested at 24 hour increments for 96 hours. Patient enrollment began in January 2016 and excluded those who might have preceding causes of elevated galectin-3 secondary to malignancy or severe chronic heart, renal, and liver disease. Study patients had galectin-3 measured at admission (baseline) and with up to two subsequent measurements prior to discharge.

Results: The Abbott method comparison results were: slope = 1.009 (0.956 to 1.061); intercept = 0.17 (-1.38 to 1.72); bias = 0.40 ng/mL (1.46%); R = 0.9944. Compared to the ELISA, results were: slope = 1.577 (1.279 to 1.875); intercept = -7.69 (-13.34 to -2.03); bias = 2.66 ng/mL (13.82%); R = 0.9253. Serum separator gel tube stability data over 96 hours produced a mean±SD of 9.26±0.65 ng/mL (CV 7.0%) at the lower concentration and 34.03±2.61 (CV 7.7%) at the higher concentration. Of the 13 study patients to date who have met inclusion criteria, baseline galectin-3 ranged from 13.7 to 43.6 ng/mL while baseline troponin I ranged from 0.03 to 19.9 ng/mL. Post-intervention CK-MB ranged from 1.8 to 300 ng/mL. No clear correlation was observed among these three cardiac biomarkers. Neither was there any concrete correlation found between galectin-3 and the number of ECG leads demonstrating ST-elevation nor with the number of coronary vessels exhibiting >50% stenosis. However, there was an emerging trend showing a sudden decrease in galectin-3 after percutaneous intervention with a mean -28.1% change from baseline in 7 of 12 patients within 27 hours and -24.7% in 7 of 9 patients within 63 hours. **Conclusion:** Our study demonstrates comparable inter-laboratory results when using the same Abbott method whereas significant proportional and fixed bias exists when compared to the ELISA method. The latter finding implies that any proposed cut-off value would depend on the particular assay used and highlights the concern that true industry wide harmonization is critical for reliable follow-up testing. Regarding our study patients, although enrollment and data acquisition is still in its early stages, we have observed a significant fall in galectin-3 immediately following cardiac catheterization in the majority of patients. This suggests that galectin-3 may yet have potential utility as an acute biomarker, perhaps associated with short-term myocyte recovery due to reperfusion, in addition to being a long-term indicator of cardiac fibrosis in chronic heart failure.

A-075

Development of a High Sensitivity Cardiac Troponin I Assay on the Siemens ADVIA Centaur® Immunoassay Systems*

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Background: The recent 2015 ESC guidelines for the management of NSTEMI patients have proposed algorithms for faster rule-in or rule-out of patients admitted in the acute care setting. Distinguishing acute from chronic c-Tn elevations using high sensitivity assays requires serial measurements to detect significant changes. High sensitivity cTn assays will allow more accurate and precise determination of serial changes or "delta's" which may afford acceptable rule-in and rule-out claims within 1 to 3 hours. The analytical performance of Siemens high sensitivity cTnI assay being developed for use on the Siemens ADVIA Centaur family of automated random access immunoassay analyzers is presented.

Methods: The Centaur TNIH assay employs streptavidin coated magnetic latex particles preformed with two biotinylated monoclonal anti-cardiac troponin I antibodies as the solid phase reagent. The detection reagent employs a recombinant sheep F_{ab} antibody covalently linked to an acridinium ester-BSA carrier. The TSPAE acridinium ester is a new generation of high yield acridinium esters developed by Siemens for enhanced chemiluminescent detection. Simultaneous addition of solid phase reagent and detection reagent to the sample forms a classic sandwich immune complex which is subsequently washed. Chemiluminescence is initiated and measured. Relative light units are directly proportional to the cTnI concentration.

Results: The assay range is from the Limit of Detection, LoD, to 25,000 pg/mL. The LoD, is determined with 2 reagent lots on 2 Centaur XP's collecting n = 60 replicate measurements for each of 10 serum samples from a normal specimen collection. The LoD was 1.18 pg/mL and 1.24 pg/mL for two reagent lots. The Limit of Blank, LoB, was determined non-parametrically by rank order calculating the 95% percentile as described by CLSI Guideline EP17. The LoB is 0.32 pg/mL and 0.51 pg/mL for the two reagent lots. The LoQ is 2.57 pg/mL defined as the cTnI concentration at 20% Total CV. The dose at 10% Total CV is 3.97 pg/mL. The 99th percentile estimate using a healthy population of n = 194 had a non-parametric value of 37.51 pg/mL in agreement with the 99th of 40 pg/mL claimed for the ADVIA Centaur TnI-Ultra method. The % normals above the LoD exceeded 90%. The Total CV at the 99th is 4.2% determined on 3 Centaur XP's using 3 reagent lots collecting 324 replicate measurements over a period of 5 days.

Conclusion: The high sensitivity cTnI in development by Siemens for the ADVIA Centaur Immunoassay Systems has a 10% Total CV at a cTnI concentration 10-fold lower than the 99th percentile, while the 100 uL sample volume is unchanged compared to the previous contemporary sensitive assay generation. This new assay represents an 8- to 10- fold sensitivity increase over current contemporary cTnI methods and will afford a new analytical window at low cardiac troponin I levels allowing for safe evaluation of clinical delta changes. *Under development. Not available for sale. Product availability will vary by country. ADVIA Centaur and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates.

A-076

Cardiac Troponin I Gender-Specific Reference Intervals and 99th Percentile Cutoffs of the Point-of-Care Assays PATHFAST™ cTnI and cTnI-II

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Background

cTn assays which have been classified as high-sensitivity assays demonstrated higher 99th percentile values in males vs. females. 1.2 – 2.4-fold higher 99th percentiles in males than in females have been reported for hs cTnI assays recently. Thus, the ability to determine sex-specific cutoffs has become an additional criterion for high-sensitivity cTn assays.

Objective

We thought to determine gender specific reference intervals including 99th percentile upper reference limits (URL) (sex-specific cutoffs) of cTnI for the POC system PATHFAST™ (LSI Medience Corporation, Tokyo).

Methods

We determined 99th percentiles for the assays PATHFAST™ cTnI (A) and PATHFAST™ cTnI-II (B) which are identical methods but standardized by using different concentrations of the NIST standard SRM 2921.

Lithium heparin plasma samples were obtained from 474 presumable healthy individuals (236 women and 238 men, mean age 51 ± 14 years, range 18 – 86 years) in whom chronic diseases or cardiac disorders were excluded by comprehensive evaluation including assessment of blood pressure, ECG registrations, oral glucose tolerance tests, TSH-, creatinine-, HbA1c-, NT-proBNP-concentration and cardiac magnetic resonance imaging without pathological findings.

Results

1. Higher cTnI values were found in males than in females: (A) mean (highest value): 5.58 (24.05) ng/L and 1.77 (14.49) ng/L; (B) mean (highest value): 6.11 (24.05) ng/L and 4.60 (42.84) ng/L, respectively.

2. Only slightly higher cTnI values were found in subjects aged >65 years compared to subjects aged <65 years. The highest values in males aged >65 years were 12.08 ng/L (A) and 27.40 ng/L (B) and didn`t exceed the manufacturer recommended 99th percentile cutoffs (A: 20 ng/L; B: 29 ng/L). Nevertheless, a slightly increasing tendency of cTnI concentration with age could be observed.

3. According to CLSI C28-A3 the following 99th percentiles were obtained: A overall 15.46 ng/L, males 16.9 ng/L, females 11.5 ng/L; B: overall 27.47 ng/L, males 31.3 ng/L, females 24.9 ng/L.

4. cTnI values were detected above the LoD (A: 1.0 ng/L; B: 3.0 ng/L) in 362 and 355 samples, respectively, demonstrating that 76.3 % and 74.9% of normal subjects revealed detectable values by using PATHFAST™ cTnI and PATHFAST™ cTnI-II, respectively.

Conclusion

PATHFAST cTnI is the first POC assay demonstrating fulfillment of the analytical criteria for high-sensitivity cTn assays: Imprecision (CV) at the 99th percentile value < 10%, detectable values above the limit of detection in 76.3% respectively 74.9% of healthy individuals.

Additionally, we could establish overall and gender specific reference intervals demonstrating significantly higher 99th percentile cutoffs in males than in females. Remarkably, the cutoffs in males differed not significantly from the overall cutoff and might be regarded as negligible in view of the biological variability. In contrast, the cutoffs in females were significantly lower than the overall cutoffs and should be taken into consideration for the diagnostic interpretation of PATHFAST cTnI/cTnI-II values to avoid underdiagnosing of acute and chronic cardiovascular diseases in women.

A-077

Plasma kynurenine-tryptophan ratio reflects metabolic inflammation, kidney function and homoarginine levels in a cardio-vascular high-risk cohort

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Background: Kynurenine (KYN) is a metabolite of tryptophan (TRP) produced by the enzyme indoleamine 2,3-dioxygenase. Apart from exerting effects on neuro-regulation and cancer immunology, KYN and its metabolites impact on vascular inflammation, endothelial integrity, and oxidative stress. KYN or the KYN-TRP ratio have recently been suggested to predict acute and chronic prognosis in cardio-vascular disease.

Methods: This study aimed at investigating plasma KYN and KYN/TRP and their dependence on various clinical and biochemical factors in 229 patients carrying automatic implantable cardioverters/defibrillators (AICD), with ~75% of them suffering from systolic heart failure (CHF), ~69% from coronary artery disease (CAD), and 25% from type-2 diabetes (T2D).

Results: Univariate linear regression analysis showed that KYN/TRP was associated with plasma high-sensitivity C-reactive protein (hsCRP), glomerular filtration rate (GFR), homoarginine, zonulin, carbonylated proteins, ADMA, and left ventricular ejection fraction, but not with other clinical routine and biochemical parameters (body mass index, sex, age, CAD severity, NT-proBNP, oxidized LDL, calprotectin, myeloperoxidase, nitrotyrosine). The subsequent multiple linear regression analysis (Generalized Linear Model) showed highly significant associations with hsCRP (positive) and GFR (negative) ($p < 0.001$ each) and a moderate negative association with homoarginine ($p = 0.030$).

Conclusion: These findings may support reports on the involvement of KYN in the phenomenon of metabolic inflammation; the negative correlations of KYN/TRP with GFR and homoarginine possibly reflect both impaired excretion and vascular dysfunction in patients with CHF and CAD.

A-078

Plasma homoarginine associates with circulating zonulin and tryptophan in a cardio-vascular high-risk cohort

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Background: Homoarginine (hArg) is an endogenous, non-proteinogenic amino acid which differs from arginine by an additional methyl group. It is mainly produced in the kidneys where the enzyme L-arginine:glycine amidinotransferase (AGAT) converts it from lysine. Recently, several epidemiological studies have identified low hArg levels as an independent risk marker for cardiovascular, cerebrovascular and renal diseases as well as for mortality. Whether hArg is a causal pathophysiological factor is a matter of ongoing debate; hArg is known to impact on NO synthesis as well as on energy metabolism.

Methods: This study aimed at investigating plasma hArg and its dependence on various clinical and biochemical factors in 229 patients carrying automatic implantable cardioverters/defibrillators (AICD), with ~75% of them suffering from systolic heart failure (CHF), ~69% from coronary artery disease (CAD), and 25% from type-2 diabetes (T2D).

Results: Univariate linear regression analysis showed that hArg was associated with plasma zonulin, tryptophan, glomerular filtration rate (GFR), and body mass index (BMI), but not with other clinical routine and biochemical parameters (sex, age, left ventricular ejection fraction, CAD severity, NT-proBNP, oxidized LDL, calprotectin, myeloperoxidase, nitrotyrosine, carbonylated proteins, ADMA, hsCRP, kynurenine). The subsequent multiple linear regression analysis (Generalized Linear Model) showed highly significant positive associations with zonulin ($p < 0.001$) and tryptophan ($p = 0.004$ each) and a moderate positive association with BMI ($p = 0.025$).

Conclusion: The hitherto unknown association of hArg and zonulin, an important tight-junction regulator, deserves further investigation and may pose a novel pathophysiological link. Regarding hArg and tryptophan, both are inhibitors of different isoenzymes of alkaline phosphatase and may be linked via the metabolism of biogenic amines.

A-079

Measurable and Undetectable Cardiac Troponin Concentrations in Men and Women Using High-Sensitivity Assays With Sex-Specific 99th Percentiles.

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Background: Using high-sensitivity cardiac troponin (hs-cTn) assays, sex-specific 99th percentiles are recommended. Our objective was to determine whether differences exist between men and women in both the proportion of measurable (≥LoD to sex-specific 99th percentiles) and undetectable (<LoD) cTn concentrations in healthy individuals.

Methods: Lithium heparin plasma samples were obtained from apparently healthy volunteers and cTn was measured across five hs-assays: 1) Abbott ARCHITECT hs-cTnI, 2) Beckman Access 2 hs-cTnI, 3) Roche Cobas e601 hs-cTnT, 4) Siemens Dimension Vista hs-cTnI, and 5) Singulex Erenna hs-cTnI. LoD and sex-specific 99th percentiles according to published peer-reviewed literature are shown in Table.

Results: The proportion of measurable (≥LoD to sex-specific 99th percentiles) and undetectable (<LoD) cTn concentrations was examined across hs-assays. Men had a higher proportion of measurable cTn concentrations than women in 4 out of 5 hs-assays. All hs-cTnI assays provided measurable concentrations above 50% for men and women, while hs-cTnT did not. The proportion of undetectable cTn values varied significantly across assays with women having a higher proportion of undetectable values in contrast to men in 4 out of 5 assays. Using the hs-cTnT assay, marked differences were observed between men and women in both the proportion of measurable (F 7% vs. M 43%) and undetectable (F 93% vs. M 57%) values.

Conclusion: Substantial variation exists between the proportion of measurable and undetectable cTn values using hs-assays among men and women. These findings have significant clinical implications, as the ability to provide measurable and undetectable hs-cTn concentrations is key when considering the use of hs-assays for both primary prevention and ruling-out acute myocardial infarction.

Manufacturer, assay	N	LoD, ng/L	99th percentile, ng/L	Excluded values above the 99th percentile, n	Measurable values ≥LoD – 99th percentile	Proportion of undetectable values (<LOD)
Abbott ARCHITECT hs-cTnI	F: 252 M: 272	1.9	F: 16 M: 34	F: 2 M: 2	F: 67% (168/250) M: 80% (215/270)	F: 33% (82/250) M: 20% (55/270)
Beckman Access 2 hs-cTnI	F: 252 M: 272	2.5	F: 9 M: 11	F: 12 M: 16	F: 73% (175/240) M: 87% (222/256)	F: 27% (65/240) M: 13% (34/256)
Roche Cobas e601 hs-cTnT	F: 252 M: 272	5	F: 14 M: 22	F: 1 M: 2	F: 7% (17/251) M: 43% (115/270)	F: 93% (234/251) M: 57% (155/270)
Siemens Dimension Vista hs-cTnI	F: 239 M: 264	0.5	F: 33 M: 55	F: 5 M: 3	F: 82% (191/234) M: 90% (234/261)	F: 18% (43/234) M: 10% (27/261)
Singulex Erenna hs-cTnI	F: 252 M: 272	0.1	F: 15 M: 27	F: 8 M: 5	F: 100% (244/244) M: 100% (267/267)	F: 0% (0/0) M: 0% (0/0)

A-081

Preliminary performance assessment of a new integrated POC biosensor for quantitative detection of BNP

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Background: Based on proprietary Magnotech biosensor technology, Philips is developing the Minicare system which consists of a handheld analyzer, the Philips Minicare I-20, and a disposable self-contained cartridge. The system is able to show test results on the reader display within minutes, at the point-of-care (POC). The Philips Minicare BNP* assay under development is for the quantitative measurement of B-type natriuretic peptide (BNP) in EDTA blood or plasma specimens using the Minicare I-20 instrument. It is targeted to be used in the emergency department as an aid in the diagnosis of heart failure. The Minicare BNP* assay is a one-step sandwich immunoassay. An anti-BNP antibody printed onto the base cartridge captures the analyte while a second anti-BNP antibody coupled to magnetic nanoparticles recognizes complexes of capture antibody and BNP thereby allowing detection by f-TIR detection optics.

Objective: To assess the preliminary performance of the Philips Minicare BNP* prototype against commercially available BNP assays.

Methods: All samples were collected at Diagnostiek Voor U, a diagnostic services lab which receives samples from hospitals and GP offices in the Netherlands. 121 fresh EDTA whole blood samples from patients who had a BNP test request were tested on the Minicare BNP* and on a commercially available POC BNP test (Alere Triage, Alere, Galway, Ireland). Next, collection tubes were centrifuged and EDTA plasma samples were run on a core lab BNP assay (BNP Centaur XP, Siemens, Marburg, Germany). Measurements on all three analyzers were performed within 8 hours after blood collection.

Results: The median BNP concentration measured on the Minicare BNP* was 62.8 ng/l with interquartile ranges (IQR) of 32.3 and 219.8 ng/L. Passing-Bablok regression demonstrated a Pearson correlation coefficient for the Minicare BNP* vs Alere Triage of R=0.979. Similarly, when the Minicare BNP* was compared to the core lab assay (Centaur XP), the correlation coefficient was R= 0.947.

Conclusion: The Minicare BNP* correlated very well with both, POC and core lab BNP assays, indicating that the future Minicare BNP* POCT will be able to deliver on-the-spot results on whole blood samples, comparable to core lab assays without the need for sample pretreatment or centrifugation.

*Minicare BNP is under development. Not available for sale.

A-083

Comparison of three troponin assays for rates of analytic false positive and negative results and percent of samples from emergency department patients exceeding 99th percentile values

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Background

Analytic false positive troponin results have been described for most methods and adversely impact interpretation of troponin results and management of patients. Use of more sensitive troponin reagents in the Emergency Department (ED) may result in more troponin values exceeding the 99th percentile value, complicating rapid evaluation of patients for acute coronary syndrome. In this study we compared the analytic false positive and false negative rates of the Roche 4th generation Troponin T STAT (cTnT), Abbott Architect Troponin I (cTnI), and Abbott Architect high-sensitive Troponin I (hs-TnI) assays. We also compared the number (percent) of samples submitted from ED patients that were above the 99th percentile cut-off value for each assay.

Methods

Rapid clot serum tubes (RST) collected from adult ED patients received for clinical cTnT testing on the Roche cobas e411 immunoassay analyzer (e411) were used for the study. Samples were centrifuged for 5 minutes at 4000 x g prior to cTnT analysis. Within 15 minutes of testing on the e411, RST samples (N=3023) were placed on an Abbott Architect 2000i for analysis of cTnI and hs-TnI, then refrigerated at 2-8°C within 2 hours of Architect analysis. Within 24 hours of initial analysis, samples were warmed to room temperature, aliquoted and re-centrifuged at 1500 x g for 15 minutes, and re-analyzed in duplicate on all three methods.

We defined analytic false positive as:

• initial value >99th percentile for assay; with both replicates ≤99th percentile ***and*** both replicates differing by >10% of initial value (failure to repeat within 10% around cut-off value) ***or***

• any replicate >99th percentile for assay and differing by >50% from any other replicate (flier or outlier)

We defined analytic false negative as:

• initial value ≤99th percentile; with both replicates > 99th percentile and both replicates differing by >10% from initial value (failure to repeat within 10% around cut-off)

The 99th percentile values used were < 0.01 ng/mL (cTnT), ≤ 0.028 ng/mL (TnI), and ≤ 26 ng/L (hsTnI).

Results

For cTnT 19/3023 (0.06%) ED samples analyzed resulted in analytic false negatives, while 21/3023 (0.07%) were analytic false positives. For cTnI only 4/3023 (0.01%) analytic false negatives were observed, while 100/3023 (3.3%) of samples were analytic false positives upon repeat analysis. hsTnI demonstrated the best analytic performance with 2/3023 (<0.01%) analytic false negatives and 9/3023

(0.03%) analytic false positives. 1997/3023 (66%) of cTnT values among ED patients were <99th percentile; compared to 2217/3023 (73%) of cTnI values and 2143/3023 (71%) of hsTnI values. Using gender-specific hsTnI 99th percentile values of ≤ 15 ng/L (female) and ≤ 36 ng/L (males), 2129/3023 (70%) of ED samples submitted were ≤ 99th percentile.

Conclusions

Abbott hsTnI had fewer analytic false positive results than Abbott cTnI, and fewer false negative and positive results than Roche cTnT. The percent of ED samples analyzed that were >99th percentile did not differ significantly between assays. The results suggest that use of hsTnI will improve analytic performance in stat measurement of troponin without impacting ED workflow in the evaluation of patients for acute coronary syndrome.

A-084

Development of an NT-proBNP Assay* Using Acridinium Ester Technology on the ADVIA Centaur Immunoassay Systems

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Background: The measurement of NT-proBNP is a useful aid in the diagnosis and assessment of severity of congestive heart failure. Siemens Healthcare is currently developing an NT-proBNP assay for serum and plasma on the ADVIA Centaur® Immunoassay Systems.

Methods: The ADVIA Centaur NT-proBNP (PBNP) assay* is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology. The development of a new acridinium ester (TSP-AE) by Siemens has enabled good precision throughout the assay range. NT-proBNP reagents include a biotinylated sheep monoclonal antibody in the Ancillary reagent, a second sheep antibody labeled with the newly developed acridinium ester in the Lite reagent, and streptavidin-coated paramagnetic latex particles in the solid phase. Samples are incubated with the Ancillary reagent and solid phase to form a PBNP/biotinylated antibody/solid phase complex. Lite reagent is added and allowed to incubate, resulting in the formation of an acridinium ester/PBNP/biotinylated antibody/solid phase complex. Separation occurs, and the signal is proportional to the concentration of NT-proBNP in the sample.

Results: The method requires 20 µL of serum or plasma. Time to first result is 18 minutes, with stable calibration for 28 days. 28-day open-well stability has been achieved. Linearity was demonstrated over the range of <LOQ to >35,000 pg/mL. With automated dilution, the measuring interval is extended to 350,000 pg/mL. Equivalent results were obtained among serum, lithium heparin plasma, and EDTA plasma. Reproducibility was assessed using the CLSI EP5-A2 protocol with serum samples ranging from 84 to 30,145 pg/mL. Repeatability CVs ranged from 1.4 to 3.5%. Within-lab CVs ranged from 2.0 to 4.3%. In accordance with the CLSI EP7-A2 protocol, no interference was observed with 75 ng/mL biotin. Split-sample correlation between this method and the Roche cobas e 411 proBNP II assay produced the following statistics by Passing-Bablok analysis: slope = 1.06, intercept = -5 pg/mL, r = 0.9940, and n = 178 over a concentration range of 31-30,542 pg/mL. Over the concentration range of 31-4819 pg/mL, these statistics are as follows: slope = 1.07, intercept = -11 pg/mL, r = 0.9965, and n = 142.

Conclusion: The ADVIA Centaur NT-proBNP assay demonstrates good precision and good correlation to the Roche proBNP II assay.*Under development. Not available for sale.

A-085

N-glycosylation of NT-IGFBP-4 does not influence its immunodetection by the neo-epitope specific sandwich immunoassay

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Background: N- and C-terminal proteolytic fragments of IGFBP-4 (NT-IGFBP-4, 14.6 kDa, and CT-IGFBP-4, 11.3 kDa) are strong predictors of major adverse cardiac events risk in patients presented with ischemia and stable patients with type 1 diabetes. The presence of these fragments in the circulation depends on the proteolytic activity of metalloprotease PAPP-A, which specifically cleaves IGFBP-4 between Met135 and Lys136. We have previously developed sandwich immunoassays based on monoclonal antibodies that are specific to proteolytic neo-epitopes on NT-IGFBP-4 and CT-IGFBP-4 and have less than 1% of cross-reactivity to the full-length IGFBP-4. It has been shown that a fraction of circulating IGFBP-4 contains glycosylated Asn104 that is located close to the PAPP-A-specific cleavage site. Our assay for NT-IGFBP-4 relies on an antibody that has a binding epitope which is in the vicinity of Asn104 and hence the NT-IGFBP-4 assay may be affected by the glycosylation of this amino acid residue. Therefore, the aims of this study were: a) To determine NT-IGFBP-4 glycosylation levels in individual EDTA-plasma samples and... b) To evaluate the influence of glycosylation on the immunodetection of NT-IGFBP-4.

Methods: IGFBP-4 and its proteolytic fragments were extracted from twelve individual EDTA plasma samples of ACS patients by immunoprecipitation and analyzed using enhanced chemiluminescence immunoblotting (ECL). Concanavalin A sepharose was used to verify the presence of glycosylated forms of IGFBP-4 and NT-IGFBP-4. The levels of IGFBP-4 and NT-IGFBP-4 were measured using sandwich HRP immunoassays. The precise masses of purified glycosylated and non-glycosylated NT-IGFBP-4 were confirmed by mass spectrometry. Increases in the concentrations of the proteolytic fragments of IGFBP-4 during the incubation with recombinant PAPP-A were used as measures of the proteolysis rates of glycosylated and non-glycosylated IGFBP-4.

Results: The investigation of individual EDTA plasma samples revealed that of the total circulating IGFBP-4, 47.2-61.7% was glycosylated. Meanwhile, of the total NT-IGFBP-4, only 9.8-23.5% was glycosylated. Mass spectrometric analysis of NT-IGFBP-4 extracted from pooled EDTA plasma revealed two peaks of 17260 and 14615 Da that corresponded to glycosylated and non-glycosylated forms respectively. The immunoreactivities of endogenous glycosylated and non-glycosylated NT-IGFBP-4 differed by less than 10% when measured using HRP or ECL based immunoassays. PAPP-A-dependent proteolysis of glycosylated IGFBP-4 was 3-4 times less efficient compared to proteolysis of non-glycosylated IGFBP-4.

Conclusion: For the first time, the presence of glycosylated NT-IGFBP-4 in human plasma was shown and the proportion of glycosylated and non-glycosylated NT-IGFBP-4 was measured. PAPP-A-dependent proteolysis of glycosylated IGFBP-4 is less efficient if compared with the proteolysis of non-glycosylated IGFBP-4, although it is not completely inhibited. The glycosylated NT-IGFBP-4 displays the same immunoreactivity as non-glycosylated NT-IGFBP-4 in the fragment-specific immunoassay. Thus, this sandwich immunoassay can be used for the reliable measurement of NT-IGFBP-4 in the blood of patients.

A-086

Influence of troponin-specific autoantibodies on measurements of cardiac troponin I in binary and ternary complexes

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Background: Autoantibodies specific to cardiac troponins (TnAAbs) have been found in the blood of 6-7% of the population. It is believed that TnAAbs may negatively affect cardiac troponin I (cTnI) measurements in the blood of patients with acute myocardium infarction (AMI) by immunoassays that utilize monoclonal antibodies (mAbs) recognizing certain epitopes in the mid-part of the cTnI molecule. In the current study we investigated the epitope specificity of TnAAbs and their influence on the cTnI immunodetection in AMI samples. **Methods:** The presence of TnAAbs in plasma samples from healthy donors was detected according to Eriksson et al., 2004. 191 plasma samples were spiked with ternary cTnI-cTnT-TnC complex and cTnI recovery was measured using an immunoassay sensitive to the presence of TnAAbs. Twelve plasma samples showing low cTnI recovery were selected and studied for TnAAbs epitope specificity. cTnT recovery was measured in the same plasma samples. The mapping of sites on cTnT that are affected by TnAAbs was performed by using eleven anti-cTnT mAbs. The effect of TnAAbs on the measurements of cTnI in blood of AMI patients was analyzed after mixing TnAAbs-containing plasmas 1:1 with the plasma samples of AMI patients (n=35; cTnI concentrations from 2.5 to 35.1 µg/L). **Results:** Human cardiac troponins (cTnI, binary cTnI-TnC or ternary cTnI-cTnT-TnC complex) were spiked into twelve TnAAbs-containing plasma samples to the concentration 50 µg/L and the recoveries of cTnI were analyzed. The well-pronounced inhibitory effect of TnAAbs on cTnI measurements (mean recovery 10.3%) was observed only when cTnI was added in the form of a ternary complex. The inhibitory effect was significantly lower with spiked cTnI-TnC complex or free cTnI (mean recoveries 71.0% and 96.5%, respectively). Since cTnT appeared to be important for the manifestation of the negative interference of TnAAbs on cTnI measurements, the influence of TnAAbs on cTnT measurements was also studied. Only one epitope (223-242 aar) of cTnT was influenced by TnAAbs. The inhibitory effect of TnAAbs on cTnT detection (mean recovery 14%) was only found with spiked ternary complex, whereas it was much less pronounced with spiked free cTnT (mean recovery 73%). Since the inhibitory effects of TnAAbs on the detection of both cTnI and cTnT were observed only for the cTnI-cTnT-TnC complex, we suggest that TnAAbs are specific to structural epitopes that are formed by closely located cTnI and cTnT polypeptide chains. The negative effect of TnAAbs on the measurements of endogenous cTnI in AMI samples was significantly less pronounced compared to measurements of spiked cTnI in the form of ternary complex. The mean recovery was 61.4% vs. 10.3%, respectively. **Conclusion:** Unlike the common notion, our study shows that the anti-cTnI TnAAbs are not specific to cTnI per se but to the structural epitopes formed by cTnI and cTnT polypeptide chains. In our experiments, added TnAAbs have a rather limited impact on the immunodetection of cTnI in AMI samples probably because TnAAbs affect the measurements of cTnI and cTnT in ternary complex, whereas the predominant form of cTnI in the blood of patients is believed to be a cTnI-TnC binary complex.

A-087

Antibodies and recombinant standards for the Lp-PLA2 fluoroimmunoassay.

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Background. Lipoprotein-associated phospholipase A2 is an enzyme that plays an important role in vascular inflammatory processes involved in the development of atherosclerosis. The blood level of Lp-PLA_2 has been shown to correlate with the risk of adverse coronary events in apparently healthy people as well as in patients with stable coronary disease. The blood concentration of Lp-PLA_2 is currently measured by immunoassays. The aim of this study was to develop a reliable and sensitive Lp-PLA2 immunoassay. To facilitate this, we developed monoclonal antibodies that detect Lp-PLA_2 with high sensitivity and are capable of recognizing Lp-PLA_2 as a part of lipoprotein complexes, as well as a recombinant antigen with biochemical and immunochemical properties similar to the native protein presented in human blood.

Methods. Recombinant human Lp-PLA_2 was produced in two different expression systems: human cell line expi293f and insect cell line HighFive. The recombinant proteins were purified by several chromatographic steps. The recombinant Lp-PLA2 was used as the immunogen in antibody development. Six murine monoclonal antibodies (mAbs) specific to human Lp-PLA_2 that was obtained were tested as capture and detection (labeled with stable Eu^{3+} chelate) antibodies in sandwich fluoroimmunoassays. Serum samples from patients with acute myocardial infarction (AMI) and healthy donors were used in the analyses. Serum dilution experiments were performed in serum specimens from healthy donors.

Results. Two mAb combinations, PL26- PL4 and PL42-PL46 (capture - detection) recognized both recombinant Lp-PLA_2 preparations in a similar manner. They demonstrated a good linearity range (2-600 ng/ml and 1-1000 ng/ml respectively) and required sensitivity (0.2 ng/ml for PL42-PL46).

Both assays also recognized native human Lp-PLA_2 in the blood of two groups of donors - healthy volunteers and AMI patients. The titration curves of serum samples and recombinant proteins were parallel, which suggests that native (from human blood) and recombinant forms of Lp-PLA_2 have similar immunoreactivity in these fluoroimmunoassays. When spiked into normal human serum both recombinant Lp-PLA_2 proteins formed complexes with serum lipoproteins and in gel-filtration studies were found in the same lipoprotein fractions as native Lp-PLA_2. In stability studies, both recombinant Lp-PLA_2 forms demonstrated consistent immunoreactivity during 6 months of incubation of the protein solution (1 mg/ml) at +4°C.

Conclusions. Monoclonal antibodies obtained in this study could be used for the development of a sandwich immunoassay for quantitative measurements of Lp-PLA_2. Both recombinant forms of human Lp-PLA_2 demonstrate immunoreactivity which is similar to that of native protein presented in human blood and could be used as standards or calibrators in an Lp-PLA_2 immunoassay.

A-088

Different susceptibility of BNP and proBNP to neprilysin cleavage suggests a limited effect of neprilysin inhibition by LCZ696 on the level of immunoreactive BNP

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Background: The new FDA approved HF drug LCZ696 (Entresto™, Novartis), which combines the neprilysin inhibitor and the angiotensin II receptor inhibitor, has stimulated the interest of the cardiology community in neprilysin. This ubiquitous protease is responsible for the degradation of various important vasoactive peptides, including natriuretic peptides (NPs). In light of this, the inhibition of neprilysin activity for the augmentation of the endogenous NPs concentrations has been considered to be a potential therapeutic strategy in HF. It was suggested that by increasing the circulating concentrations of B-type natriuretic peptide (BNP), LCZ696 could make BNP measurements ambiguous and misleading from a diagnostic perspective. However, the major form of plasma BNP-immunoreactivity in HF patients is represented by its uncleaved precursor proBNP, which differs from BNP due to the presence of the 76 amino acid N-terminal extension. Therefore, the inhibition of neprilysin in HF patients should affect the level of proBNP rather than BNP. To the best of our knowledge, neprilysin mediated degradation of proBNP has never been analyzed. Furthermore, susceptibility of different BNP epitopes to neprilysin-dependent proteolysis is unknown. Hereby, the aim of the present study was to compare the susceptibility of two different epitopes within the central region of BNP and proBNP to cleavage by neprilysin.

Methods: BNP 1-32 (synthetic) as well as non-glycosylated (expressed in E. coli) and glycosylated (expressed in mammalian cells) forms of proBNP 1-108 were incubated with human recombinant neprilysin for different time periods. The susceptibility of two different epitopes of BNP and proBNP that are recognized by antibodies in commercial BNP assays to neprilysin cleavage was analyzed using two sandwich immunoassays. In the first assay, the mAb KY-BNP-II (epitope 14-21) was used as a capture antibody and the mAb 50E1 (epitope 26-32) was used as a detection antibody. The second assay was the Single Epitope Sandwich BNP assay (SES-BNP™) that is specific to the epitope 11-17. Mass spectrometry was applied to determine the sites of BNP cleavage by neprilysin.

Results: Both forms of proBNP, glycosylated and non-glycosylated, were resistant to the degradation by neprilysin. As follows from both immunochemical and MS analysis, proBNP remained intact even after prolonged incubation with neprilysin. In the case of BNP, the epitope 14-21 that contains the known Arg_{17}-Ile_{17} neprilysin cleavage site was much more susceptible to cleavage by neprilysin than the epitope 11-17 that was utilized in the SES-BNP assay.

Conclusion: Our findings demonstrate that the major BNP-immunoreactive form, proBNP, is not susceptible to neprilysin cleavage. On this basis, we speculate that modulation of neprilysin activity by specific inhibitors (e.g. LCZ696) may not greatly affect the circulating concentrations of immunoreactive BNP, which in HF is mostly represented by intact proBNP. The different stability of BNP epitopes highlights the importance of the choice of antibodies for reliable BNP immunodetection: BNP immunoassays that utilize antibodies with epitopes comprising the site Arg_{17}-Ile_{18} are expected to be more sensitive to proteolysis by neprilysin than immunoassays that utilize antibodies with other specificity.

A-089

Younger age and women lower the upper reference limit for high-sensitivity troponin T in a large multi-ethnic population

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Purpose: We sought to ascertain the effect of age on the 99th percentile upper reference limit (99PURL) for high-sensitivity cardiac troponin-T (hs-cTnT) from a large multi-ethnic population as the current recommended cut-point of 14 ng/L is derived from younger subjects while patients evaluated for chest pain are older.

Methods: Previously we found the hs-cTnT all-subject, male, and female 99PURL to be 16.0, 18.0 and 11.0 ng/L respectively from 1659 (816 men) apparently healthy subjects (questionnaire) aged 30-65 (mean 46.6±10.12). An additional 572 healthy persons aged 20-29 (241 men) and 233 individuals aged 66-98 (124 men) were studied. The hs-cTnT assay has a limit of detection (LoD) of 5 ng/L. and a 10% assay CV corresponding to 13 ng/L.

Results: Hs-cTnT concentrations (range: <3–64 ng/L) are shown in the Table. Detectable hs-cTnT concentrations (>LoD) were seen in 32.1% of all participants, and higher in men (51.1%) than women (14.7%). In both the older group (<98y) and the younger cohort (<65y) successive inclusion of younger subjects below 50 years resulted in a lowering of the hs-cTnT 99PURL.

Conclusion: Younger age decreases the troponin-T 99PURL due to a higher proportion of undetectable (<LOD) hs-cTnT values especially in women as the hs-cTnT assay may not exhibit high sensitivity performance in these subjects. Composition of the reference population, especially age and sex, impacts the determination of the hs-cTnT 99PURL. Separate hs-cTnT cut-points for chest pain evaluation and community screening may be needed. As troponin 99PURL is a key decision metric in cardiology, requisite guidelines for constituting a reference population should be provided.

AGE GROUP		50-98	40-98	35-98	30-98	20-98	50-65	40-65	35-65	30-65	20-65
Males	n	465	704	821	940	1181	341	580	697	816	1057
	Mean Age y	61.2	55.5	52.8	50.2	45.1	56.9	51.7	49.2	46.7	41.8
	TnT>LoD %	77.6	65.8	60.8	56.7	51.1	67.4	57.4	52.9	49.4	44.6
	99PURL ng/L	34.0	29.9	27.6	25.6	24.2	19.6	18.2	18.0	18.0	17.0
Females	n	458	701	827	952	1283	349	593	718	843	1174
	Mean Age	60.8	55.2	52.4	49.7	43.3	56.7	51.8	49.2	46.6	40.5
	TnT>LoD %	35.6	25.2	21.9	19.5	14.7	24.9	16.9	14.5	12.9	9.4
	99PURL	21.4	19.0	16.9	16.0	16.0	15.0	12.0	11.0	11.0	10.0
ALL	n	923	1405	1648	1892	2464	690	1173	1415	1659	2231
	Mean Age	60.9	55.3	52.6	49.9	44.2	56.8	51.8	49.2	46.6	41.1
	TnT>LoD %	56.8	45.6	41.3	38.0	32.1	45.9	36.9	33.4	30.9	26
	99PURL	25.8	23.9	23.0	23.0	21.0	18.1	17.0	16.8	16.0	14.2

A-090

A whole blood high-sensitivity cardiac troponin assay for POCT

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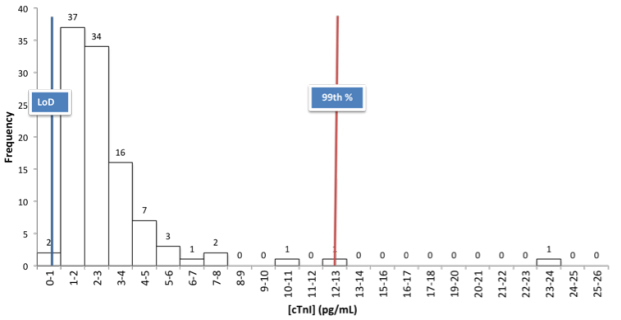
Background: The potential life-saving and cost-reduction value of high-sensitivity cardiac troponin (hs cTnI) in the emergency room, in particular as a rule-out, has captured wide attention. We are developing a fully automated hs cTnI assay using the Pylon™ technology designed to deliver central lab performance in the point-of-care setting (abstract submitted). A Pylon test strip with a quartz-glass probe tip, coated with analyte-specific antibody, moves through a test strip as it picks up the sample and goes through the steps of a classic sandwich assay. The assay uses epitopes for capture and signal antibodies common to hs cTnI assays reported in the literature.

Methods: The reference range study used 500 EDTA plasma samples from presumably healthy individuals and calibration values assigned by NIST SRM 2921. To assess analytical performance using whole blood, samples from 105 healthy blood bank donors were tested against corresponding EDTA plasma samples.

Results: The Pylon cTnI assay detected more than 95% of the healthy group samples. Preliminary data showed that 99th percentile is estimated to be 12 pg/mL, with precision of <10% CV. LoD is within the range of 0.5-1.0 pg/mL; LoQ is ~2 pg/mL. There is no interference from samples containing human anti-mouse antibodies. Spike recovery of whole blood samples is >90% at 10, 100 and 1000 pg/mL cTnI. There is good correlation between whole blood samples and corresponding plasma samples (R=0.92); precision is comparable. Quantified value of >95% whole blood samples is higher than LoD. Time to results is about 20 minutes.

Conclusions: The Pylon cTnI assay met the definition of a fourth-generation hs cTnI assay, with <10% CV at the 99th percentile and detection in >95% of reference population. This will be confirmed by testing the assay using the AACC normal range sample bank when it becomes available.



Whole blood cardiac troponin I distribution in healthy individuals (n=105)

A-092

Serial Changes in High-Sensitivity Cardiac Troponin I in Emergency Department Patients Without Acute Myocardial Infacrtion

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Background: Limited studies have reported serial changes of cardiac troponin (cTn) in patients presenting to the emergency department (ED) in the absence of acute myocardial infarction (AMI) according to cTn concentrations above or below the 99th percentile. High sensitivity (hs) cTn assays have improved total imprecision (%CV) compared to contemporary assays, allowing a better assessment of serial cTn changes with minimal analytical influence.

Methods: The objective of our study was to examine serial changes in hs-cTnI measured by a research assay (Abbott ARCHITECT; gender-specific 99th percentile cutoffs (GSCs): F 16 ng/L (%CV=5.3), M 34 ng/L (%CV=3.5); analytical limit of detection (LOD): 1.9 ng/L). 1159 patients presenting to the ED who underwent serial cTnI measurements on clinical indications (UTROPIA, NCT02060760) at presentation (baseline) and at 2 to 6 hours and had both concentrations above the LOD were included. Log normal percent reference change values (RCVs, %) based on the median of within-individual cTnI variations were assessed among patients with or without AMI and with baseline cTnI concentrations above or below the GSC.

Results: Patients with AMI (74 female, 83 male) had greater absolute and percent hs-cTnI changes compared to non-AMI patients (368 female, 634 male), independent of the baseline cTnI concentration (Table). RCVs were smaller in non-AMI patients with hs-cTnI at presentation above GSC (F -21 to +26%; M -21 to +27%), compared to non-AMI patients presented with below GSC hs-cTnI concentrations (F -34 to +51%; M -26 to + 35%).

Conclusion: In patients without AMI, RCVs were smaller among those with increased hs-cTnI concentrations above the GSC at presentation in comparison to those with concentrations below the GSC. Future studies assessing serial hs-cTn

changes according to the presence or absence of increased cTn concentrations and its clinical implications needs to be explored.

Changes in troponin levels in ED patients diagnosed with AMI or non AMI.								
	Female				Male			
	Baseline hs-cTnI < GSC		Baseline hs-cTnI > GSC		Baseline hs-cTnI < GSC		Baseline hs-cTnI > GSC	
	AMI[2]	Non AMI	AMI	Non AMI	AMI[2]	Non AMI	AMI	Non AMI
Number	3	260	71	108	2	516	81	118
hs-cTnI at presentation (ng/L) [1]	13.4	5.0 (3.0-7.1)	29.5 (15.8-78.5)	24.1 (17.9-48.5)	26.6	6.7 (3.8-12.3)	59.8 (22.5-155.8)	50.5 (37.7-94.9)
Changes in hs-cTnI within 2-6 hrs (ng/L) [1]	21.8	1.1 (0.5-2.0)	24.4 (4.7-227.2) [3]	3.5 (1.3-10.9) [3]	148.3	1.2 (0.5-2.6)	47.0 (11.0-283.9)	8.2 (3.7-20.4)
Changes in hs-cTnI within 2-6 hrs (%) [1]	163	21 (10-46)	51 (15-304)	12 (5-26)	574	15 (7-34)	101 (14-324)	12 (6-25)
Percent RCV limit (%, non AMI group)		(-34-51)		(-21-26)		(-26-35)		(-21-27)
[1] Values are expressed as median (interquartile range, IQR).								
[2] IQRs not shown and comparisons with the non-AMI group not performed due to limited sample size in the group.								
[3] $P < 0.05$ from 2-tailed unpaired T test indicates significant difference between the AMI and non-AMI group.								

A-093

Regional distribution of 99th percentile of high sensitivity troponin I in China

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Background and Objective

Currently, the 99th percentile value of serum high sensitivity troponin I(hs-ThI) of the healthy population is used as a critical criteria for acute chest pain diagnosis. However, little information has been reported in Chinese population. Thus, the aim of this study is to establish the 99th percentile value for hs-ThI in a healthy Chinese population in different regions.

Patients and Methods

A total of 1,537 cases of healthy subjects were recruited from different regions of China. All patients were carefully scanned for any diseases or factors that might influence cardiac troponin levels. The measurement of hs-ThI was standardized and analyzed by ARCHITECT *i2000*. Quality Controls were monitored to ensure that the differences in regional centers were not due to the analytical system.

Results and Discussion

There were significant differences in the regional distribution of the 99th percentile as seen in Table 1, wherein for the Harbin region, the total value, the value for males and the value for females were 29.8pg/ml, 35.8pg/ml and 27.5pg/ml respectively. It was higher than the value of the corresponding groups in other regions($P < 0.05$). The total value, the value for males and the value for females in Beijing were 13.5pg/ml ,23.9 pg/ml and 8.8pg/ml respectively, which were lower than in other regions ($P < 0.05$). The overall value for Shanghai, Zhejiang, Chongqing and Xi'an showed no statistical significance. The 99th percentile was higher in males as compared to females which was also noted in many other studies conducted abroad.

Conclusion

The present study may provide a preliminary basis for the differentiation of cut-off values of 99th percentile in the Chinese population.

Table 1. Regional distribution of 99th percentile of hs-TnI in China

Region	Study Subjects	99th percentile (pg/ml)		
		Total	Male	Female
Beijing	238	13.46	23.85	8.8
Fujiang	208	26.62	28.40	13.60
Harbin	144	29.76	35.80	27.50
Lanzhou	206	22.15	29.60	19.20
Shanghai	191	19.84	22.00	14.40
Zhejiang	210	19.72	29.07	17.20
Chongqing	164	16.50	17.20	13.60
Xi'an	176	16.16	16.17	13.74
Total	1537	26.42	29.40	19.46

Tuesday, August 2, 2016

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

Clinical Studies/Outcomes

A-095

Vitamin D status of women with risk factors for gestational diabetes mellitus in Abuja, Nigeria

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Objective: Gestational diabetes mellitus is associated with maternal and perinatal morbidity and mortality. Studies have suggested that vitamin D plays a role in pancreatic beta-cell function and insulin sensitivity and has been associated with type 2 DM. Despite conflicting reports on the association between GDM and vitamin D insufficiency, vitamin D supplementation in pregnancy is practised in some developed countries. The study was aimed at determining the relationship between vitamin D status and GDM in this environment. **Methodology:** This was a case-control study involving pregnant women at 24 - 28 weeks gestation referred for oral glucose tolerance testing at the Department of Chemical Pathology of a tertiary healthcare facility over a period of 10 months (February - November, 2014). A standard 75 g OGTT was administered on subjects after 8 - 10 hours of previous overnight fast. Cases were defined based on the current WHO (2013) criteria for the diagnosis of GDM requiring a fasting-, 1 hour- or 2 hours-plasma glucose value ≥ 5.1 mmol/L, ≥ 10.0 mmol/L or ≥ 8.5 mmol/L respectively, while controls were subjects whose corresponding plasma glucose levels fell below the above stated values. One hundred cases and a hundred controls that met the eligibility criteria were recruited into the study. Socio-demographic and clinical characteristics were obtained via semi-structured interviewer-administered questionnaire/data collection form. Plasma glucose, calcium, phosphate, albumin were measured with Cobas c311 (Roche Diagnostics, GmbH) analyzer. Plasma parathyroid hormone (PTH) and 25-hydroxycholecalciferol were assayed on the Cobas e411 (Roche Diagnostics, GmbH) analyzer. Univariate, bivariate and multivariate analyses were performed using Statistical Package for Social Sciences version 20 (SPSS, Chicago, USA). **Results:** The overall mean age was 31.73 ± 4.32 years and pre-pregnancy BMI was 28.02 ± 5.12 kg/m^2. The mean age of cases was higher than that of controls with a difference of 1.95 years (95% CI: 0.227 - 3.627 years; *p*-value = 0.023). Mean pre-pregnancy BMI was higher in cases with a difference of 3.79kg/m^2 (95% CI: 1.87 - 5.70; *P*-value < 0.001). The overall mean values of plasma 25-hydroxycholecalciferol, PTH, corrected total calcium and phosphate were 28.77 ± 12.42 ng/mL, 7.69 ± 8.36 pg/mL, 2.12 ± 0.15 mmol/L, and 1.00 ± 0.25 mmol/L respectively. Overall, 58% of the subjects had plasma 25-hydroxycholecalciferol levels below 30 ng/mL (defined as vitamin D insufficiency). The proportion of cases with vitamin D insufficiency was 62%, while the proportion of controls with vitamin D insufficiency was 54%. Odds ratio for GDM was 1.39 (95% CI: 0.79 - 2.44) and *p*-value = 0.3159. Adjusted odds ratio for GDM, after logistic regression using age, pre-pregnancy BMI, history of DM in first-degree relative, average time spent outdoors daily, skin exposure, use of vitamin supplements, and fish diet as possible confounding variables was 0.984 (95% CI: 0.944 - 1.025) and *p*-value = 0.438. **Conclusion:** The results indicated that was no association between vitamin D insufficiency and gestational diabetes mellitus. However, the high proportion of vitamin D insufficiency among the study population requires appropriate attention and possible intervention.

A-096

Method Comparison Improvements: A Case Study Using Serum Free Light Chain

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BACKGROUND: Assay validation is a required process before any new test is approved for clinical testing. Patient sample comparisons between an existing and a new assay are used to determine method accuracy. Kappa and lambda free light chains (FLC) are important for diagnosis and prognosis of malignant plasma cell proliferation disorders. Historically, FLC were performed at our institution on an IMMAGE 800 analyzer (Beckman/Coulter) which was replaced by a SPAPLUS instrument (The Binding Site Company). The original method comparison indicated a high bias for kappa FLC which was deemed to be clinically significant. Due to this fact, it was decided to test all patients using both methods for a period of time to determine if a new baseline was required for our patient population. **METHODS:** Two months of serum kappa and lambda FLC samples ordered in our laboratory were assayed prospectively using IMMAGE 800 and SPAPLUS instruments**.** Both methods employ the same test principle, which depends on antigen-antibody interactions that form insoluble immune complexes. A beam of light passes through the sample and light scattering is monitored. Data analysis was performed using Excel (Microsoft) and/or EP Evaluator Release 10 (Data Innovations). EP Evaluator uses a complex iterative algorithm to identify outliers and defines outliers as points whose distance from the regression line exceeds 10 times the standard error of estimate (SEE). The SEE is computed from the data set with the outliers removed. **RESULTS:** Primary analysis of the original kappa FLC validation (N=95) using Excel revealed a high bias ~56%. A secondary analysis of the original validation data using EP evaluator including outliers yielded a slope of 0.778, an intercept of 22.4 mg/L with a bias of 9.3% for kappa FLC while excluding outliers (N=84) the slope was 2.333, the intercept was -13.0 mg/L and the bias was 57.0%. Analysis of post-validation kappa FLC samples including outliers (N=1560) determined a slope (0.842), intercept (18 mg/L) and bias (-7%) excluding outliers (N=1517) the slope (1.055),

intercept (10.5 mg/L), and bias (14.7%). Similar results were seen for lambda FLC. **DISCUSSION:** One of the first things noticed was when outlier accounted for ~12% of the original data set. All the samples that were removed were above 100 mg/L and yielded a similar bias as the Excel data. The marked difference between the original and post-validation data sets would suggest a flaw in the collection parameters for the original sample set. Although the original specimen number (N=95) exceeded our requirements for comparison (N=40), the sample distribution was significantly skewed towards the lower end of the reference interval. The subsequent data set with the increased number of patient specimens that covered the whole reference interval produced a much reduced bias. Indicating a proper comparison required not only an adequate number of samples but also an appropriate distribution of samples due to statistical sampling errors. Erroneous or incomplete validation processes have both monetary and clinical consequences that need to be critically evaluated. A consistent statistical method should be used and any deviation must have prior approval from the technical director.

A-097

MicroRNA-218 is a prognostic indicator in colorectal cancer and enhances 5-fluorouracil-induced apoptosis by targeting BIRC5

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Background: One major reason for the failure of advanced colorectal cancer (CRC) treatment is the resistance to fluoropyrimidine(FU)-based chemotherapy. The enhanced ability of tumor cells to undergo anti-apoptosis process is the main contributor to drug resistance. Previous studies have found that miR-218 was significantly down-regulated in cancer patients and had a role in cancer progression. However, the functional significance of miR-218 in CRC chemoresistance remains unknown. To further explore the possible mechanisms and promote chemosensitivity of CRC treatment, we evaluated the prognostic effect of miR-218 in patients received 5-FU-based treatment and investigated the pro-apoptotic role of miR-218 *in vitro*.

Methods: Paired resected surgical specimens from primary tumor and adjacent non-tumor sites were selected and utilized from 63 CRC patients received 5-FU-based treatment with histological diagnosis. All patients received standard follow-up with computed tomography of abdomen after operation. Reverse transcription quantitative PCR (RT-qPCR) was performed to quantify miRNAs and mRNAs expression. The apoptosis assay was performed by using the flow cytometry after staining with annexin V FITC and propidium iodide (PI). Besides, apoptotic cells were also analyzed using the One Step TUNEL Apoptosis Assay Kit to get a more visualized image. Dual-Luciferase reporter assay was carried out to find the target genes. Western blot analysis was used to detect the thymidylate synthasev (TS) and BIRC 5 protein expression.

Results: The expression of miR-218 was downregulated in HT29 and HCT116 cell lines and significantly decreased in tumor tissues compared with paired normal tissues (*P*<0.001). High miR-218 expression was associated with positive response to first-line 5-FU treatment in CRC patients. A Kaplan-Meier survival analysis indicated that patients with high miR-218 expression was associated with long OS (*P*=0.0002) and PFS (*P*=0.002). Flow cytometry showed that miR-218 increased apoptosis in both CRC cell lines and the TUNEL assay indicated that cells transfected with miR-218 showed significantly elevated fluorescence level of DNA cleavage. Cell proliferation was significantly inhibited in miR-218-transfected CRC cells. At day 5, the cell proliferation of miR-218-transfected HT29, HCT116 cells were reduced by 23% and 55% respectively. We also found that *BIRC5* mRNA and protein expression was

significantly reduced in cell lines transfected with miR-218 when compared with negative control. Moreover, luciferase assay showed that cells transfected with miR-218 significantly inhibited the luciferase activity while miR-218 did not inhibit the luciferase activity of the reporter vector containing mutated 3'UTR of *BIRC5* mRNA. We also found that miR-218 decreased the IC_{50} value of 5-FU in both cell lines. MiR-218 suppressed the expression of 5-FU-targeted TS protein, which explained the potential regulatory mechanism of miR-218 effects on enhancement of 5-FU-induced apoptosis.

Conclusion: The present work has identified miR-218 as a non-coding RNA related to the response to 5-FU based treatment in CRC patients and revealed that miR-218 promotes apoptosis by suppressing *BIRC5* protein expression in CRC. miR-218 also induces inhibition of cell proliferation and enhances 5-FU cytotoxcity *in vitro*. Thus, restoration of miR-218 levels could be a potential novel strategy to enhance chemosensitivity to 5-FU based treatment.

A-100

Combined assessment of plasma D-dimer concentration and estimated glomerular filtration rate for predicting the risk of all-cause and cardiovascular mortality in outpatients with stable coronary artery disease

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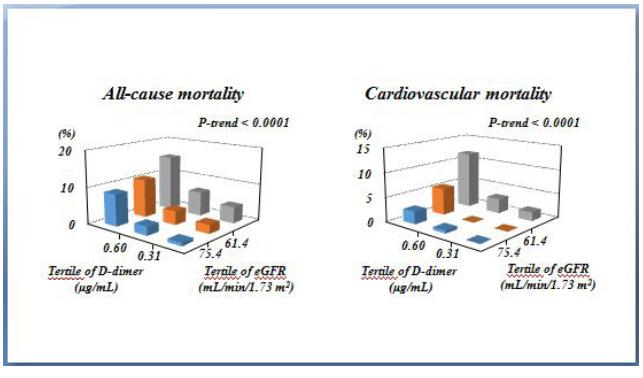
Background: Renal dysfunction is an important risk factor for cardiovascular mortality in patients with coronary artery disease (CAD); it may accelerate hypercoagulability in them. Whether the combined assessment of D-dimer level and glomerular filtration rate estimated by creatinine-based equations (eGFR) is useful for predicting mortality in outpatients with stable CAD was prospectively investigated. **Methods:** Plasma D-dimer level and eGFR were measured in 1526 outpatients (median age: 66 years; male, 77%) with angiographically documented significant coronary artery stenosis (> 50%) and/or a history of myocardial infarction, of whom 42% had a history of old myocardial infarction; 46%, history of coronary revascularization; and 32%, diabetes. **Results:** D-dimer levels significantly correlated with eGFR ($r = -0.26$; $p < 0.0001$). During a mean follow-up period of 41 months, 97 (6.4%) all-cause mortality cases were recoded, including 48 cardiovascular mortality cases. The patients who died were older (median: 73 vs. 66 years; $p < 0.0001$) and exhibited higher D-dimer (0.90 vs. 0.42 μg/mL; $p < 0.0001$) and high-sensitive C-reactive protein levels (2.0 vs. 1.9 mg/L; $p = 0.02$) but had lower left ventricular ejection fraction (52% vs. 57%; $p = 0.003$) and eGFR (59.2 vs. 68.5 mL/min/1.73 m^2; $p < 0.0001$) than those who survived. Multivariate Cox regression analysis, including 9 clinical, biochemical, and echocardiographic variables, identified D-dimer (relative risk: 2.38 per 10-fold increment; $p = 0.0002$) and eGFR (relative risk: 0.86 per 10 mL/min/1.73 m^2 increment; $p = 0.02$) as independent predictors of all-cause mortality. Similar results were obtained for cardiovascular mortality. The combination of D-dimer and eGFR tertiles was significantly associated with all-cause and cardiovascular mortality rates (Figure). **Conclusions:** The combined assessment of D-dimer and eGFR may improve the prediction of mortality in outpatients with stable CAD.



A-101

Multicenter Evaluation Supports Accuracy of the Beckman Coulter Gram-Negative Identification Product with Improved Database for Clinically Significant Bacteria

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Background: Bacterial identification is essential for determining effective antimicrobial therapies against infections. The MicroScan Gram-negative Identification (NID) organism database was revised and includes an additional 39 new taxa - 22 fermentative and 17 non-fermentative Gram-negatives - along with updated nomenclature. A multicenter study was performed to evaluate the accuracy of the updated NID database.

Methods: MicroScan NID panels were evaluated at two sites with 609 fresh clinical isolates comprised of 55 fermentative and non-fermentative Gram-negative taxa. MicroScan panels were inoculated using both the turbidity and PROMPT® System inoculation methods. Reference testing was performed following manufacturer's instructions and sequencing of 16S rDNA was included for discrepant isolates. All NID panels were incubated in a WalkAway instrument, and the NID panel identifications were generated using the updated NID organism database. Percent correct and incorrect results were used to assess accuracy of the updated NID panel. Thirteen isolates could only be identified to genus-level using the reference methods available.

Results: A correct identification was obtained for 98.6% (414/420) of the fermentative Gram-negative taxa. A correct identification was obtained for 94.7% (179/189) of the non-fermentative Gram-negative taxa. Therefore, an overall correct species-level identification was obtained for 97.4% (593/609) of the isolates. Only 3.1% of all isolates (19/593) required additional tests to confirm a low-probability correct identification, including 1 *Alcaligenes faecalis*, 5 *Achromobacter xylosoxidans/denitrificans*, 1 *Citrobacter freundii*, 2 *Chryseomonas indologenes*, 1 *Enterobacter aerogenes*, 2 *Escherichia coli*, 1 *Klebsiella oxytoca*, 1 *K. pneumoniae*, 1 *Morganella morganii*, 2 *Pseudomonas aeruginosa*, and 2 *Proteus mirabilis*. Furthermore, the clinically significant species *A. baumannii/haemolyticus*, *E. coli*, *P. aeruginosa*, and *P. mirabilis* were correct at 100%, 97.4%, 97.8%, and 96.4% respectively. Incorrect species-level identifications were obtained for 2.5% (15/609) of the isolates, including 1 *A. xylosoxidans,* 1 *E. hormaechei*, 2 *Achromobacter* species, 4 *Acinetobacter* species, 3 *Enterobacter* species, 1 *Pantoea* species, and 3 *Pseudomonas* species. The 13 isolates with genus-level reference identifications were counted as incorrect species-level identifications. A very rare biotype was obtained for 0.2% (1/609) - a single *E. aerogenes* isolate.

Conclusions: The results of this evaluation with fresh clinical isolates show that the MicroScan Dried Gram-negative ID panel with an updated database provides accurate identification results for clinically important Gram-negative bacteria.

A-102

Multicenter Evaluation of Clindamycin MIC Results for Staphylococci Using MicroScan Dried Gram Positive MIC Panels

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Background: Clindamycin has activity against Staphylococci both *in vitro* and in clinical infections. A multicenter study was performed to evaluate the accuracy of a revised formulation of clindamycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to frozen CLSI broth microdilution reference panels.

Methods: For efficacy, MSDGP panels were evaluated at four sites by comparing MICs obtained to MICs using a CLSI broth microdilution reference panel. A total of 784 *Staphylococcus* spp. clinical isolates were tested using the turbidity and Prompt™* methods of inoculation. For reproducibility, a subset of 10 organisms was

tested on MSDGP panels at each site. MSDGP panels were incubated at 35 ±2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGP panels were at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ±2°C and read visually. Frozen reference panels were read and reported at 18 hours for all organisms. CLSI M100-S25 breakpoints (µg/ml) were used for MIC interpretation (i.e., ≤ 0.5 S, 1-2 I, and ≥ 4 R).

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

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors %	
	T	P	T	P	T	P	T	P	T	P
Visual	98.7 (774/784)	97.7 (766/784)	99.1 (777/784)	98.9 (775/784)	0.6 (1/168)	0.6 (1/168)	0.5 (3/608)	0.2 (1/608)	0.4 (3/784)	0.9 (7/784)
Walk Away	98.2 (770/784)	98.1 (769/784)	98.9 (775/784)	98.5 (772/784)	0.0 (0/168)	0.0 (0/168)	0.3 (2/608)	0.5 (3/608)	0.9 (7/784)	1.1 (9/784)
auto SCAN-4	96.7 (758/784)	96.6 (757/784)	98.9 (775/784)	98.6 (771/784)	0.6 (1/168)	0.6 (1/168)	0.7 (4/608)	0.5 (3/608)	0.5 (4/784)	1.1 (9/784)
T = Turbidity inoculation method, P = Prompt™* inoculation method										

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

Conclusion: The MicroScan Dried Gram Positive MIC panel showed excellent correlation with MICs obtained using a CLSI broth microdilution reference panel for susceptibility testing of a revised formulation of clindamycin and *Staphylococcocus* spp.

* PROMPT™ is a registered trademark of 3M.

A-103

Multicenter Evaluation of Vancomycin MIC Results at 18 hours for Staphylococci and Enterococci Using MicroScan Dried Overnight Performance Evaluation Device Panel

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Background: With increasing rates of antibiotic resistance, MIC determination is important to initiate effective antimicrobial therapy. Vancomycin has been shown to be active against most strains of Enterococci and Staphylococci, both *in vitro* and in clinical infections. A multicenter study was performed to evaluate the accuracy of a revised formulation of vancomycin on a MicroScan Dried Gram Positive MIC (MSDGP) Panel when compared to CLSI broth microdilution reference panels.

Methods: For efficacy, MSDCP panels were evaluated at four sites with 947 clinical isolates. For reproducibility, a subset of 11 organisms was tested on MSDGP panels at each site. MSDGP panels were inoculated using both turbidity and Prompt™* inoculation methods. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All panels were incubated at 35 ±2°C and visually read. Frozen reference panels were read and reported at 24 hours for all organisms. Read times for the MSDGP panels were 18 hours for all species. FDA/CLSI breakpoints (µg/ml) were used for interpretation of MIC results.

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

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors %	
	T	P	T	P	T	P	T	P	T	P
Visual	99.4 (941/947)	96.2 (911/947)	99.5 (942/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.1 (1/882)	0.5 (5/947)	0.7 (7/947)
Walk Away	99.5 (942/947)	95.8 (907/947)	99.6 (943/947)	99.2 (939/947)	0.0 (0/62)	0.0 (0/62)	0.0 (0/882)	0.0 (0/882)	0.4 (4/947)	0.8 (8/947)
auto SCAN-4	99.0 (938/947)	96.2 (911/947)	99.4 (941/947)	99.3 (940/947)	0.0 (0/62)	0.0 (0/62)	0.1 (1/882)	0.0 (0/882)	0.5 (5/947)	0.7 (7/947)
T = Turbidity inoculation method, P = Prompt™* inoculation method										

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

Conclusion: This multicenter study showed that vancomycin MIC results read at 18 hours for *Enterococcus* and *Staphylococcus* species obtained with the MSDGP panel with a revised vancomycin formulation correlate well with MICs obtained using frozen reference panels read at 24 hours.

* PROMPT is a registered trademark of 3M.

A-104

Correlation of Anti-Pneumococcal Capsular Polysaccharide IgM, IgG and IgA specific antibodies in adult blood donors.

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Background: Post vaccination serum IgG antibody measurements are used to assess immune system competence, recovery and are included in guidelines for the assessment of antibody deficiencies. Recently, the measurement of PCP IgM and IgA has been reported in patients with common variable immunodeficiency (CVID). At present, the measurement of antigen-specific IgM and IgA antibodies is not routinely performed for the assessment of immunocompetence or risk of infection. We hypothesise that the simultaneous measurement IgM and IgA in addition to PCP IgG may give the clinician a more detailed antibody profile for the assessment of immunocompetence.

Objectives: Anti-pneumococcal capsular polysaccharide (PCP) IgM, IgG and IgA ELISAs have been developed (VaccZyme™ PCP ELISA, The Binding Site Group Limited, UK) to aid assessment of the adaptive immune system. The relationship between the concentrations of PCP IgM, IgG, and IgA was investigated.

Methods: The concentrations of PCP IgM, IgG, and IgA were measured in serum samples obtained from 231 adult blood donors (125 males and 106 females) aged 18-90 years. Only subjects who were free of recurrent infections or inflammation and whose C-reactive protein concentrations were <10mg/L were included in the analysis.

Results: Concentrations of each isotype were not normally distributed. The median concentration for PCP IgM was 54 U/mL (range 37-75 U/mL), IgG 40 mg/L (range 26-79 mg/L) and IgA 21 U/mL (range 13-44 U/mL). The median PCP IgM titres decreased with age and there was a significantly lower median PCP IgM titre in patients aged 81-90 years compared to those aged 18-80 years (27 *vs* 55 U/mL, p=0.0017). By contrast, there was a significantly higher median serum PCP IgG titre in the 61-90 years (61-70 years: 98 mg/L; p=0.0004, 71-80 years:110 mg/L; p<0.0001 and 81-90 years:67 mg/L; p=0.018) compared to those aged 18-60 years (median 35 mg/L). For PCP IgA, there was a significantly higher median titre in the 51-60 years (28 U/mL; p=0.036), 61-70 years (35 U/mL; p=0.0078), 71-80 years (43 U/mL; p=0.0065), and 81-90 years (55 U/mL; p=0.0034), compared to those aged 18-50 years (20 U/mL). The correlation between PCP IgG and IgA was more significant than between IgM and IgA and between IgM and IgG. Correlation of PCP IgA and IgM concentrations identified four immunophenotypes: those with i) high PCP IgM and IgA, ii) high PCP IgM only, iii) high PCP IgA only and iv) low PCP IgM and IgA. A significant number of individuals with a PCP IgG concentration >50mg/L had low PCP IgA (10-94%) and IgM (7-53%) concentrations.

Conclusion: The additional measurement of PCP IgA and PCP IgM, alongside PCP IgG, in individuals investigated for a compromised immune system may provide a more detailed antibody profile.

A-105

A Comparison of Two Compact Nucleic Acid Extractors, The Qiagen EZ1 Advanced XL And The Roche Magnapur Compact.

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Background: Nucleic acid extraction and purification is a fundamental and important prequel to successful and accurate downstream analytical processes. While numerous methods/manufacturers are available, contemporary considerations such as complexity of operation, breadth of application, cost and environmental impact become increasingly significant in choice consideration.

Via open tender process we shortlisted and evaluated two instruments fitting our modus operandi. Performance of extractors were evaluated based on outcomes of two viral hepatitis quantitation assays, influenza swabs in transport media, human genomic DNA and stool for *Clostridium difficile* testing.

Materials and methods: Plasma, stool, whole blood and where applicable, External Quality Assurance samples, with previously tested outcomes were used as evaluation reference samples. EZ1 Advanced XL (EZ1) (Qiagen, Germany) and MagnaPure Compact (MPC) (Roche, USA) were compared against current method, Qiagen QiaSymphony SP.

Outcomes were evaluated using regression analysis or binary classification testing for specificity and sensitivity against current/reference extraction methods.

Results: 41 anonymised stool samples were processed according to respective instrument protocols then analysed for *C.difficile tcdC* gene using Lightmix *C.difficile* kit (TibMolbiol, Germany). Specificities and sensitivities were 86% and 75% for MPC and 92% and 100% for EZ1 respectively. In addition, 2 samples negative for *C.difficile* processed by MPC displayed absence of Internal Control.

Both extractors showed equivalent quality when 32 UTM samples with some spiked with Influenza A virus were fed through respective instruments. Upon analysis, mean cycle threshold value was 29.88 and 30.50 for MPC and EZ1 respectively, indicating both instruments had extracted equal amounts of target RNA, based on 'M' protein assay developed by the Chinese National Influenza Center.

For extraction of human genomic DNA, concentration, yield and A260/280ratio for EZ1 were 44.3ng/uL, 9.2ug and 1.9 compared to MPC which yielded 62.5ng/uL, 12.5ug and 2.0 and Qiagen QiaSymphony SP, which yielded 43.5ng/uL, 8.7ug and 1.8.

MPC was unable to process samples to be tested by Roche Hepatitis B and C assays as the start and elution volumes were not compatible with kit Quantitation Standard concentration. MPC thus did not qualify for this segment of evaluation.

Conclusion: Both extractors use established magnetic bead capture techniques on contemporary compact instruments. User interaction is minimal, intuitive and requires only rudimentary technical skill. Extraction times are fairly quick with largely high quality eluate. Complex starting materials such as stool require pre-processing and may have significant influence on downstream testing outcomes.

The two instruments showed marginal difference in human genomic DNA extraction and Influenza A detection, but MPC was challenged with stool samples not only in specificity and sensitivity but also in number of definitive results. 2 stool samples which were successfully resulted on EZ1 and our current method were negative on MPC for target and internal control, indicating likely inhibition. However, the most decisive outcome was the fact that we were unable to incorporate, into a single extraction run, Hepatitis viral load assays because the start and eluate volumes were not compatible with the kit reagents. As such, it was clear that EZ1 was more suited to the operational and diagnostic needs of our laboratory.

A-106

Both Proinflammatory (IL-6) and Anti-Inflammatory (TGF-beta1) Cytokines Were Elevated in Clinicaly Stable Schizophrenia Patients

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Background: Pro-inflammatory cytokines, microglial cells, astrocytes, and invading immune cells mediate inflammation in the CNS. Inflammatory mechanisms should be well regulated, as excessive response can be a source of injury for the host cells. Besides several hypotheses, overactivation of inflammatory mechanisms is proposed for the etiology of schizophrenia. Increase in some proinflammatory cytokines have been shown in the blood and CSF of these patients and the therapeutic benefit of anti-inflammatory medications in some studies. However, there were a lot of controversies. In this study, we aimed to measure the serum levels of some pro-inflammatory (IL-6, TNF-alpha) and anti-inflammatory (TGF-beta1) cytokines and reveal their effects on disease mechanism.

Methods: The study was conducted in Marmara University Pendik E&R Hospital Department of Psychiatry and Biochemistry Laboratory. The study was approved by the ethical committee of Marmara University School of Medicine and informed consent was obtained from each case. Clinically stable patients with diagnosis of schizophrenia (n=30) and 29 healthy controls were enrolled. Broad neuropsychological test battery was conducted to assess cognitive functions. Patients with neurologic diseases, known acute or chronic inflammatory or allergic diseases, those using anti-iflammatory or immunosuppressive drugs were excluded. Serum IL-6, TNF-alpha, TGF-beta1 levels were measured with ELISA.

Results: Serum IL-6 and TGF-beta1 levels were found to be significantly higher in the patient group when compared to the controls. However, TNF-alpha levels were not significantly different (Table 1). Multiple logistic regression revealed a positive association between the disease state, IL-6 and TGF-beta1 (for IL-6 OR=2.52, P=0.031; for TGF-beta1 OR=1,00, P=0.027).

Conclusion: We concluded that inflammatory response in clinically stable schizophrenia patients is increased compared to controls. To clarify the significance of inflammation, further research both on serum cytokine levels in different schizophrenia patient groups and the relation between cognitive functions and symptom clusters in homogeneous patient groups on a broader scale is needed.

	Patient	Control	P
IL-6 (pg/mL)	1.2 (0.8-2.0)	0.9 (0.5-1.2)	0.048
TNF-alpha (pg/mL)	6.0 (4.0-7.5)	5.9 (3.9-8.1)	0.726
TGF-beta1 (pg/mL)	1891 (1185-7944)	1320 (984-1723)	0.012

A-107

A Queuing Model Analysis to Evaluate the Impact of High-sensitive Troponin I on Emergency Department Management Metrics

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Objectives: Emergency departments (ED) are important entry points to hospital care. Access to treatment and patient flow is however frequently challenged by ED crowding that is a key issue for many hospitals. Crowding could negatively impact clinical endpoints as well as efficiency of health care processes. Different approaches have been made in order to alleviate crowding issues and improve patient flow. A new diagnostic assay (high-sensitive troponin I, hsTnI) has been recommended to facilitate faster clinical decisions for patients presenting with chest pain (CPP) in the ED suggestive of having myocardial infarction. The objective of the study was to develop a model and evaluate the impact of an accelerated hsTnI driven chest pain triaging pathway on ED patient flow and ED performance metrics from a hospital perspective. **Methods:** An economic model based on a queuing theory has been developed and applied to data from a major emergency department in Rijadh. The management of CPP is based on serial measurements of troponin at defined time points. The standard scenario running a contemporary troponin (cTnI) with a standard protocol time of 6 hours was tested against the use of a high-sensitive troponin (hsTnI) supported scenario with a 3 hours protocol time as recently recommended by clinical guidelines. In order to measure the impact of a shorter protocol time on ED metrics (Waiting time, ED time, risk for waiting, diversion rate, required beds per day, total costs of ED stay) both, a deterministic base case analysis and a probabilistic analysis (5,000 iterations) were performed to test robustness and reflect variability and uncertainty. **Results:** Deterministic and probabilistic analysis showed significant improvements in all ED performance indicators for the hsTnI scenario: ED waiting time (> -7hrs), ED time (> -10hrs), required beds for CPP (-9.9 beds per day). In addition, results suggested that the hsTnI scenario would be less affected by variation in demand and service. Despite increased test costs for high-sensitive troponin, total costs could be expected to be significantly reduced from an average of 726€ (95%CI 724-728) per CPP in the cTnI scenario to 429€ (428-430) per CPP in the hsTnI scenario. **Conclusions:** The management of CPPs requires a substantial amount of ED resources and time. This economic model suggests that a switch to hsTnI with shorter protocol times for risk stratification would significantly improve ED flow metrics. It would stabilize the CPP pathway which would become more predictable and manageable. It would release resources that could be devoted to other ED patients, thus alleviating ED crowding issues and generating benefits for the overall hospital system.

A-108

Autoantibodies against CD74 - A new diagnostic marker for Spondyloarthritis

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Background: Spondyloarthritis (SpA) is a common debilitating inflammatory disorder. Pathogenesis of axial SpA (axSpA) including ankylosing spondylitis (AS) is still largely unclear. Diagnosis is difficult, since abnormalities in conventional X-rays develop with a latency of several years and only HLA-B27 is used as laboratory marker. The presence of radiographic sacroiliitis is essential for SpA diagnosis. To prevent destructive effects early diagnosis and intervention in SpA patients may be important. To evaluate antibodies to the human leukocyte antigen class II-associated invariant chain peptide (anti-CD74) as a diagnostic marker of SpA.

Methods: Sera of 117 patients with axial SpA and 38 non-SpA patients were analyzed for IgA and IgG antibodies against CD74 by ELISA. HLA-B27 status was available in 112 patients. All donors provided informed consent for the study approved by the local ethics committee (project number 4928).

Results: Anti-CD74 antibodies were detected in 85.1% of SpA patients but only in 5% of non-SpA patients ($p \leq 0.0001$). Detection of both IgG and IgA anti-CD74 antibodies for diagnosing SpA revealed a sensitivity of 77% and a specificity of 90%. Remarkably, IgA autoantibodies against CD74 alone had a sensitivity of 67% and a specificity of 95%. IgA anti-CD74 antibodies were even more frequent in SpA patients with short disease duration and significantly correlate with more advanced radiological sacroiliitis and reduced spinal mobility.

Conclusion: Anti-CD74 IgA antibodies were strongly associated with SpA. Antibodies against CD74 could provide an important additional tool for diagnosis of SpA.

A-109

Industrial food additive microbial transglutaminase is immunogenic in children with celiac disease

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Background: Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in CD. Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking.

Methods: he serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: *AESKULISA®* tTg (tTg), *AESKULISA®* tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). Marsh criteria were used for the degree of intestinal injury.

Results: Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones ($p<0.0001$). All mTg-neo and tTg-neo levels were higher ($p<0.001$). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG ($p<0.0001$). The levels of tTg-neo IgA/IgG were higher than tTg IgA/IgG ($p<0.0001$). The sequential antibody activities reflecting best the increased intestinal damage were: tTg-neo IgG ≥ mTg-neo IgG > mTg-neo IgA+IgG > tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology ($r^2=0.989$, $r^2=0.989$, $p<0.0001$, $p<0.0001$, respectively).

Conclusion: mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

A-110

Correlation between age and semen quality in healthy Brazilian men

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Although the effect of maternal age on fertility is well known, it is unclear whether paternal age also affects fertility. This retrospective study sought to characterize the association between age and semen quality, a well-known point of fertility status. Samples data of 54 men (36±9.0years old, range 20 - 58 yo) cryopreserved between 2010 and 2015 without known fertility problems and considered healthy by initial trials and attested by an urologist were evaluated at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). The cohort was divided in two groups, group 1 (G1 n=28) 20-36 yo and group 2 (G2, n=26) 37-58 yo. Cellularity (concentration of sperm cells) and motility were assessed by Makler chamber and vitality was measured by eosin Y. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests. The comparison between the G1 and G2 was evaluated by Student's t-test for parametric data or by Mann-Whitney test for non-parametric analysis. Further, the correlation between age and the different parameters of sperm was evaluated by Spearman coefficient. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. No correlation was observed between age and cellularity ($r = 0.2658$, $p = 0.0521$), motility ($r = 0.1679$, $p = 0.2249$) and viability ($r = 0,1704$, $p = 0,2179$). Evaluating the population in separated groups based on the median of the age of the cohort (36 ± 9.0 yo), G1 showed semen with less cellularity (4.93 ± 11.0 x 107 per mL) compared to the older group G2 (7.63 ± 14.6 x 107 per mL), but the difference was not statistical significant ($p = 0.0965$). Spermatozoa motility was measured according with World Health Organization (WHO) classification, being the frequency of motile spermatozoa classified as "a" (sperm with progressive motility) plus "b" (non-linear motility). G1 showed 52% of motility and G2 60% ($p = 0.1297$), and the viability showed similar difference between G1 (54%) and G2 (58%) ($p = 0.2646$). Therefore, no statistical significant and impact were observed between age and sperm quality in our cohort. Further studies with a large cohort are needed to confirm these findings.

A-111

Quality evaluation of umbilical cord blood cryopreserved from vaginal and cesarean deliveries

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Umbilical cord blood (UCB) is largely employed as an alternative source of stem cells in the treatment of hemato-oncological diseases. In this study, we determined the impact of the modeof delivery, maternal factors and laboratory parameters of hematopoietic potential, such as viability, cell recovery after processing and percentage of $CD34^+$ cells. Data of 170 cryopreserved UCB samples from live births of 34 - 41 weeks collected between 2013 and 2015 from cesarean and vaginal deliveries were evaluatedand processed by Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests and the comparison between cesarean and vaginal deliveries data were measured by Student's *t*-test for parametric data or by Mann-Whitney test for non-parametric analysis. Data was shown as median ± SD and a *p* value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. Maternal and neonatal parameters include age of the mother, gestational weeks, mode of delivery and baby's gender and laboratory parameters include cord blood volume, frequency of $CD34^+$cells, viability and recovery after processing. Amongst the 170 UCB samples evaluated here, 85 (50%) correspond from cesarean deliveryin which 46% of the babies correspond to female gender. The median age of the mother of this group was 36.0 ± 4.0 years old (yo) and gestational age was 38 ± 0.9 weeks. On the other hand, 85 UCB samples (50%) were from vaginal birth and 59% of the neonates were female. The median age of the mother was 34.0 ± 4.0 yo and gestational age was 39.0 ± 1.0 weeks. The total of umbilical cord blood volume collected was higher in cesarean delivery (80 ± 33.3 mL) compared to vaginal delivery (69 ± 27.8 mL, $p = 0.0417$). No difference was observed between cesarean and vaginal delivery in the follow parameters: percentage of cell viability (97.7 and 96.8, respectively, $p = 0.1639$),percentage of recovery (92.4 and 91.4, respectively, $p = 0.4843$) and percentage of $CD34^+$cells ($9.2 \times 10^{-6}$and 8.9×10^{-6},

respectively, $p = 0.5434$). Thus, the study concludes that cesarean delivery provide a higher volume of UCB but no difference in samples quality was observed between cesarean and vaginal deliveries inthe cohort here evaluated.

A-112

Semen quality of men infected by *Mycoplasma hominis*

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This study was undertaken to determine the prevalence of *Mycoplasma hominis* infection among men and to study the effects of these infection on semen quality. A total of cryopreserved samples data from 54 men (36.0 ± 9.0 years old(yo), range 20 - 58yo), being 6 (11%) infected with *M.hominis* and 48 (89%) negative for sexually transmitted diseases and considered healthy by initial trials and attested by an urologist were evaluated before and after sample freezing at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil). *M.hominis*was detected by polymerase chain reaction and sperm quality was assessed by cellularityand motility through Makler chamber and viability by eosin Y. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests.The comparison between infected and uninfected groupswas evaluated by Student's *t*-test for parametric data or by Mann-Whitney test for non-parametric analysis. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. The men positive for *M.hominis*had semen sampleswith lower spermatozoa viability than those uninfected and showed statistically significant ($p = 0.0447$). In addition, the percentage of reduction of sperm motility after freezing were higher in samples of men infected (38%) compared to those uninfected (27%, $p = 0.0279$).No statistically difference was observed in sperm cellularity from infected men ($5.4 \pm 3.4 \times 10^7$) from those uninfected ($6.9 \pm 0.1 \times 10^7$,$p = 0.2418$). Therefore, these results showed that *M. hominis* infection interfere on semen quality negatively, but not in cellularity of the sperm.

A-113

The effects of cryopreservation on sperm cellularity, motility and vitality

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The effects of cryoinjury were determined simultaneously on cellularity, motility and viability of ejaculated human sperm. This retrospective study included 54 men (36yo± 9.0, range 20 - 58yo) without known fertility problems and considered healthy by initial trials and attested by anurologist at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil).Semen cellularity and spermatozoa motility were measured byMakler Counting Chamber andviability was assessed by eosin Y. Data are presented as a percentage of the difference between median of the values obtained before and after freezing. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests and the comparison between data before and after freezing was measured by Student's *t*-test for parametric data or by Mann Whitney for non-parametric analysis. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6.Freeze-thawingcaused 42% (p= 0.0005) reduction in cellularity. Spermatozoa motility was measuredaccording with World Health Organization (WHO) classification, being thefrequency of motile spermatozoa classified as "*a*" (sperm with progressive motility) plus "*b*"(non-linear motility). Motility and vitality of the sperm showed similar reduction after freezing (31%, p = 0.0002 and 32%, p< 0.0001, respectively). Semen cellularity, motility and vitality are equally susceptible to cryopreservation-induced damage. To avoid critically reducing sperm quality after thawing, it is necessary criticism at the time of cryopreservation, ensuring high rates of motility and viability or high cellularity of the sample.

A-114

Association between umbilical cord blood volume and CD34 positive cells.

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Umbilical cord blood (UCB) has been recently considered as an alternative source of hematopoietic progenitor cells for clinical application. Some of the parameters commonly used to evaluate an UCB unitand predict transplant outcomes have been $CD34^+$cells concentration, which is a hematopoietic stem cells marker, and total cord blood volume collected. Thus, the aim of the study was to find the correlation between umbilical cord blood volume and $CD34^+$ cells concentration derived from cord blood. For this study, 2787 UCB samples that were processed and cryopreserved at Criovida - Hermes Pardini Institute (Belo Horizonte-MG/Brazil) were evaluated. $CD34^+$ cells were detected by Flow Cytometry at Hermes Pardini Institute (Vespasiano-MG/Brazil) and were expressed in percentage, considering the ratio between the number of $CD34^+$ cells and the total number of collected cells x 100. The total cord blood volume was noted at the moment that the bag arrivedin the technical area. The normality of the data was assessed by Kolmogorov-Smirnov and Shapiro Wilk tests. The correlation between umbilical cord blood volume and $CD34^+$ cells was evaluated by Spearman correlation coefficient. Data was shown as median ± SDand a p value < 0.05 was considered statistically significant. All the statistical analyses were performed using the software GraphPad Prism 6. Here, only cesarean deliveries were evaluatedandthe median of gestational age was 39 weeks (39 ± 1.5 weeks). The median of mother age was 38 years old(38 ± 27) and the cohort was composed of 1431 UCB samples of male gender (51%) and 1356 (49%) of female. The median of the total cord blood volume was 85 mL (85 ± 30 mL, range 22 - 227 mL) and the percentage of $CD34^+$cells were 9.9×10^{-6}% (± 0.18). We found that $CD34^+$ cells concentration was higher in greater volume of collected cord blood ($r = 0.4343$, $p < 0.0001$). Our study concludes that higher volume of cord blood should be preferred for processing and stem cell infusion.

A-115

Bone marrow cryopreservation: recovery due to the freezing solution concentrations and storage temperatures

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The transfusion of autologous cryopreserved bone marrow (BM) cells is a well-established procedure in the management of both hematological and non-hematological malignant diseases. The good quality of cryopreserved BM depends on storage temperature and cryopreservant concentration (dimethyl sulfoxide - DMSO).Thus, the aim of this study was to evaluate the effect of different of DMSO concentrations and temperatures of storage on cryopreservation of BM among distinct storage time points. For this study, 40 mL of BM that would be discarded from medical procedures was collected from posterior iliac crest by a hematologist at Criovida - Hermes Pardini Institute (Belo Horizonte/Brazil). BM was processed by SEPAX® with a single-use kit CS-900. The material was concentrated to 30 mL with 75% of recovery. Cells were cryopreserved using 2.5% or 5% of DMSO for -80°C storage and 10% or 20% of DMSO for cryopreservation at -196°C (nitrogen liquid). Cryopreserved BM cells were evaluated 1, 30 and 90 days after freezing. The cell quality was assessed by CD34 positive cells and the percentage of viable cells using 7-AAD as a vital exclusion marker by flow cytometry at Hermes Pardini Institute (Vespasiano-MG/Brazil).The results obtained show that frequency of $CD34^+$ cells and cell viability had, in all conditions, better results stored at -196°C compared to -80°C (Table 1). However, DMSO concentration was decisive to the number of $CD34^+$ cells.The value was proportional to DMSO concentration. On the other hand, the viability was higher at 10% DMSO concentration condition, even better than 20%, probably by the cell toxicity of the cryopreservant. Therefore, cryopreservation of BM at -196°C with 10% of DMSO could be more effective to ensure the quality of the cells, however, more samples are required to confirm this result.

Evaluation of bone marrow among distinct storage and DMSO concentrations time points							
		1 day		30 days		90 days	
Storage Temperature	[DMSO]	CD34+ (%)	Viability (%)	CD34+ (%)	Viability (%)	CD34+ (%)	Viability (%)
-80°C	2,5%	0,64	62,9	0,30	61,3	0,06	56,0
	5%	1,00	62,5	0,29	59,5	0,33	56,0
-196°C	10%	1,31	99,8	1,31	70,8	0,24	62,0
	20%	1,57	61,8	1,21	56,0	0,54	49,1

A-116

Evaluation of serum biomarker assays for mild traumatic brain injury in a cohort of mixed martial arts fighters

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Background: Identifying mild traumatic brain injury (mTBI) in sports is a continuous clinical challenge. Assessments are dominated by self-reported symptoms and cognitive function tests, but lack good sensitivity and specificity to detect mTBI injury. Biomarker tests may improve discrimination between injured and non-injured. There is increasing participation in mixed martial arts (MMA), a sport similar to boxing in which participants have increased exposure to head injury. The **objective** of our clinical study was to assess pre- and post-fight serum concentrations of two mTBI biomarkers, S100 and neuron-specific enolase (NSE), in a cohort of otherwise healthy amateur MMA fighters (mean age 25 y.o., range 16-46). All fighters were eligible to participate and completed informed consent approved by the university's IRB.

Methods: *S100 and NSE measurements*: Blood samples were collected in a serum-separator tube on 58 fighters (97% male) both before (pre-) and within 30 minutes after the fight ended (post-). Blood was centrifuged after clot formation following standard protocol, and serum was frozen and kept at minus 80° C until analysis. S100 and NSE tests were performed on a Roche Diagnostics Modular E170 instrument using manufacturer-supplied reagents, calibrators, and controls. Both tests are sandwich immunoassays with chemiluminescent signal detection proportional to protein concentration of S100 (ug/L) or NSE (ng/mL). All subject test results fell within the analytical measurement range of the assays. We confirmed agreement with manufacturer reported precision values with repeated tests of low, mid, and high level samples. *Statistics:* Descriptive statistics and paired Wilcoxon-rank sum test were analyzed using Minitab software. ROC curve analysis was performed using MedCalc software.

Results: For this initial analysis, we adopted serum concentration cut-offs provided by the manufacturer of S100 > 0.105 ug/L or NSE > 16.3 ng/mL to indicate evidence of mTBI. For S100 test, 86% had evidence of mTBI within 30 minutes post-fight, whereas only 19% had pre-fight results greater than the cut-off value. All but 3 fighters (95%) had increased S100 post-fight *versus* pre-fight, while 90% had increased NSE post-fight. Median test value differences in fighters were statistically significant between pre- and post-samples for S100 (0.056 *vs* 0.199 ug/L) and NSE (12.1 *vs* 17.1 ng/mL), both $p<0.001$. Importantly, observed post-fight increases are unlikely explained by chance alone since changes were > 3 SD at these test levels (based on precision experiments). ROC curve discriminate analyses were excellent for both post-fight S100 levels (AUC = 0.921) and NSE levels (AUC = 0.900) in this cohort.

Conclusions: Increased levels of S100 and NSE serum markers were observed in the majority of subjects post-fight, suggesting mTBI injury may be common following MMA fights. In particular, S100 protein levels above cut-off value were found in 86% of subjects within 30 minutes post-fight. Moreover, increased S100 and NSE levels after the fight compared with baseline were observed in the majority of fighters. To our knowledge, this is the first study demonstrating early increases in serum levels of S100 and NSE biomarkers in a group of mixed martial arts fighters.

A-117

Gene deletion is associated with Second Toe Signal phenotype in Neurofibromatosis Type 1

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The early diagnosis of Neurofibromatosis types 1 (NF1), based on consensus criteria, is useful for themanagement of clinical aspects and genetic counseling. Additional specific congenital lesions might assist in the early diagnosis of NF1. Our group previously reported, through questionnaire (12%) and photographic register (5.8%), the prevalence of a not yet described NF1 phenotype component: bilateral superposition of the second toe over the first and the third toes, which we referred to as the "Second Toe Signal" (STS) (Figure 1). Regarding the fact that the most severe NF1 phenotypes are associated with microdeletions (the former whole-gene deletions), we assessed the association between STS and microdeletions. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for 21 NF1 patients presenting at least three NIH diagnostic criteria. The kits used were P081 e P082 - version C1. The samples' results were analyzed by Coffalyser v.140721.1958 software. Statistical analysiswas performedwith the open source calculator OpenEpi (version 3, www.openepi.com), using the Fisher exact test. Results: We found three microdeletions, including the regions of the flanking probes of the NF1 gene. All three microdeletion subjects have STS,one patient has STS but not microdeletion and the 17 others have neither STS nor microdeletion (P=0.006). These three microdeletions patients with STS present the generally accepted microdeletion clinical phenotype. As we increase our sample size, we suggest that STS, particularly regarding its presence at birth, is a useful clinical sign of NF1 microdeletion.

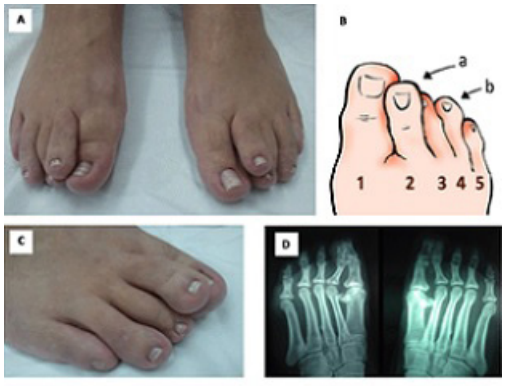

Figure 1 – Pictures (A, C) of one NF1 volunteer (three NIH criteria ate least) with bilateral superposition of the up of second toe (diagram B, a) and enlargement of the forth (diagram B, b) and feet XR (D). The patient presents the NF1 gene microdeletion phenotype and gene deletion at MLPA.

A-118

Cerebrospinal Fluid Angiotensin-Converting Enzyme Activity Levels of Patients With Alzheimer's and Parkinson's Disease

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Background: Angiotensin-converting enzyme (ACE) is an endopeptidase expressed by endothelial, epithelial and neuronal cells. It is found as both in membrane-bound and soluble forms. ACE is also one of the enzymes degrading amyloid-β (Aβ) in central nervous system. Accumulation of Aβ has an important role in the pathogenesis of Alzheimer's disease (AD). The angiotensin-converting enzyme may also be involved in the pathogenesis of Parkinson's disease (PD). There have been several studies investigating the association between several ACE gene polymorphisms and

PD risk. The aim of this study is to measure activity level of ACE in cerebrospinal fluid (CSF) samples of AD and PD patients and compare with the results of control group.

Method: 20 patients with AD, 17 patients with PD and 25 control cases. Control cases had no neurodegenerative disease but had diagnostic lumbar puncture for headache or peripheral nervous system disorders. All subjects were recruited from Hacettepe University Hospitals Neurology Clinics in the period of September 2012-June 2014. Participants were assessed by their age and CSF ACE activity. ACE activity was measured by spectrophotometric method from all CSF samples and calculated according to the total protein content of samples. Groups were assessed with Mann-Whitney U test. SPSS 21.0 was used for statistical analysis.

Results: Mean ages of AD, PD and control groups were 67.5±1.96, 59.76±2.71 and 50.68±3.4, respectively. The mean age of control group was lower than both AD and PD groups ($p<0.05$). CSF ACE enzyme activities of AD, PD and control groups were measured as 0.30±0.06, 0.33±0.07 0.53±0.11 U/L, respectively. CSF ACE specific enzyme activities of AD, PD and control groups were measured as 0.75±0.24, 0.82±0.29 and 1.29±0.47 U/g protein, respectively. According to CSF ACE enzyme activity and specific enzyme activity levels, there was statistically significant difference between AD and control group ($p<0.05$). However, the differences of enzyme activities and specific enzyme activities between PD and control group were not found to be statistically significant ($p>0.05$).

Conclusion: In this study, it is shown that CSF ACE activity was found to be significantly decreased in AD patients than control whereas the decrease in PD patients' CSF ACE activity was not significant. Further new studies with large-scale patient groups should be planned to assess CSF ACE as a biomarker for neurodegenerative diseases thoroughly.

Keywords: Alzheimer's Disease, Parkinson's Disease, Angiotensin-converting enzyme, Cerebrospinal fluid

A-121

Graft-derived cell-free DNA - a promising Rejection Marker in Liver transplantation - Results from a prospective Multicenter Trial

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Background: There is a need for cost-effective, non-invasive biomarkers of graft integrity with a short turn-around time that can be used with therapeutic drug monitoring (TDM) to personalize post-transplant immunosuppression. Graft-derived cell-free DNA (GcfDNA) has shown promise as such a biomarker for the detection of graft injury.

Methods: This is the first report on a prospective multicenter trial that monitored plasma GcfDNA in 106 adult liver transplant (LTx) recipients followed over at least one year post transplant. A total of 128 Patients were recruited at 3 German transplant centers (Charité/Berlin, UKE/Hamburg-Eppendorf, UMG/Göttingen), of which 22 were lost to follow up or censored based on exclusion criteria. cfDNA was extracted from ≥ 1 ml EDTA plasma, obtained in Cell-free DNA-BCT tubes. GcfDNA was determined as described elsewhere (Clin Chem 2013; 59: 1732-1741). The turn-around time for an initial sample is about two days and one working day for any consecutive sample.

Results: The GcfDNA percentage was highly elevated (>50% of total cfDNA) on the first day after transplantation, evaluated in a subset of 23 patients; presumably due to ischemia/reperfusion damage. The median GcfDNA percentage decreased in stable patients with no signs of graft injury within the first week to a baseline level below 10%, where it remained throughout the one year observation period. In otherwise stable patients with positive HCV virus detection (N=17, n=60 samples), GcfDNA values were generally only slightly elevated (median: 7.2%, 95% CI 4.9%-13.0%) compared to stable, HCV negative patients (N=87, median: 3.3%, 95% CI 3.0%-3.7%). In patients (N=20), with samples (n=34) drawn during biopsy-proven acute rejection periods, values (median: 30.7% CI 25.4%-45.0%) were about 10-fold higher than median values observed in samples (n=279) from stable patients without rejection (N=87). Interestingly, in five otherwise clinically stable patients with samples available 7-15 days prior to a diagnosis of biopsy proven acute rejection, also elevated GcfDNA values (>20%) were already observed (median: 38.7%, range: 21.1%-51.3%) at that time. Aminotransferases, γ-glutamyltransferase, glutamate dehydrogenase, and bilirubin showed low overall correlations with GcfDNA (correlation coefficients (r) ranged from 0.32 to 0.59) and these conventional liver function tests (LFTs) had greater overlap in acute rejection, HCV positive, and stable patients. The diagnostic sensitivity and specificity of GcfDNA ≥10% was 91.2% (CI 76.3%-98.1%) and 92.5% (CI 88.7%-95.3%) respectively, comparing stable HCV negative samples (n=279) versus biopsy-proven acute rejection samples (n=34). The AUC under the ROC curve was overall highest for GcfDNA (0.97, 95% CI:0.93-1.0), compared to LFTs; of which ALT was best with an AUC of 0.93 (CI: 0.85-1.0).

Conclusion: Plasma GcfDNA determinations allowed for better discrimination of liver transplant patients with acute rejection or allograft injury, compared to conventional LFTs and may be helpful to personalize post-LTx immunosuppression.

A-122

Presepsin Predicts Acute Kidney Injury and Mortality in Cardiac Surgery Patients

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Background

Presepsin (sCD14-ST) represents a 13 kDa fragment of sCD14 which is released after conversion of CD14++ monocytes into CD14+/16+ monocytes upon monocyte activation. Presepsin has been shown powerful prognostic validity in inflammatory related conditions like sepsis and SIRS and association with disease severity, multi organ dysfunction syndrome and outcome.

Objective

Preoperative mortality risk assessment should support optimizing peri- and postoperative care of patients. We thought to evaluate the prognostic value of presepsin for outcome prediction in patients undergoing elective cardiac surgery in comparison with EuroSCORE 2 and cardiac, inflammatory, and renal diagnostic markers (NT-proBNP, CRP, procalcitonin (PCT), Leucocytes, Cystatin C).

Methods

We included 856 consecutive patients having cardiac surgery and measured preoperative plasma concentration of presepsin, NT-pro-BNP, PCT, leucocytes, and cystatin C as well as postoperative presepsin levels. Presepsin was determined by using the PATHFAST Presepsin assay (LSI Medience corporation,Tokyo). The diagnostic markers NT-proBNP, CRP, PCT, Leucocytes, and Cystatin C were measured in the central laboratory by using routine clinical chemistry methods. Outcome measures were in-hospital mortality, 6- month mortality and occurence of acute kidney injury (AKI) during hospitalization. Areas-under-the-curves (AUCs) were compared using the tests of DeLong and Clarke-Pearson. Logistic regression analysis was used to calculate univariable and multivariable odds ratios.

Results

Patients with in-hospital mortality (n=27, *3.2%*) and 6-month mortality (n=49, *6.1%)* had higher preoperative presepsin levels than survivors: 1166±1453 pg/mL vs. 258±391 pg/mL; $p<0.001$ and 913±1215 pg/mL vs. 231±194 pg/mL; $p<0.001$, respectively. C-statistics showed elevated presepsin level to accurately predict occurrence of in-hospital mortality (AUC 0.88) and 6-month mortality (AUC 0.87) whereas the EuroSCORE 2 showed

significantly less predictive power (AUC values 0.74 and 0.76) as well as NT-pro-BNP (AUCs 0.77 and 0.79), PCT (AUCs 0.59 and 0.56), leucocytes (AUCs 0.58 and 0.63), and cystatin C (AUCs 0.76 and 0.74).

222 patients (25.9%) who developed AKI (AKI classification: 1 (n=122), 2 (n=54), 3 (n=46)) revealed higher mortality and higher presepsin values compared to patients without AKI (in-hospital mortality: 8% vs 1.4%; preoperative presepsin: 441±585 vs 233±436 pg/mL; $p<0.001$; postoperative presepsin: 927±926 vs 426±583 pg/mL; $p<0.001$). ROC analysis of postoperative presepsin showed the highest discriminatory power for risk prediction of AKI occurence (AUC 0.78) and renal replacement therapy (AUC 0.88) during hospitalization in comparison with the other diagnostic markers and EuroSCORE 2. Even after adjustment for confounding factors (i.e. EuroSCORE 2, age, glomerular filtration rate, and operation duration) presepsin remained an independent risk predictor.

Conclusion

Preoperative plasma presepsin level is an predictor of post-operative mortality and AKI in elective cardiac surgery patients, and is a stronger predictor than several other commonly used factors. Presepsin has proven as an independent risk indicator to predict outcome and may be used for risk stratification in patients scheduled for surgery already preoperatively.

A-124

Clinical Value of Bioactive Hepcidin-25, Soluble Transferrin Receptor and Their Ratio in Predialysis Patients Chronic Kidney Disease: Correlation with the Response to Intravenous Ferric Carboxymaltose

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Background: Anemia of inflammation, also known as anemia of chronic disease, is a complicating factor of a broad spectrum of inflammatory disorders, including chronic renal failure, autoimmune diseases, infections and certain cancers. Hepcidin is elevated in these conditions because of the increased production of cytokines (e.g. interleukin-6, BMP2), although in chronic kidney disease (CKD), decreased kidney clearance of hepcidin may also contribute. No reliable biomarker exists to predict responsiveness to intravenous (IV) iron (Fe) in iron deficient patients with CKD. We aimed to investigate the clinical value of bioactive Hepcidin-25 and soluble Transferrin Receptor (sTfR) levels in predialysis patients.

Patients and Methods: In this prospective study 78 stable stage III-IV CKD predialysis patients with (responders) and without (non-responders) adequate erythropoiesis after IV administration of ferric-carboxymaltose (FCM). Patients were divided in two groups according to their response to IV administration of ferric-carboxymaltose (FCM). Group R (responders) included 40 patients who had true iron deficiency and increased their Hg concentration by > 1g/dl from baseline. Group NR (non-responders) included 38 patients, who failed to respond. Along with measurements of common hematologic and blood chemistry parameters, determinations of sTfR (immunonephelometric technique, BN Prospec Nephelometer-Siemens Healthcare Diagnostics, Liederbach, Germany) and bioactive Hepcidin-25 (ELISA, DRG Instruments GmbH, Marburg, Germany) were performed.

Results: The main results of the study showed that: Hepcidin-25 levels (mean±SEM) were lower in the responders 1.4±1.2ng/mL (range from 0.5-33.6ng/mL) compared to non-responders 3.4±1.7ng/mL (range from 0.4-38.6ng/mL), (p=0.03), while sTfR and sTfR/Hepcidin-25 ratio were higher (p<0.01 and p=0.002 respectively). Diagnostic efficacy was analyzed by ROC analysis. Cut off point of 1.49 for Hepcidin-25 had sensitivity 84% and specificity 48%, while cut off point of 1.21 for sTfR/Hepcidin-25 ratio had sensitivity 82% and specificity 52% to predict correctly response to iron supplementation therapy. Furthermore, log sTfR/Hepcidin-25 correlated significantly with hs-CRP (r=-0.462, p=0.005) and IL-6 (r=-0.335, p<0.04) in non-responders, while such correlations were not found in responders (p>0.05).

Conclusions: These results suggest that lower Hepcidin-25, as well as higher sTfR and sTfR/Hepcidin-25 ratio were significant predictors of favorable hemoglobin response within a month after IV administration of FCM in patients with CKD. Further experiments and clinical studies in other groups of patients are needed to better elucidate the role of Hepcidin-25 and sTfR/Hepcidin-25 ratio as predictors of response to intravenous iron administration.

A-129

Do samples without HbA have dectected HbA1c?

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Background: Hemoglobin (Hb)A1c is a widely used biochemical marker for the management of diabetes mellitus and can be quantified by many methods. Some methods were reported least interference from those factors. However, those methods may present incorrect HbA1c results. The current study evaluated the five different HbA1c systems for five patients with no Hemoglobin A

Methods: Fresh no variants blood samples and 5 patients without Hemoglobin A were screened by capillary electrophoresis technique (Capillarys 2 Flex Piercing, Sebia), and identified by genetic sequence analysis. Those samples are analyzed for HbA1c by ion exchange HPLC(Variant II, Variant IITurbo 2.0, Bio-Rad), boronate affinity HPLC(Ultra2,Trinity Biotech), CE(Capillarys 2 Flex Piercing, Sebia), and Tinaquant immunoassay(Moddular PPI 800, Roche). The Bio-Rad Variant II(Bio-Rad, USA) has certified as NGSP Level I Laboratory and was used as Comparative method. The results of normal fresh samples for each method were used to eliminate any inherent calibration bias.

Results: The HbA1c values of normal samples obtained from VII-T 2.0, Ultra2,C2FP and PPI system were well correlated with VII system. Bias of each method to VII system were between -6% and 6% which met the standard of NGSP. But the samples without Hemoglobin A fraction that do not contain HbA1c analyte were unexpectedly obtained HbA1c results from different systems (Table 1). The VII and VII-T 2.0 randomly detected HbA1c of 2 and 3 samples without HbA; the C2FP system only detect HbA1c of the fifth patient with abnormal HbA2 value sign; the Ultra2 system and PPI system reported the HbA1c values of all samples

Conclusion: The evaluated HbA1c methods are affected to different extents by samples without HbA. Only VII,VII-T 2.0 and CE technique partially provides the abnormal sign in its profiles. The laboratories must be carefully selecting HbA1c analyzing methods and reporting HbA1c results in the higher Hb disorder prevalence area.

Table 1. The results of Hemoglobin electrophoresis and HbA1c for five blood samples

Genotype	HbA(%)	HbF(%)	HbA2(%)	Others(%)	Bio-Rad VII	Bio-Rad VII - T 2.0	Sebia C2FP	Trinity Biotech Ultra²	Roche PPI
αα/αα;$\beta^{CD26}/\beta^{CD41-42}$	0	45	5.1	49.9	-	-	-	4.30%	5.10%
								23mmol/mol	32mmol/mol
αα/αα;$\beta^{IVS2-654}/\beta^{New-York}$	0	1.5	5	93.5	4.30%	4.50%	-	4.10%	4.20%
					23mmol/mol	26mmol/mol		21mmol/mol	22mmol/mol
αα/αα;$\beta^{CD41-42}/\beta^{New-York}$	0	1	5	94	4.50%	4.60%	-	4.30%	4.80%
					26mmol/mol	27mmol/mol		23mmol/mol	29mmol/mol
αα/αα;$\beta^{CD41-42}/\beta^{E-Bangkok}$	0	0.6	4.6	94.8	-	4.70%	-	4.70%	3.80%
						28mmol/mol		28mmol/mol	-
--SEA/--$^{4.2-Q-Thailand}\beta/\beta$	0	0	0	100	-	-	3.7%	5.3%	5.7%
							-	34mmol/mol	39mmol/mol

A-130

Clinical lab data of High-Risk HPV genotyping

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Persistent infection by high-risk human papillomavirus (HRHPV) contributed to cervical cancer. Accurate HRHPV genotyping helps identify the women with real risk of developing cervical cancer. The Centers for Disease Control and Prevention (CDC) also calls HPV genotyping to evaluate efficacy of HPV vaccine. We developed a multiplex PCR to amplify E6, E7 or L1 region for each of all 13 HRHPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Validation of the genotyping compared with FDA approved Digene Hybrid Capture 2 (HC2) yielded an analytical specificity of 100%, and an analytical sensitivity of 98.3%. The type specific amplification has been confirmed by DNA sequencing.

The test has been applied in our reference lab for 3532 reflex or duel cases in conjunction with pap cytology. Total of 549 HRHPV positives were identified, in which single infection (one genotype) is 408 cases, 74%; double infection (two genotypes) is 115 cases, 21%; triple infection or more is 26 cases, 5%; HPV 16 and/or 18 infection is 49%. Surprisingly, only 25% of total HRHPV positive cases are HPV 16 and/or 18, counted either as single infection or double infection, which will be covered by the quadruple HPV vaccine. Almost half of 16, 18, the two most common types, co-infect with other types.

In the 581 reflex atypical pap cases, 189 were identified as HRHPV positives (32.5%). Among the HRHPV positive cases, 18 were cervical cancer or high-grade squamous intraepithelial lesion (HSIL). Single HRHPV infection has been found in all 10 cancer cases (carcinoma in situ), including HPV 16 in 7 cases; HPV 18 in one case; HPV 52 and HPV 35 in the other two cases, respectively. Among the 8 cases of HSIL and/or atypical glandular cells (AGC), five were single infection including three HPV 16, one HPV 59, and one HPV 33; the remaining cases are multiple infections, with HPV 16 and 59; HPV 33 and 35; HPV 16, 39, 52 respectively. Among the positive HRHPV cases, 89 pap cytology graded CIN1 and above were tested HRHPV positive, yielding a clinical sensitivity of 100%. Referenced with the pap cytology result as standard, our genotyping test yielded a clinical specificity of 86.6%.

Only persistent infection of HRHPV contributes to the progression to cervical high-grade lesions and cancer, while most women can remove the transient HPV infection by their own immune system. Accurate HPV genotyping can distinguish the two scenarios, to identify the women who are really at high risk as persistently infected with specific HRHPV('s). By our HRHPV genotyping, multiple infections can be detected simultaneously, which is not practical by Hybrid Capture 2 assay or other commonly available tests. Approximately 25% of HRHPV positive patients were infected by multiple types according to our data. It has been noted that patients with multiple infections are at risk for treatment failure, which further validates the clinical significance of accurate genotyping.

A-131

Comparative Study of Jaffe Kinetic And Enzymatic Creatinine In Indian Origin

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Introduction: Jaffe kinetic method is the most commonly used for measurement of serum creatinine. It is cost effective and easy to perform. However it is affected by endogenous metabolites or exogenous substances. Enzymatic method is a better alternative where creatinine and derived metabolites are converted by the combined use of creatinase, creatininase and sacrosine oxidase; the liberated hydrogen peroxide is used to form colorimetric indicator.

Aims and objectives: To compare serum creatinine concentration obtained by Jaffe kinetic with enzymatic method, to standardize the reference range in Indian population and to assess the interference of bilirubin in measurement of the same.

Materials and methods: This prospective study was carried out over perioed of six months in semi urban locality. 122 individuals with age 19 to 75 years were included. Out of these 25 had normal kidney function, 72 were known cases of chronic kidney disease and 25 had altered hepatic function (serum billirubin 2 to 9 mg%). 2 cc blood sample was collected in plain bulb and serum used to estimate creatinine by Jaffe Kinetic Autospan and Meril india kits were used. For Enzymatic method AGAPPE reagents were used. All the methods were standardised as per the pack insert and the quality control tests were run on daily basis and validated externally. Tests performed on semi automated analyzer.

Results: Following results were noticed for Enzymatic and Jaffe for the above said groups of normal, chronic kidney disease, hepatic dysfunction. In the normal group comparison, enzymatic creatinine with the mean Patient value of 0.79 mg % ± 0.21 and Jaffe with mean Patient value of 0.86mg% ± 0.25 were yield the p value > 0.09 CKD Group Enzymatic creatinine with the mean patient value of 5.68 mg% ± 2.78 and Jaffe with mean patient value of 6.25 ± 2.81 yield the P value > 2.06 altered hepatic function patients with the mean Bilirubin of 5.33 mg% ± 7.28 yield enzymatic results 0.8 mg% ± 0.22 and Jafe 0.44 mg% ± 0. 45 with P value > 0.9

Reference range female 0.7 to 0.9 mg % Male 0.8 to 1.1 mg % The hypotheses with the P values > 0.05 clearly prove better utility of enzymatic creatinine method.

Conclusion: Enzymatic creatinine shows better accuracy and precision and ease of use. Reagent & Calibration stability is > 20 days, smaller volume of sample is required.

A-132

A Retrospective Review of Paraneoplastic Panel Utilization at a Tertiary-Care Academic Hospital to Determine Predictors of Diagnostic Yield

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Background: Despite the recent advances in understanding of paraneoplastic neurologic syndromes (PNS), no clinical guidelines for diagnostic evaluation of PNS currently exist. Recommendations for the work-up of PNS include radiologic, CSF, and electrophysiologic studies in combination with onconeural antibody detection. However no consensus on the integration of these studies into an algorithmic approach has been made. Our goal for this study was to determine if the patient's prior history of malignancy or results of radiologic, CSF, and electrophysiologic studies could predict the detection of onconeural antibodies and guide utilization review.

Methods: The results of all patients that had either serum or CSF paraneoplastic panel testing sent to a reference lab between February 2015 and February 2016 were reviewed. Chart review was conducted for all patients that had detectable onconeural antibodies and a subset of patients that did not. The following information was collected during chart review: age, sex, and presenting symptoms at time of testing; performance and findings of MRI/CT studies of the CNS, chest, and abdomen, electrophysiological studies, and CSF analysis including electrophoretic evaluation conducted prior to testing; prior or current history of malignancy; and treatment modification following reporting of results.

Results: A total of 97 serum and 24 CSF studies were sent for paraneoplastic panel evaluation over the one year timeframe. Antibodies were detected in 18 of 97 (18.6%) serum samples and 0 of 24 CSF samples. Eleven of the CSF studies had paired serum samples. The ordering preference for and increased sensitivity of serum analysis mirrors findings in the primary literature. All 18 patients with detectable onconeural antibodies and a random subset (n=15) of patients with negative results underwent chart review. The rate of performance of MRI/CT CNS, MRI/CT chest and abdomen, FDG-PET, EEG, EMG, and CSF analysis for patients that underwent chart review was: 65.6, 15.6, 9.4, 9.4, 59.4, and 59.4%, respectively. The rate of onconeural antibody detection among tests ordered by neurologists was 16.5% (n=97), whereas the rate for all other providers combined was 8.0% (n=24). The sensitivity and specificity of abnormal findings in CSF analysis to predict detection of onconeural antibodies were both 54.5% with no significant correlation between abnormal CSF findings and positivity for onconeural antibodies found by chi-squared analysis with Yates correction (X^2 1.66, p=0.44). The sensitivity and specificity of a prior history of cancer to predict detection of onconeural antibodies performed even worse at 5.6 and 34.6%, respectively. Investigation of treatment outcomes in the 18 onconeural antibody positive patients revealed that 5 (28%) were lost to follow-up, 6 (33%) had no follow-up with appropriate imaging studies, and 7 (39%) were followed appropriately. As of this time no malignancy has been detected in the patients that have undergone appropriate follow-up.

Conclusion: Our results demonstrate that history of prior malignancy and abnormal CSF studies do not predict detection of onconeural antibodies in patients being evaluated for PNS. CSF evaluation for PNS provides no increase in sensitivity in our patient population. There is significant variability among providers on follow-up of patients with positive results.

A-133

A qRT-PCR Diagnostic System to Monitor Toca 511, a Cancer-Selective Gene Therapy for the Treatment of Patients with Recurrent High Grade Glioma

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OBJECTIVE: This study determines if Toca 511 can be effectively detected in multiple body fluids to support Toca 5, a Phase 2/3 clinical trial in recurrent high grade glioma (NCT01470794).

RELEVANCE: Toca 511 (vocimagene amiretrorepvec) is an investigational retroviral replicating vector that encodes the transgene cytosine deaminase. Toca 511 may be delivered by multiple routes and selectively infects and spreads in tumor cells. Subsequent oral administration of investigational extended-release 5-fluorocytosine (Toca FC) results in formation of the antineoplastic drug, 5-fluorouracil, within infected tumors. 5-FU kills cancer cells and myeloid derived suppressor cells resulting in durable and selective immune activity against the cancer in preclinical models. Monitoring levels of this virus in bodily fluids is intended to detect non-tumor-specific viremia if it were to occur.

METHODOLOGY: Multiple manufacturing lots of Toca 511 were spiked into Tocagen Buffer (TB), Base Pool (BP; Siemens modified formulation of the SeraConII human plasma product from SeraCare), saliva (SV), or urine (UR). Viral RNA was purified from each diluent utilizing the Siemens VERSANT kPCR Sample Prep instrument and measured using a Siemens optimized RT-qPCR assay targeting the Toca 511 polymerase gene (*pol*). Differences in mean RNA recovery from matrices were tested using ANOVA on the mean quantitation cycle (Cq) +/- standard deviation. Due to viscosity differences, SV samples were diluted 4-fold in TB before processing. Stability after freeze-thaws and incubation at room temperature were also evaluated.

VALIDATION: There was no statistically significant difference observed for mean Cq in TB and BP (27.614 Cq +/- 0.614 in TB; 27.554 +/- 0.992 Cq in BP). Viral samples in UR generated higher Cq values (28.3743 +/- 0.379 Cq; $P<0.05$). After adjusting the mean Cq of SV samples for this additional dilution factor, SV was found to be equivalent to TB and BP. The effect of 1-3 freeze thaws and incubations at room temperature up to 24 hours did not affect mean Cq in any matrix.

CONCLUSION(S): Toca 511 virus is stable and detectable when spiked into various matrices. Lower recovery was observed for the virus in urine. Dilution of SV was required for automated processing due to increased matrix viscosity. These results indicate that Toca 511 levels may be accurately and efficiently monitored in multiple sample types from clinical trial subjects being treated for recurrent high grade glioma.

A-134

Comparison of Presepsin and Neutrophil Gelatinase-Associated Lipocalin in Predicting Acute Kidney Injury in Cardiac Surgery Patients

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Background Acute kidney injury (AKI) is a common complication after cardiac surgery. Neutrophil gelatinase-associated lipocalin (NGAL) has been reported to be a promising marker for cardiac surgery-associated AKI (CSA-AKI). Also sepsis has consistently been shown to be a contributing factor for the development of AKI. Presepsin has proven as a sepsis marker with high diagnostic and prognostic validity in assessment of disease severity and association to kidney function in septic patients.

Objective The aim of the present study was to evaluate the diagnostic validity presepsin and NGAL to predict CSA-AKI in patients undergoing elective cardiac surgery in comparison with inflammatory, cardiac and renal markers (CRP, procalcitonin (PCT), NT-proBNP, Cystatin C, and creatinine).

Methods The marker concentrations were measured in pre- and postoperative plasma samples which were drawn in the late afternoon before and the early morning after surgery from 235 patients undergoing elective cardiac surgery. Outcome measures were occurence of acute kidney injury (AKI) during hospitalization. Presepsin was determined by using the PATHFAST Presepsin assay (LSI Medience corporation,Tokyo). NGAL was measured by using the NGAL Rapid ELISA Kit, BioPorto Diagnostics A/S, Gentofte, Denmark. CRP, PCT, NT-proBNP, Cystatin C, and creatinine were measured using routine clinical chemistry methods in the central laboratory.

Results AKI has been assessed according to AKIN classification: stages 1 (n=41), 2 (n=21), 3 (n=9). Patients who developed AKI (n=71, *30.2%*) had higher pre- and postoperative presepsin and NGAL levels than patients without AKI. Both markers revealed higher levels postoperatively than preoperatively. The postoperative values of NGAL and presepsin exceeded the preoperative values 1.8fold and 1.7fold in patients without and 3.0fold and 1.9fold in patients with AKI, respectively, but these factors showed less discriminatory power than the marker levels. Receiver operator curve (ROC) analysis of postoperative values for prediction of AKI occurrence revealed AUC values of 0.813 and 0.828 for NGAL and presepsin, compared to AUC values of 0.808, 0.785, 0.636 0.624 and 0.529 for NT-proBNP, cystatin C, creatnine, PCT and CRP, respectively. Examination of the predictive value of marker combinations by logistic regression demonstrated superiority for the combined postoperative presepsin and NGAL values compared to all other possible combinations. The increased AUC of 0.856 showed that the simultaneous assessment of NGAL and presepsin performed better than the markers alone.

Conclusion Presepsin and NGAL demonstrated comparable predictive power to identify patients who were at risk of developing CSA-AKI. Moreover, the combination of both markers was found to improve the diagnostic performance. The simultaneous assessment of NGAL and presepsin allows early diagnosis of AKI already at the first day after surgery and may enable individual risk stratification with appropriate individualized patient care.

A-135

Plasma neuron derived exosomal protein biomarkers in the diagnosis of Alzheimer's Disease

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Background: Alzheimer's disease results in brain neuronal plaques composed of amyloid beta peptide (Aβ42) and neurofibrillary tangles composed of phosphorylated tau proteins (P-T181-tau and P-S396-tau). P-T181-tau and P-S396-tau are present at higher than normal concentrations and Aβ42 at lower than normal concentrations in the cerebrospinal fluid of AD patients. These proteins are not high in plasma samples of AD patients in part due to poor blood brain barrier transport and protease activities. Exosomes are shed by brain neurons, freely cross the blood brain barrier and protect and carry proteins from their cellular origin into plasma. We validated ELISA assays for Aβ42, P-T181-tau and P-S396-tau, and used them to quantify these proteins in neuron-derived exosomal extracts from normal and AD plasma samples.

Methods: Plasma samples were obtained from patients with mild cognitive impairment (MCI) and dementia due to Alzheimer's disease (AD), as well as matched normal controls. Exosomes were precipitated from the plasma samples using the ExoQuick preparation. Following centrifugation, they were suspended in a buffer containing protease inhibitors and phosphatase inhibitors. Antibody affinity purification with a solid phase mouse anti-human CD 171 antibody was used to enrich the content of neuron-specific exosomes. ELISA were validated for the biomarkers Aβ42, P-T181-tau and P-S396-tau including accuracy, precision, sensitivity, and specificity.

Results: ELISA assays for Aβ42, P-T181-tau and P-S396-tau were reproducible and the Inter-assay CVs were less than 15%. The sensitivity of the biomarker ELISAs varied from 2 - 10 pg/ml. Neuron-specific exosomes were prepared from the plasma of normal controls, MCI and AD patients. The reproducibility of the exosome preparations and biomarker levels were monitored in each ELISA. All biomarkers were elevated in MCI patients and AD patients compared to normal.

Conclusion: We have validated a reproducible procedure to isolate specific neuron-derived exosomes for quantification of specific protein biomarkers in plasma samples. The concentration of the biomarkers are high in patients with early dementia and Alzheimer's Disease. This procedure may be useful in the early diagnosis of Alzheimer's disease.

A-136

Noninvasive assessment of liver fibrosis staging using biomarkers in HCV carrier patients

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Background: Liver fibrosis involves excessive accumulation of extracellular matrix proteins (e.g., collagen) on liver cells, resulting in scar tissues. It occurs in most chronic liver disease, such as metabolic liver diseases and those associated with hepatitis B or C infection and alcohol consumption. Advanced liver fibrosis leads to cirrhosis, liver cancer, liver failure and portal hypertension. Currently liver biopsy is an optimal approach for detecting liver fibrosis and determining its severity. However, patient acceptance of this kind of examination is low, only less than 5% hepatitis patients are willing to perform biopsy, and thus many patients missed the appropriate treatment point. BioFibroScore® (General Biologicals Corporation, Taiwan) have been developed to predict liver fibrosis stages based on objective data from assays from three novel serum biomarker (urokinase-type plasminogen, matrix metal-loproteinase 9 and B-2-microglobulin) and the suitable algorithm. The present study aimed to analyze the accuracy of BioFibroScore® predicting liver fibrosis stages in HCV carrier patients.

Methods and results: A total of 100 frozen serum samples and their liver biopsy were provided by National Taiwan University Hospital, collected from HCV carriers. Numbers of these samples in each liver fibrosis stages were shown below: 12 samples of F0, 13 samples of F1, 25 samples of F2, 25 samples of F3 and 25 samples of F4. After analyzed by BioFibroScore® kits, we used two statistical strategies, logistic regression and SVM+KNN (suport vector machine + k-nearest neighbors algorithm), to construct prediction models that predict liver fibrosis stages of these 100 HCV carriers. The predicted results were compared with biopsy stages result. The accuracies with one stage tolerance of logistic regression model and SVM+KNN model were respectively 87% and 86%. The percentage of predicted liver fibrosis stages by logistic regression model lower and higher than biopsy stages were respectively 26% and 25%. The percentage of predicted liver fibrosis stages by SVM+KNN model lower and higher than biopsy stages were respectively 9% and 28%.

Conclusion: We obtained great accuracies by using these 100 HCV carriers samples to construct prediction models that could determine liver fibrosis stage. The potential clinical applications of BioFibrScore® are:

1. Reference for treatment decision
2. Monitor disease progression and prognosis
3. Outcome measurement for clinical trials.

A-138

CircRNAs in metabolic disease during pregnancy

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Background: Metabolic disease during pregnancy (MDP) includes mainly hypertensive disease of pregnancy (HDP) and gestational diabetes mellitus (GDM). Recently, it reported circular RNAs (circRNAs) may play important roles in the regulation of gene expression by acting as competing endogenous RNAs in diseases. So it may be a more effective early screening of circulating markers of disease. The lack of effective prediction scheme of MDP is the barrier of complications in prevention and management obstetrical severe cases. Here, we aim to investigate whether circRNA expression profiling can predict the metabolic disease during pregnancy before clinical diagnose.

Methods: A nested case-control study was performed at Guang Zhou Maternal-children Medical Center in 2015. Eighteen blood corpuscles were collected from groups (HDP, GDM and age and sample time matched control) before 24 weeks gestation (before clinical diagnose). The expression profile of circRNAs in blood corpuscles was performed using a human circRNA microarray which designed to simultaneously detect 5396 circRNA(Fig. 1).s. The express data of circRNAs was analyzed by volcano plot and heat map. The enrichment analysis and pathway annotation of these circRNAs' target genes were respectively done by DAVID.

Results: Volcano plot analysis shows the differentially expressed circRNAs among three groups using the parameters (P<0.01, fold change >50). We found that 293 circRNAs were differentially expressed among the three groups, in which 4 circRNAs were down-regulated and 15 circRNAs were up-regulated in the HDP group; 59 circRNAs were down-regulated and 215 circRNAs were up-regulated in the GDM group (Fig. 2). GO analysis of the circRNAs in the MDP group showed significant enrichment of biological processes, such as cell process, biological regulation (cell death, regulation of apoptosis, immune response, positive regulation of developmental process, positive regulation of cell differentiation, and regulation of angiogenesis). ; signaling (cell surface receptor linked signal transduction). The result of hierarchical clustering shows a distinguishable circRNA expression profiling among HDP, GDM and control group (Fig. 3).

Conclusion: CircRNAs may participate in the pathogenesis of MDP by involving in molecular function of ion binding, protein binding; glucuronosyltransfer et for HDP and of binding; cytoskeletal protein binding; Small GTPase binding; Ras GTPase binding,heterocyclic compound binding;organic cyclic compound binding et for GDM. HDP and GDM are distinguishable from gravidas by circRNA expression profiling of blood corpuscles before clinical diagnose. However, the results are preliminary and need to be validated in larger studies and other population. Here, hierarchical clustering was performed based on "All Targets Value - CircRNAs". The experiment consists of 18 different samples. The result of hierarchical clustering shows a distinguishable circRNA expression profiling among samples.

A-139

Combination of Urinary Liver-Type Fatty-Acid Binding Protein and Albumin Improves Prediction of Acute Kidney Injury in Patients Hospitalized to CCUs

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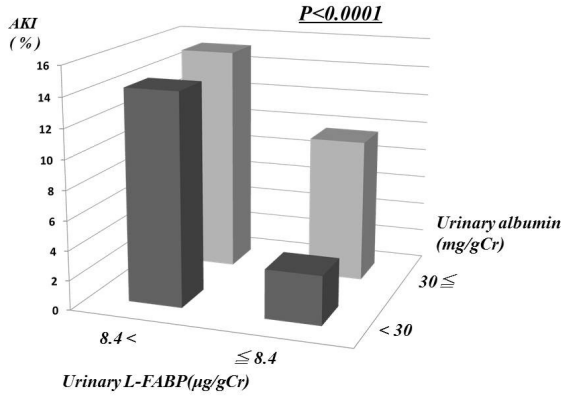
Background: Acute kidney injury (AKI) detected after admission to CCU is associated with very poor outcomes. Urinary albumin concentration reflects glomerular damage, and urinary concentration of liver-type fatty acid-binding protein (L-FABP) reflects tubulointerstitial damage.

Methods: We prospectively investigated the predictive value of combined urinary L-FABP and albumin on admission for AKI in 1,119 patients (mean age, 71 years) hospitalized to CCUs.Among these patients, 60% had heart failure; and 39.3%, acute coronary syndrome (ACS). AKI was defined as an increase of >50% in creatinine from baseline or an absolute increase of ≥0.3 mg/dL within 48 h after admission.

Results: AKI was detected in 177 (16%) patients. Multivariate logistic analysis identified urinary L-FABP (p=0.03) and albumin (p=0.002) as independent predictors of AK. Combined urinary L-FABP and albumin was associated with AKI incident rates (**Figure**).

Conclusion: The combination of urinary L-FABP and albumin could improve the prediction of AKI in patients hospitalized to CCUs.



A-140

The Discordance Between Serum and Vitreous Vascular Endothelial Growth Factor Levels in Proliferative Diabetic Retinopathy

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Background: In proliferative diabetic retinopathy (PDR), neovascularization occurs via hypoxia-induced vascular endothelial growth factor (VEGF), which is thought to be the major angiogenic agent. Vitreous VEGF level in eyes with PDR has been found higher than that in eyes with non-proliferative diabetic retinopathy (NPDR). There are conflicting results, however, regarding serum VEGF level in patients having PDR. Some studies indicate higher serum VEGF concentrations while others report that serum VEGF levels are not important. The objective of the present study was to find correlations between the vitreous and serum levels of VEGF in patients with type 2 diabetes mellitus that developed PDR.

Methods: Totally 88 subjects (63 diabetic and 25 non-diabetic) were enrolled. Diabetic patients were subdivided into 3 following categories: no diabetic retinopathy (noDRP, n=20), non-proliferative diabetic retinopathy (NPDR, n=20) and proliferative diabetic retinopathy (PDR n=23). Pars plana vitrectomy was performed with PDR while 15 out of 25 non-diabetics underwent vitrectomy for various reasons. Vitreous and serum samples were obtained during the vitrectomy procedures. Only serum samples were collected from the remaining participants. Serum and vitreous VEGF levels were analyzed using enzyme-linked immunosorbent assay (ELISA) by using Human VEGF-A Platinum ELISA kit from (BMS277/2, EBioscience). Total vitreous proteins and total serum proteins were also measured. The results were statistically compared. Statistical significance was accepted as P<0.05.

Results: There was significant difference for serum VEGF levels (P<0.001) and VEGF/total protein ratio (P<0.001) among 3 diabetic groups and non-diabetic control group. Higher vitreous VEGF levels were observed in eyes with PDR compared to non-diabetics (524.19±492.90 pg/mL [mean±standard deviation] *vs.* 97.97±108.72 pg/mL, P<0.001). The difference was still remained after correcting it for total vitreous protein levels (VEGF/total protein ratio) (P=0.001). Contrarily, serum VEGF levels did not differ between PDR and non-diabetic control group (493.15±372.02 pg/mL *vs.* 336.88±321.35 pg/mL, P=0.122). In non-diabetics, VEGF level in vitreous was significantly lower than that in their serum (P=0.001) while serum and vitreous VEGF levels were similar in subjects with PDR (P>0.05). However, there was no correlation between serum and vitreous VEGF levels of PDR and non-diabetic control group (P>0.05 and P>0.05, respectively).

Conclusion: Vitreous VEGF levels did not correlate with serum VEGF levels neither in patients with PDR nor in non-diabetics. It seems that the disruption of blood-retinal barrier was not the only part of proliferative retinopathy process. Elevated vitreous VEGF levels were mostly resulted of endogenous VEGF released from hypoxic retina. The increased VEGF level in serum may not be used as a significant marker for PDR development.

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A-141

Does heat shock protein 70 play a role on pathogenesis of proliferative diabetic retinopathy?

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Background: Diabetic retinopathy is the most common cause of blindness in productive age group of developed societies. Proliferative diabetic retinopathy, the severe stage of the pathology, manifests itself with retinal neovascularization and vitreoretinal proliferation. The exact mechanism of proliferative diabetic retinopathy has not yet been fully identified. An impaired antioxidant defense system probably contributes to the condition. The objective of the study was to find possible correlations between vitreous humor and serum levels of heat shock protein 70 (Hsp70) in eyes with proliferative diabetic retinopathy and compare them with non-diabetic subjects.

Methods: Overall 63 diabetic patients and 25 non-diabetic control subjects were enrolled in the study. Diabetic patients were divided into following 3 sub-groups according to their stage of retinopathy: no retinopathy (n=20), non-proliferative diabetic retinopathy (n=20) and proliferative diabetic retinopathy (n=23). Twenty-two eyes of 20 patients with proliferative diabetic retinopathy complicated with tractional retinal detachment and intravitreal hemorrhage as well as 15 eyes of 15 non-diabetic control group patients having macular hole, epiretinal membrane, rhegmatogenous retinal detachment underwent pars plana vitrectomy procedure. Vitreous humor and serum samples were collected from 37 patients at the time of vitrectomy. Serum samples were obtained from the rest of the subjects. The levels of Hsp70 in vitreous and serum were analyzed using enzyme-linked immunosorbent assay (ELISA). For this detection, Hsp70 high sensitivity ELISA kit was used (ADI-EKS-715, Enzo Life Sciences). Total vitreous proteins and total serum proteins were also measured. Kruskal-Wallis, Mann-Whitney U and Spearman correlation tests were performed. Statistical significance was set to $P<0.05$.

Results: There was a significant difference among 3 diabetic sub-groups and control group for serum Hsp70 levels ($P=0.024$) and for Hsp70/total protein ratio ($P=0.013$). Eyes that underwent vitrectomy for proliferative diabetic retinopathy had a significantly higher serum Hsp70 level (mean±standard deviation) compared to those of non-diabetic control group (201.06±80.44 pg/mL *vs* 85.43±27.47 pg/mL, $P=0.003$). Similar tendency was not observed between eyes with proliferative diabetic retinopathy and non-diabetic controls for vitreous Hsp70 level (149.79±56.60 pg/mL *vs* 184.41±154.40 pg/mL, $P=0.546$), and neither after adjusting it for total vitreous protein levels (Hsp70/total protein ratio) ($P=0.353$). In eyes with proliferative diabetic retinopathy, Hsp70 levels in serum and vitreous humor was similar ($P>0.05$). No correlation was found between serum and vitreous Hsp70 levels in non-diabetic control subjects ($P>0.05$).

Conclusion: The increased Hsp70 level in serum may be used as a significant marker for proliferative diabetic retinopathy development and may contribute to the elucidation of the pathogenesis of proliferative diabetic retinopathy.

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A-145

Multicenter characterization of a fully automated electrochemiluminescence immunoassay for the quantitation of serum periostin, a promising biomarker for guiding treatment in Type 2 asthma

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Objective: Periostin is a biomarker that may be effective in guiding treatment for Type 2 asthma with the drug lebrikizumab. This study aimed to characterize the Elecsys® Periostin immunoassay on the cobas e 601 analyzer under field conditions in 3 routine (field) laboratories. **Relevance:** Asthma, a chronic inflammatory airway disease with an increasing worldwide incidence, is a heterogeneous disorder that has at least two distinct molecular phenotypes defined by degree of Type 2 inflammation. The latter is caused by various cytokines, including the multifunctional and pleiotropic mediator IL-13. The physiological importance of IL-13 has made it a treatment target for Type 2 asthma using the monoclonal antibody lebrikizumab, and Phase II studies have indicated that lebrikizumab improves outcomes in patients identified with moderate-to-severe uncontrolled Type 2 asthma. Identifying Type 2 patients who will likely benefit from lebrikizumab treatment is important. Periostin is a surrogate biomarker of IL-13 activity in the airway that helps pinpoint asthma patients who are most likely to benefit from lebrikizumab therapy; periostin has potential for guiding therapy and improving both clinical and economic value of lebrikizumab treatment. However, for periostin to be used for guiding lebrikizumab therapy, an assay with potential for wide availability must be properly validated. **Methods:** Repeatability, intermediate precision, reproducibility and lot-to-lot variability were assessed according to CLSI-EP5-A3 guidelines, using sample panels consisting of 11 human serum samples and 3 concentration levels of quality control materials (14 samples in all) at 3 field laboratories. Materials were shipped to the 3 sites; each site used the cobas e 601 system (Roche Diagnostics) and a combination of 2 out of 3 available assay lots for measurements. The 11 individual sample pools and 3 control levels were run in randomized order. Study design included 1 run per day for each lot, with 5 replicates of each sample over 5 separate days. The total reproducibility and variance components were calculated for each of the samples and expressed as %CV using variance components analysis that included site, lot, day, run and within-run precision. **Validation:** For all 14 samples analyzed, reproducibility ranged between 1.7% and 3.1%. Intermediate precision was 1.2-1.7% CV. Repeatability ranged between 0.9% and 1.5% CV. **Results and Conclusion:** The Elecsys® Periostin immunoassay on the cobas e 601 system demonstrated the specified level of reproducibility in the field, with %CV values of <3.1% that are required for effective use in guiding lebrikizumab treatment. Lot-to-lot variability was satisfactory at 0.6–2.5% CV. The Elecsys® Periostin immunoassay has been used in the lebrikizumab Phase III LAVOLTA trials (NCT01867125/NCT01868061) to classify patients as periostin low (<50ng/ml) or periostin high (≥50ng/ml) based on pretreatment levels. This cut-off was previously established in the lebrikizumab Phase II study MILLY (NCT00930163) and confirmed in the Phase IIb studies LUTE (NCT01545440) and VERSE (NCT01545453). Our findings indicate that the Elecsys® Periostin immunoassay has the appropriate performance characteristics for use in identifying patients who may benefit from lebrikizumab treatment. **Disclaimer:** This product is not cleared or approved for use in the USA or elsewhere.

A-146

Evaluation of Astute Medical NEPHROCHECK © Test System as a risk assessment device for moderate to severe acute kidney injury (AKI)

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Background: Acute kidney injury (AKI) is defined as abrupt decrease in kidney function as measured by an increase in serum creatinine or decrease in urine output. However in the non-steady state setting of AKI, serum creatinine does not accurately reflect the glomerular filtration rate (GFR), and may lead to a delay in the diagnosis of AKI. Additionally, serum creatinine often does not increase until kidney function has declined by at least 50%. AKI is independently associate with increased morbidity and mortality in critically ill intensive care unit (ICU) patients. A sandwich immunoassay has been developed to measure urinary concentration of insulin-like growth factor binding protein 7 (IGFBP-7) and tissue inhibitor of metalloproteinase 2 (TIMP-2). These markers have been shown to be elevated upon renal tubular injury and prognosticate the future development of AKI. The objective of this study was to evaluate the performance of this new assay for risk assessment of human AKI.

Methods: The study examined urine samples of 43 patients who are 21 years of age or older without gender specification. The inclusion criteria for this study are at least one of the following two acute conditions within 24 hours prior to enrollment: 1.) Respiratory sepsis-related organ failure assessment (SOFA) score of ≥ 2 (PaO_2/FiO_2 <300). 2.) Cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required). Patient had not been diagnosed with AKI or had stage 1 AKI (50% or 0.3 mg/dL increase in serum creatinine from baseline) based on the Kidney Disease Improving Global Outcomes (KDIGO). During evaluation, NEPHROCHECK® Calibration Verification Kit was used as linearity material and Liquid Control Kit for precision study. Both kits contain TIMP-2 and IGFBP7 markers used to derive the AKIRISK™ Score.

Results: The Astute Medical NEPHROCHECK® Test System demonstrated good linearity for the calculated measurement of IGFBP-7 and TIMP-2 concentration converted to a risk score range of 0.06-6.21 $(ng/mL)^2/1000$ with an $R^2>0.999$. The total precision displayed CVs at <15% for all QCs. Interference study showed assay was not affected by hemolysis index up to 200 mg/dL and bilirubin index up to 16 mg/dL. Comparison between two NEPHROCHECK® devices showed the equation $y= 1.03 x + 0.005$ ($R^2> 0.99$, $n= 43$), with an acceptable overall bias of 0.05.

Conclusion: The data demonstrates good analytical performance of precision and correlation and is acceptable for clinical implementation.

A-147

BIOCHEMICAL EVALUATION OF SEMINAL PLASMA IN AZOOSPERMIC SAMPLES

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Background: Infertility is considered one of the main public health issues, as it affects about 15% of the couples of reproductiveage. The male factor is involved in 40% - 50% of infertility cases and azoospermic men constitute approximately 10 to 15% of all infertile men . The aim of our study is to determine the relationship between biochemical parameters in seminal plasma and the complete absence of sperm in the ejaculate.

Methods: Prospective study, patients older than 18 years who asked for a spermiogram were included, those who did not follow the pre-analytical guidelines recommended by the WHO and lack of Informed Consent were excluded. Patients were classified into two categories: one group with presence of sperm in the ejaculate and the other group with azoospermia. Several biochemical parameters (glucose, creatinine, urea, AST, ALT, CK, lactate dehydrogenase, CRP, total protein, alkaline phosphatase, Na, K, chloride, calcium, cholesterol) and hormones (TSH, testosterone, FSH, LH, prolactin, cortisol) were determined in the seminal plasma and serum on the COBAS MODULAR 711 (Roche Diagnostic). **Results:** Forty-six men with a mean age of 36.65 (range between 23 and 54) years were studied. Six cases had azoospermia. Azoospermia patients had significantly lower levels of urea 54 (42-55) mg/dl vs. 62 (58 to 74.5) mg/dl; p=0.026, ALT 32 (8-39) IU/L vs. 47 (38-58) IU/L; p=0.013 and AST 172 (131-192) IU/L vs. 285 (231-357) IU/L; p=0.01. TSH was significantly higher in the azoospermic samples 0.14 (0.12-0.16) mU/L vs. 0.08 (0.052-0.1) mU/L; p=0.008. The serum parameters did not correlate with the seminal plasma ones. The following table shows the areas under the ROC curves (AUC) and the optimal cutoff points with corresponding sensitivity and specificity.**Conclusion:** Decreased urea, ALT and AST levels and high TSH value in seminal plasma may predict the presence of azoospermia.

	AUC (CI 95 %)	Cutoff	Sensitivity (CI 95 %)	Specificity (CI 95 %)
Urea	0.892 (0,765-0,964)	55 mg/ml	100% (100-100)	85% (70.2-94.3)
ALT	0.860 (0.722-0.945)	39 UI/L	100% (100-100)	73.7 % (56.9-86.6)
AST	0.925 (0.808-0.981)	192 UI/L	100% (100-100)	85 % (70.2-94.3)
TSH	0.948 (0.823-0.992)	0.1 mU/L	100% (100-100)	81.3 % (63.6-92.7)

A-148

S100B protein as serum marker of brain damage after general anesthesia

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Background. S100B protein is a serum marker of cerebral damage. The objective was to evaluate the brain damage caused for general anesthesia, by determining the concentration of serum S100B protein before and after of general anesthesia. **Methods.** Patients with chronic adenotonsillar hypertrophy and indications for tonsillectomy were included. Venous blood sample was taken from patients before general anesthesia (basal sample). The patients were anesthetized using the following intravenous anesthetic drugs: midazolam, fentanyl and propofol; and sevoflurane inhaled. Second venous blood sample (sample postoperative) was taken from patients after the surgery, in the operating room. The concentration of serum S100B protein was determined in the basal sample (S100Bb) and postoperative sample (S100Bp) by immunoassay electro-chemiluminescence in MODULAR E-170 (Roche Diagnostics®). **Results.** 76 patients were included, 46 males and 30 females, with age between 3 to 14 years (median = 5 years). Descriptive statistics are showed in following table (CI: confidence interval; IR: interquartile range):

	Lowest	Highest	Median (95% CI)	IR
S100Bb (ng/L)	41,0	205,0	94,5 (89,0-103,0)	41
S100Bp (ng/L)	57,0	1052,0	164,0 (145,0-189,0)	149
S100Bp-b (ng/L)	7,0	955,0	58,0 (42,0-75,0)	136

In all patients, serum S100B protein levels have increased after general anesthesia. The values of S100Bp (median = 164.0 ng/L) were significantly higher than values of S100Bb (median = 94.5 ng/L). The median of difference between S100Bp and S100Bb was 58.0 ng/L. There were statistically significant differences between S100Bb and S100Bp using the Wilcoxon test (p<0.0001). **Conclusions.** The concentration of serum S100B protein has increased significantly after general anesthesia. This indicates that general anesthesia may cause brain damage.

A-151

Stabilization of glucose concentration in the new VACUETTE® FC Mix blood collection tube for diagnosis of gestational diabetes

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Background: Reliable detection of Gestational Diabetes Mellitus (GDM) is required to prevent maternal and fetal complications. With transport times of up to 48h, the aim of the study was to demonstrate long term stability of initial glucose concentration in specimens centrifuged directly after collection and compared to whole blood specimens stored at room temperatures. Recent guidelines from the German Diabetes Association recommend use of tubes with an additive composed of citrate, EDTA and sodium fluoride, as in VACUETTE® FC Mix tubes, to effectively stabilize glucose levels for the diagnosis of GDM due to the incomplete inhibition of glycolysis by sodium fluoride alone.

Methods: The current study was conducted at ISALA Hospital (Zwolle, Netherlands) using VENOSAFE™ FC Mixture® versus VACUETTE FC Mix blood collection tubes. Altogether, 43 pregnant donors who were healthy (n=19) or diagnosed with gestational diabetes by 75g-Oral Glucose Tolerance Test (n=24) were recruited. Informed consent was given by all donors and the study was approved by EC Netherlands. Venous blood was drawn from each donor into four tubes (two tubes each tube type). One tube of each type was centrifuged directly after blood collection according to manufacturer recommendations and the second one after whole blood storage for 48h at room temperature. Following collection, plasma was measured immediately after centrifugation to obtain initial values (fasting) and after 48h for evaluation of glucose stability using the Hexokinase method on a COBAS 8000 (Roche Diagnostics, Mannheim, repeatability VC 1%, total precision VC 1.7%). Statistical evaluation was done by STATISTICA 12.

Results: Evaluation of all clinical results for glucose concentration and any deviations was done on the basis of maximal allowed deviation for a single value (for glucose 11%) according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of both tubes for performance testing did not reveal any clinically nor statistically significant deviations (p<0.05). The values of both tubes resulted in an initial highest deviation of 5.5%, and 6.4% after 48h (both healthy). Comparable highest deviations for initial values in relation to 48h values were obtained for VENOSAFE and VACUETTE tubes with 5.4% (healthy) and 6.6% (GDM), respectively. The storage of whole blood specimens for 48h showed no significant deviation (10.5%, healthy).

Conclusion: Based on these results, the VACUETTE FC Mix blood collection tube is suitable for reliable determination of blood glucose, one of the most frequently measured laboratory analytes and of primary importance in diagnosis, monitoring and therapy of GDM. The stability of glucose concentration in whole blood specimens drawn in the VACUETTE FC Mix tube and stored up to a 48h at room temperature has been shown. Use of this tube will improve the stability of glucose with extended transport times up to 48h, which is more common with centralization of laboratory testing, negate the need for sample aliquotting and allow for good obstetric practice.

A-152

Use of Selected Clinical Laboratory Tests - Claims Data from a Sample of Commercially Insured and Medicare Supplemental Enrollees, 2009-2014

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Background: Identifying reimbursement changes for specific clinical laboratory tests may provide information to assess the effectiveness of test recommendations to screen for or treat diseases, inform practice guidelines, and provide needed information for health policy decisions. Laboratory reimbursements rates for Medicare Part B enrollees in 2000-2010 for the most commonly ordered clinical laboratory tests were previously evaluated (Shahangian S, et al. *Arch Pathol Lab Med*. 2014;138:189-203). However, that study did not include the younger, commercially insured U.S. population. The objective of this study was to provide information useful for more effective implementation of evidence-based recommendations by evaluating test ordering trends of specific clinical laboratory tests or test panels in 2009-2014. Methods: Claims data were collected using Truven Health Analytics' MarketScan databases for commercial claims and encounters and Medicare Supplemental in 2009-2014 (92 million). These populations constituted an average of ~30% of privately insured U.S. population, as well as ~5% of Medicare enrollees. The ratio of the most recent (2014) divided by the oldest (2009) reimbursed test utilization rate (rate ratio or RR) was used as a measure of trend. A two-sided Poisson regression, adjusted for potential overdispersion, was used to determine *P* for trend. Trends were considered significant at $P < 0.050$. Results: Of the 77 laboratory tests and 6 test panels examined, 59 (77%) showed very significant ($P < 0.0001$) trends; and in all but five of them utilization rate increased. Exceptions were tests for myoglobin (RR, 0.62), phenytoin (RR, 0.64), electrolytes (RR, 0.68), carbohydrate antigen (CA) 125 (RR, 0.83), and carbamazepine (RR, 0.86). There were also less significant downward trends for two tests with RR < 0.85: digoxin (RR, 0.76; $P = 0.002$) and cervical cytology (RR, 0.83; $P = 0.0003$). The greatest RR values were seen for testing of drugs with potential for abuse or overuse from ethanol (RR, 2.31) to nicotine (RR, 15.09), $P < 0.0001$. Seventeen other tests showed significantly increasing use over time (RR, ≥1.50; $P < 0.0001$): natriuretic peptide (RR, 1.50; $P = 0.0005$), human papilloma virus (HPV) DNA (RR, 1.52), D-dimer (RR, 1.53), IgM (RR, 1.59), herpes in women (RR, 1.65), folic acid (RR, 1.67), herpes in men (RR, 1.75), free PSA (RR, 1.76), glycohemoglobin (RR, 1.76), vitamin B-12 (RR, 1.84), testosterone in men (RR, 1.97), fibrinogen (RR, 1.99; $P = 0.001$), hepatitis C virus antibody (RR, 2.18), chlamydia in men (RR, 2.29), gonorrhea in men (RR, 2.35), vitamin D (RR, 2.49), and apolipoproteins (RR, 3.11). Conclusion: These results show changes in utilization practices for laboratory tests over a 6-year time frame, with some trends likely to have been positively impacted by evidence-based recommendations such as decreasing CA 125 screening for ovarian cancer, while others showing that recommendations have not been as effective such as increasing vitamin D testing. Given that some laboratory utilization trends do not follow evidence-based recommendations for certain laboratory tests, there is a need to understand the underlying factors so that measures can be adopted to promote better laboratory utilization practices.

A-153

Stabilization of organisms in the VACUETTE® CCM urine collection tube for diagnosis of urinary tract infections

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Background: Reliable test results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract infections (UTI), one of the most common healthcare associated infections. With regard to delays in delivery to the laboratory, an increase in microbial counts due to lack of preservative or inappropriate transport temperatures may lead to false results. The new VACUETTE® Urine CCM Tube contains a novel preservative that stabilizes urine samples at room temperatures (20-25°C) for up to 48 hours making it appropriate for collection, transport, storage and microbiology testing. Urine culture results showing microbial counts of $\geq 10^5$ CFU/ml are indicative of a UTI. Counts below usually indicate contamination of the urine sample.

Methods: A study was designed to evaluate the stability of organisms (bacteria and yeast), which occur frequently in urine, for a storage period of up to 48h at room temperature relative to initial counts in the specimen. Urine samples (n= 170, partly spiked) from clinically inconspicuous and conspicuous (nitrite and leucocyte positive with dipstick urinalysis) specimens were used. Midstream urine was collected from healthy subjects and clinical patients using a urine beaker (100ml) with integrated transfer unit. All specimens were transferred to the CCM tube and examined within 2 hours (initial), 24h and 48h after filling. On the basis of adjusting the McFarland, spiked-in urine samples were prepared with a target concentration of 10^3 CFU/ml (low) and 10^5 CFU/ml (high) from 120 healthy subjects. The organisms were selected based on CLSI, M40-A. Samples were assessed for stability of the following pathogenic organisms: *Escherichia coli (ATCC®25922), Enterococcus faecalis (ATCC®29212), Pseudomonas aeruginosa (ATCC®BAA-427), Staphylococcus saprophyticus (ATCC®15305), Proteus mirabilis (ATCC®7022),* and *Candida albicans (ATCC®24433)*. Agar test plates were innoculated with 10µl of urine and incubated for 24 to 48h at 37°C, followed by visual counting of colonies. Counts were recorded as CFU/µl. This innoculation method yields a detection limit of 0.1 CFU/µl, equivalent to 100 CFU/ml.

Results: Of all clinically inconspicuous urine specimens, 26 contained <1,000 CFU/mL at the initial time point, 15 showed a bacterial count between 1,000-10,000 CFU/mL, 6 between 10,001-100,000 CFU/mL, and 3 specimens >100,000 CFU/mL. Of all clinically conspicuous urine specimens, 18 contained <1,000 CFU/mL at the initial time point, another 8 between 1,000-10,000 CFU/mL, 2 between 10,001 - 100,000 CFU/mL, and 22 specimens >100,000 CFU/mL. None of the tubes showed a significant deviation in relation to the initial bacterial count. Additionally, none of the urine tubes spiked with facultative pathogenic organisms in either concentration ($1*10^3$ CFU/mL or $1*10^5$ CFU/mL) showed significant deviation.

Conclusion: These results demonstrate the suitability of the VACUETTE Urine CCM Tube for microbial testing. This tube stabilizes the tested organisms responsible for urinary tract infections for 48h at room temperature. The VACUETTE Urine CCM tube is a urine sampling and transport system suitable for microbiologic diagnostics and improves preanalytic variables in urine culture.

A-155

Modified fasting glucose cutpoints reduce un-necessary tolerance testing in pregnant women from a large urban and rural population

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Background: Calgary Laboratory Services (CLS) is the sole provider of laboratory testing for Calgary and surrounding communities in Alberta, Canada (>1.4 million people). Glucose tolerance testing is performed by CLS at outpatient laboratories in hospitals and patient service centers (PSCs) in Calgary, rural hospitals and community health centers. At each site, the 75 gram glucose drink is administered only if a fasting glucose is <7.8 mmol/L unless otherwise requested. This is because a fasting glucose ≥ 7.0 mmol/L combined with clinical symptoms is compatible with a diagnosis of diabetes. This decision is made by rapid fasting venous glucose at all sites except PSCs, where a capillary glucose by glucometer is used to decide whether to proceed while a venous sample is sent for later testing at a central lab. Glucose tolerance testing (75 gram) is performed in pregnant women if their gestational diabetes (GDM) screening test is inconclusive (1-hour glucose post 50 gram load = 7.8-11.0 mmol/L). GDM is diagnosed by either a fasting glucose ≥ 5.3 mmol/L, a 1-hour glucose ≥ 10.6 mmo/L, or a 2-hour glucose ≥ 9.0 mmol/L. Reducing the fasting cutpoint value from 7.8 to 5.3 mmol/L would reduce un-necessary testing and potential harm. However because diagnosis cannot be made by glucometer alone, a glucometer cutpoint must be chosen with adequate sensitivity and specificity for diagnosis by the fasting venous result which is returned later. Our objectives were to determine how many pregnant women will be saved from glucose tolerance testing by (1) changing the fasting cutpoint from 7.8 to 5.3 in urban hospitals and rural sites, and (2) selecting a glucometer cutpoint with a false positive rate that would minimize the number of patients required to repeat the tests. **Methods:** Two years of glucose tolerance testing results (venous fasting glucose, 1-hour glucose, 2-hour glucose) for pregnant women were extracted along with accession number from the CLS lab information system. The number of GDM diagnoses made by diagnostic criteria were determined. Venous results were linked by accession number to a fasting glucometer result from a separate server. Receiver operating characteristic (ROC) curve analysis was used to select a glucometer cutpoint with a false positive (GDM) rate of 1 patient per month.

Results: Across CLS, 4400 GDM tolerance tests were performed annually resulting in 1314 GDM diagnoses, 409 (31%) of which were by fasting glucose alone. As 11% of tests were performed at urban hospitals and rural sites, approximately 45 patients would be saved annually from testing by changing the fasting cutpoint to 5.3 mmol/L. ROC analysis of PSC capillary (glucometer) glucose and GDM diagnosis by fasting venous glucose yielded a c-statistic of 0.97. A glucometer cutpoint of ≥ 5.8 mmol/L resulted in a sensitivity of 42% and specificity of 99.7%, which if implemented would save 153 patients annually from having to undergo tolerance tests, and result in 1 patient per month having to be re-tested.

Conclusion: Modifying fasting glucose cut-points for pregnant women will eliminate un-necessary tolerance testing in 198 patients annually, resulting in only 1 re-test per month.

A-157

A Comparison Between Hemolyzed Specimen Rejection Rates and Blood Collection Techniques In Emergency Department

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Background:

The employment of industry standard blood collection techniques greatly impacts the quality of patient care (QPC) delivered at medical facilities, as improper techniques can lead to hemolysis thus decreasing the accuracy of clinical results by producing various testing interferences[1]. Moreover, QPC is affected through subsequent re-draws leading to an increase in patient turnaround time. The objective of this study was to identify the primary *in vitro* factors resulting in the hemolysis of blood samples from the Emergency Department (ED) at Southlake Regional Health Centre (SRHC). This was accomplished through a two phase study in which initial retrospective analysis of rejected specimens' database was carried out to determine the rate of rejection due to hemolysis in the ED compared to other in-patient units, followed by observation of techniques deemed through literature to be factors that may result in hemolysis of blood samples[1].

Methodology:

Collection date and time, ordering unit, patient accession number and reason for rejection were extracted from the laboratory information system of Southlake Regional Health Centre (March to June 2015). The rate of rejection due to hemolysis was compared between the ED and other in-patient units followed by development of an observation checklist which included observations for: phlebotomy site, equipment used, needle gauge, length of tourniquet placement, number of punctures, filling of tubes, degree of mixing, and mechanical trauma. Observations of 114 randomly selected phlebotomy procedures were conducted in the ER from July 18 to August 25 followed by an analysis to determine correlation between the observed techniques and hemolysis of blood samples.

Results:

Of the 274 specimens rejected due to hemolysis from March to June 2015, 191 were rejected due to hemolysis in the ED (70%). Of the 114 randomly selected phlebotomy observations in the ED, 27 produced some degree of in-vitro hemolysis (24%). The greatest correlation was found between the use of 20 gauge IV catheters for blood collection and the presence of hemolysis in the blood sample. Use of IV catheters observed to produce 67% of hemolyzed samples (n=18), remaining 33% of blood samples were collected using straight or butterfly needles (n=9). All other factors were not found to correlate significantly with the presence of hemolysis in the blood samples.

Conclusion:

The results of the study support previous findings indicating that collection through IV catheters is the leading cause of hemolysis[2]. Other factors were determined to be insignificant contributors to the high rate of hemolysis in the ED at SRHC, providing insight to the possibility that the leading *in vitro* causes for hemolysis varies between hospitals as a result of differing blood collection techniques. Future research can be aimed at studying the components of the IV catheter and their potential effects on hemolysis, in order to improve QPC.

1. Lippi G et al. Critical Reviews in Clinical Laboratory Sciences 2011; 48:143-153.

2. Wollowitz A et al. Academic Emergency Medicine 2013; 20:1151-1155.

A-159

Monocyte Chemoattractant Protein-1 Levels are Associated with Reduced Myocardial Reperfusion after Primary Percutaneous Coronary Intervention for ST-Segment Elevation Myocardial Infarction

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Background: : Inflammation is an important factor in atherosclerosis and the development of coronary heart diseases. The CC chemokine monocyte chemoattractant protein (MCP)-1 is involved in the formation, progression, and destabilization of atheromatous plaques. Emerging evidence suggests that MCP-1 plasma levels have prognostic value in the acute and chronic phase following acute coronary syndrome, providing information independent of standard clinical variables. The aim of this study was to investigate the time course and possible associations between MCP-1 levels and myocardial reperfusion after primary percutaneous coronary intervention (pPCI) for acute ST-elevated myocardial infarction (STEMI).

Methods: A total of 43 consecutive patients with the first anterior STEMI successfully treated by pPCI (< 20% of residual stenosis and TIMI flow 3) within 6 hours after the onset of the chest pain were included. Serum was sampled at baseline, 4, 12, 24 hours after pPCI, 2 days and 7 days after pPCI. Samples were stored at -80°C until analysed. Quantification of MCP-1 concentration was performed by Randox, Ltd. (Crumlin, UK), by using a biochip array analyzer (Evidence Investigator®). Coronary angiograms post-PCI were analysed for myocardial blush grade (MBG) as indicator of myocardial reperfusion injury and reflects the microvascular damage. Myocardial blush grade was categorized as follows: 0 (no myocardial blush, or contrast density), 1 (minimal myocardial blush), 2 (moderate myocardial blush but less than that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery) and 3 (normal myocardial blush comparable to that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery). The primary analysis was MBG (reduced vs normal, 0, 1, 2 vs 3). Coronary angiograms were analysed by a physician blinded to clinical data. Univariate and multivariate logistic regression analyses were used to explore the association between MCP-1 levels and myocardial blush grade.

Results: The average age was 56 ± 11 years and 80% were male. Seventy percent of the patients had hypertension, 27% had diabetes mellitus, 56% hyperlipidemia, 30% a positive family history of coronary heart disease, and 40% were current smokers. The median time from symptom onset was 2.5 h and did not differ significantly between groups (p>0.05). Clinical characteristics did not significantly differ between patients with reduced and patients with normal MBG. MCP-1 level at baseline was 202 (153-237) pg/mL in patients in MBG normal group and 324 (IQR 239-457) pg/mL in MBG reduced group. In both MBG groups, MCP-1 level decreased over time. This decrease was more pronounced in patients with optimal reperfusion compared to patients with reduced reperfusion. After multivariate adjustment, baseline MCP-1 was an independent predictor of the optimal MBG (OR 1.012, 95% CI 1.002-1.023, P=0.021).

Conclusion: Our study shows an association of MCP-1 with MBG after pPCI in STEMI patients.

A-160

The incidence of *Streptococcus pneumoniae* strains in patients with otitis media in hospital and ambulatory

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Introduction: Otitis media is common especially in children, representing an important socio-economic issue both through expanded use of financial and human resources. The development of mechanisms of bacterial resistance to antibiotic treatment is a problem in otitis therapeutic management.

Aim: To assess the incidence of pneumococcal strains involved in the etiology of otitis media and the development of prophylactic regimens in a population group of hospital and ambulatory.

Material and methods: We performed a prospective study of pathogens isolated from patients with acute otitis media, diagnosed on the basis of otoscopic findings of either middle ear effusion or purulent otorrhea with a duration of less than 24 h. Isolation on conventional culture media (blood and chocolate agar) and identification of germs were performed at the hospital laboratory. Identification of *Streptococcus pneumoniae* and extensive antimicrobial tests (by dilution antimicrobial susceptibility tests) were performed using the BioMerieux® VITEK2 automated microbiology system, with Vitek 2 GP and Vitek 2 AST P533 cards (EUCAST standards). Quality control strains used in the testing was *Streptococcus pneumoniae* ATCC 49619.

Results: We analysed 50 patients samples. Out of these 40%(20 patients) were *Streptococcus pneumoniae*, 20%(10 patients) nontypable *Haemophilus influenzae*, 3%(6 patients) *Moraxella catarrhalis*, 8%(4 patients) *Streptococcus pyogenes* positive. *Staphylococcus* spp. and *Enterobacteriaceae* strains were interpreted as a contaminant 19%(10 patients). Of 20 isolates of *S. pneumoniae* tested in our study,

35% were intermediately or fully resistant to penicillin. Finally, 47% of intermediately and fully penicillin-resistant isolates were resistant to multiple antimicrobial classes in our study. Only 1% of the isolates of *S. pneumoniae* were resistant to amoxicillin in our study.

Conclusions: Our findings may have clinical implications because the selection of antibiotic therapy for intermediately or fully penicillin-resistant *S. pneumoniae* infections. The need for parenteral agents in patients with acute otitis media is especially doubtful because of the low morbidity, high frequency of spontaneous recovery, and frequent resolution of clinical signs and symptoms, despite the persistence of bacteria in middle ear fluid.

Tuesday, August 2, 2016

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

Endocrinology/Hormones

A-161

Systemic inflammation affects human osteocyte signaling

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Background: Bone remodeling, phosphate homeostasis, and cardiovascular function are disturbed in rheumatoid arthritis (RA), possibly as a result of elevated levels of circulating inflammatory cytokines. Osteocyte signaling plays a vital role in bone remodeling, phosphate homeostasis, and cardiovascular function. Therefore we aimed to investigate the effect of RA-serum or inflammatory cytokines on human osteocyte signaling.

Methods: Human trabecular bone chips containing live osteocytes in their native matrix were cultured with RA-serum or inflammatory cytokines for 7 days. Live-dead staining was performed to assess cell viability. Gene expression of osteocyte signaling proteins and cytokines was analyzed by qPCR. Immuno-staining was performed for osteocyte-specific markers.

Results: Approximately 60% of the osteocytes on the bone chips were alive at day 7. Cells in or on the bone chips did express the gene for osteocyte signaling molecules *SOST*, *FGF23*, *DMP1*, and *MEPE*, and the cytokines *IL-1β*, *IL-6*, and *TNFα* at day 0 and 7. Treatment with RA-serum, IL-1β, or TNFα enhanced gene expression of *IL-1β* (8 to 15-fold) and *TNFα* (2 to 3-fold). Treatment with IL-1β or TNFα, but not RA-serum, also enhanced gene expression of *IL-6* (25 to 32-fold) and *IL-8* (24 to 58-fold). The stimulatory effect of the combination of IL-1β, TNFα, and IL-6 on gene expression of *IL-1β*, *IL-6*, and *IL-8* was significantly higher (80 to 120-fold) than the effect of the individual cytokines. IL-1β, TNFα, and the combination of IL-1β, TNFα, and IL-6 enhanced *FGF23* expression (2 to 4-fold). *SOST* expression was

enhanced by IL-1β (5-fold), while RA-serum increased both *SOST* (2.5-fold), and *DKK1* expression (2-fold).

Conclusion: Osteocyte is not just a bone cell, it also has endocrine function. Osteocyte signaling, was affected by RA-serum, individual exogenous recombinant cytokines, and a combination of IL-1β, TNFα, and IL-6 suggesting that osteocytes could provide a new target to prevent inflammation-induced bone loss, disturbed phosphate homeostasis, and cardiovascular diseases.

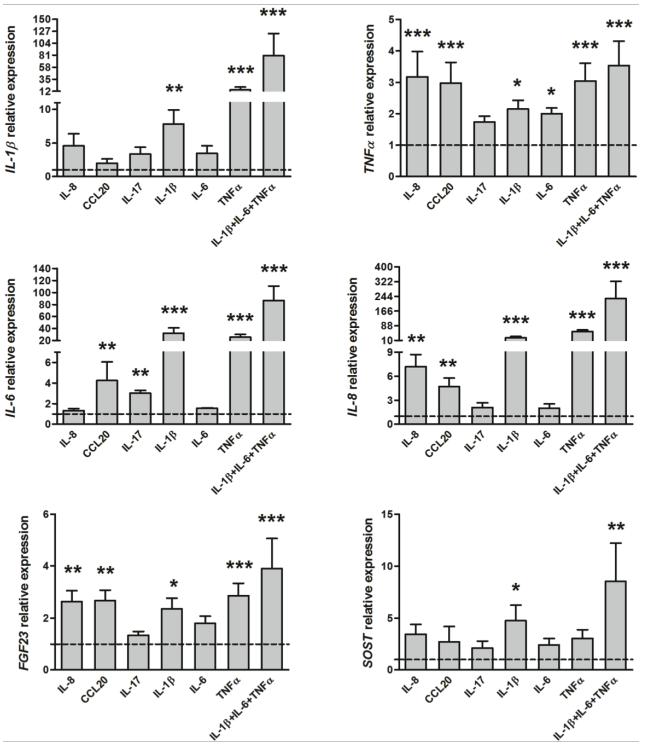

FIGURE. Inflammatory cytokines and chemokines, enhanced gene expression of cytokines (IL-1β,TNFα, IL-6 and IL-8) and signaling molecules (FGF23 and SOST) by osteocytes

A-162

Information content of paired measurements of A1c and 1,5-anhydroglucitol relative to post-prandial glucose excursions: a model simulation study

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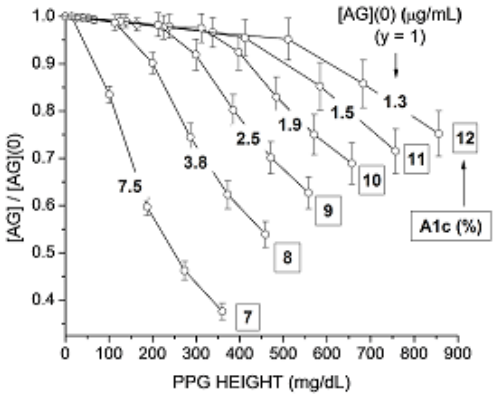
BACKGROUND: The magnitude of post-prandial glucose excursions (PPGEs) is arguably a factor contributing to diabetes complications beyond its influence on average glucose (G) as evaluated by A1c. 1,5-anhydroglucitol (AG; diet-derived; reference interval: 7-33 µg/mL) is in principle a high-side marker for hyperglycemia, responsive via decreased reabsorption when G exceeds a threshold of approximately 130 mg/dL. However, there are no established guidelines to interpretation of AG measurements. Our objective was to examine the predicted information content of paired A1c and AG measurements with respect to PPGEs. METHODS: We used an established mass balance model for AG (PMID: 9357814), assuming a basis of fixed normal GFR (100 mL/min) and population-average AG ingestion rate (4.6 mg/d). PPGEs were characterized as a fixed waveform (shape) characteristic of diabetes (maximum at 2h; half-maximum at 3h; duration 5h) with variations in specified height (H, maximum mg/dL). PPGEs were added to fasting plasma glucose (FPG) 3 times per day at 0600, 1200 and 1800 hours to form 24-h waveforms, G(t). For a given average H, individual PPGEs were varied according to a normal distribution of H(average) ±20% (1sd). The AG mass balance model was used to simulate changes in AG as a function of FPG and H(average). Simulations (n=100 per condition; 1 min intervals) were conducted until daily average AG was invariant (120 days). Average glucose was calculated from cumulative G(t) to determine associated %A1c. RESULTS: *Figure* shows simulated model relationship between AG (±1sd; y-axis normalized to baseline AG (H=0)) and H(average), with constant %A1c as a parameter. AG decreased with increased H. Resolution (ΔAG/ΔH) decreased substantially with increasing %A1c, due primarily to decreased baseline AG. CONCLUSIONS: Results provide a resource for interpretation of paired A1c/AG measurements relative to PPGEs. Caveats, however, include restriction of analysis to basis parameters (average AG ingestion rate, normal GFR) and a representative but fixed PPG waveform.



A-163

Serum Total Testosterone and Dehydroepiandrosterone levels in Osteoporosis males above the age of 60 years

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Background: There is much debate regarding osteoporosis in elderly males and its relation to the declining level of Testosterone and Dehydroepiandosterone (DHEA) in males after the age of 60 years which is responsible for fragile fractures. The purpose of this study is to determine the incidence of osteoporosis in males above the age of 60 years and their correlation with Testosterone and DHEA.

Methods: It's a prospective, cross sectional study in which 100 male patients of 60 years and above were evaluated for Osteoporosis. Patients were selected based on age, clinical signs and symptoms, who had visited as a participant of Orthopaedic camps for the assessment of Osteoporosis by Bone Mineral Density (BMD) machine organized by Universal College of Medical Sciences and Teaching Hospital. BMD were done for all males patients above 60 years at distal radius using quantitative ultrasounds. Blood samples were taken simultaneously after BMD record and sent for determination of Total Testosterone and DHEA level. The Total Testosterone and DHEA were assessed by competitive immunoassay technique using Human ELISA kit, Germany.

Results: Out of enrolled 100 men above 60 years suspected of Osteoporosis, 46 % is diagnosed as Osteoporosis, 32% as Osteopenia and 22% were observed to be normal based on BMD T-Score value. The average level of Serum Total Testosterone and DHEA in entire suspected osteoporosis patients were 2.74 ± 1.04 ng/ml and 1.45 ± 1.08 µg/ml respectively. The level across Osteoporotic cases (n=46), the average Total Testosterone and DHEA were 2.20 ± 0.77 ng/ml and 1.55 ± 0.91 µg/ml respectively. The cut off value by the International Society of Andrology considers abnormally low serum testosterone <2 ng/ml were found in 19 (41%) cases out of 46 osteoporotic men compared with normal testosterone level men. Testosterone deficiency was defined as a level of total testosterone <3 ng/ml which includes 39 (84%) cases of osteoporosis. This study has shown that the men with low testosterone levels had decreased BMD T-scores across entire cases which was statistically significant ($p<0.001$). The men with decreased DHEA level also had decreased BMD T-scores across entire cases but were statistically insignificant. BMI was not significant but inversely associated with testosterone and DHEA levels. In this study male above 60 years old osteoporotic patients with decreased plasma testosterone had a 14 fold higher risk for decreased BMD compared with their peer with normal testosterone level. The Correlation analysis of testosterone levels has shown significant association with BMD ($r=0.57$, <0.001) and BMD with age ($r=-0.24$, <0.01) respectively.

Conclusion: In this study, the incidence of osteoporosis increases with advancing age. It can be concluded from the statistical evidence that Testosterone play an important role in the progress and maintenance of osteoporosis but DHEA shows no such evidence in osteoporosis. But still further study is needed to clearly identify the role of testosterone in osteoporosis with interventional approaches in a large population. There was significant decline in Testosterone and DHEA with advancing age and moreover decreased in BMD indicating the pathogenesis of osteoporosis in the patients.

A-164

Determination of salivary cortisol: preliminary validation of the LIAISON® XL method

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Background: The measurement of analytes in matrices such as blood and urine is commonly used to gain useful clinical information, but also other biological fluids can give important clinical information. However, especially in immunochemistry, methods' performances as declared by the producers for conventional matrices, can't be entirely transferred when using not validated sample types. Therefore, whenever a laboratory has no availability of validated reagents for a specific matrix, here comes the need to validate internally those commonly used for conventional matrices. Among the biological specimens with increasing interest, saliva presents significant advantages: in fact, in addition to the advantage of a non-invasive collection procedure, it has been observed that concentration of some hormones measured in this matrix correlates better to the corresponding circulating blood free fraction than the total one. Two main applications are particularly relevant: Cushing syndrome diagnosis through nocturnal salivary cortisol determination and the monitoring of athletes' performances from morning samples, when higher concentrations are expected.

Methods: We did a preliminary evaluation for the salivary cortisol determination in our laboratory using the LIAISON® Cortisol assay (DiaSorin S.p.A., Saluggia (VC), Italy), validated by the producer for serum, plasma and urine samples. To fulfill the validation procedure we followed also the CLSI C49-A guideline *(Analysis of Body fluids in Clinical Chemistry; Approved Guideline)* measuring cortisol in 44 saliva samples, collected from apparently healthy donors between 11 pm - 12 pm (n= 25) and 8 am - 9 am (n= 19). Again the same samples have been measured with another method validated from the producer for saliva samples: Cortisol II on Elecsys® 2010 (Roche, Mannheim - Germany). The samples have been collected by spontaneous salivation with the Salivette® device (Sarstedt, Nümbrecht - Germany) and following centrifugation with no further treatment analyzed. The morning untreated samples which cortisol concentrations were detectable by both methods, underwent also measurement after extraction with dichloromethane, evaporation and resuspension in an adequate matrix for immunochemical analysis.

Results: Measured concentrations ranged from <0.054 to 1.19 mg/dL and from 0.16 to 3.18 mg/dL with both Elecsys® e LIAISON® XL systems, respectively. Comparing the results from the two analytical methods, a high correlation is evident (R^2=0,973, P<0,0001), both for direct measurement and with extractive methodology, but the regression equation of Passing & Bablok (y= 2,14x + 0,07) shows a significant difference between the methods, proportional to the measured sample concentration, even if the intercept is negligible. Regarding the nocturnal collected samples, whose concentration were undetectable by the Elecsys® system (< 0,054 mg/dL), meanwhile with the LIAISON® XL system the concentrations ranged between 0,16 and 0,29 mg/dL. The imprecision of the measured samples by the LIAISON® XL system resulted lower than 6%.

Conclusion:

From the obtained results of measured salivary samples by the LIAISON® Cortisol assay on the LIAISON® XL system we can conclude that further investigations are needed. Both the analytical and the reference ranges should be defined compared to the clinical status of patients.

A-165

Correlation of Thyroid function and biochemical parameters in type II diabetic subjects of Western Nepalese population

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Background: The prevalence of type II DM is continuously increasing worldwide. These disorders are shown to be associated with, among many other abnormalities and with thyroid dysfunctions. However these association have so far not been explored among Nepalese type II DM. Main aim of the present study was done to evaluate thyroid function in type II diabetics.

Methods: It was a hospital based case control study including 100 type II DM patients and 100 control. All subjects having no any known thyroid and other chronic illness. All the demographic and anthropometric data were collected using a preformed set of questionnaire and biochemical data were obtained from the laboratory analysis of the patients' blood samples. Statistical analysis was done with SPSS version 17.

Results: In this study we found overall of 17% prevalence in DM cases with Thyroid disorders and amongst this, 11.76% with Primary Hypothyroidism, 76.48% with Sub clinical Hypothyroidism and 11.76% with Sub clinical hyperthyroidism. A statistical significant difference was noted between cases-DM and controls with respect to BMI (p<0.000), arm circumference (p<0.000), FT3 (p<0.004), TSH (p<0.000), FBS (p<0.000), PP(P <0.000), HbA1C (p<0.000), TC (p<0.000), TG(p<0.005) and LDL (p<0.018) respectively. In this study, the mean±SD of FT3, FT4 and TSH in control and DM were found to be (2.43±0.64 and 2.67±0.93 with p-value 0.004), (1.06±0.27 and 1.15±0.31 with p-value 0.31) and (2.62±1.42 and 3.70±5.13 with p-value 0.00). Analysis between serum FT3, FT4 and TSH with respect to baseline characteristics and biochemical parameter of the study subjects showed negative significant correlation (p<0.05) between FT3 with region in DM, positive significant correlation between FT4 with Age in DM, positive significant correlation (p<0.05) between TSH with TC in DM.

Conclusion: This study confirms that thyroid dysfunctions is also common among Nepalese type II DM patients. Our study also reveal that prevalence of thyroid dysfunction is more common in type II DM. It is thus recommended that these group of population should be routinely screened for asymptomatic thyroid dysfunctions besides their usual treatment.

A-166

Graves disease: Patients with hyperthyroid status have a higher risk of developing type 2 diabetes

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Background. Graves' disease (GD) is a multi-systemic autoimmune disorder caused by thyroid stimulating antibodies that bind to and activate the thyroid stimulating hormone (TSH) receptor on thyroid cells (TRAbs). Common findings are low serum concentration of TSH, positive TRAbs, and high concentrations of anti-thyroid peroxidase antibodies (ATPO). In insulin-dependent diabetics, hyperthyroidism may aggravate glucose intolerance by multiple mechanisms, decreasing responsiveness to insulin. An association between type 1 diabetes mellitus (DM) and autoimmune reaction to thyroid antigens, including anti-thyroid antibodies (ATPO) in pediatric patients with positive TRAbs, was recently reported. The objective of this study is to investigate the association between thyroid status, serum TSH levels, positive TRAbs and ATPO, and the potential risk to develop type 2 DM based on insulin levels in adults. Methods. The study was conducted in 64 patients between May 2014 and October 2015. The mean subject age was 47 ± 18 years old and the male/female ratio was 11 (17.7% male):51 (82.3%female). Pregnant women and patients under 25 years of age were excluded. We measured TRAbs, ATPO, TSH, and insulin concentrations in euthyroids (TSH = 1.10 to 9.00 µU/mL) and hyperthyroids (TSH between 0.01 to 0.44 µU/mL). TRAbs were measured by second generation thyrotropin-binding inhibitor immunoglobulin (TBII) assay (DiaMetra, Italy). The cut-off for positive TRAbs was 1.50 UI/L. ATPO, TSH and insulin concentrations were determined by chemiluminescent microparticle immunoassay (CMIA) using a Advia Centaur (Siemens, USA). The cut off for positive ATPO was &gt 37 UI/mL, reference interval for TSH was 0.4 to 4.4 µU/mL and for insulin was 5 to 20 µU/mL. Data obtained for all measurements of TRAbs, ATPO, TSH and insulin in both groups was analyzed using the Student's t-test. A p value &lt 0.05 represented a significant difference. Data was expressed as mean ± standard error of the mean (SEM). Results. As expected, TSH serum concentrations were significantly decreased in hyperthyroid patients (0.13 ± 0.03) compared with euthyroid patients (3.31 ± 0.48) (t=12.79; p &lt 0.05). We observed a significant increase in TRAbs levels in hyperthyroid patients (7.67 ± 1.91) compared with euthyroid patients (2.23 ± 0.40) (t=2.07; p &lt 0.05). In addition, we reported a significant enhacement on ATPO levels in hyperthyroid patients (650.8 ± 84.82) versus euthyroid patients (296.2 ± 85.30) (t=3.03; p &lt 0.05). Similarly, higher insulin levels were observed in hyperthyroid patients (15.35 ± 1.94) versus euthyroid patients (9.94 ± 1.43) (t=2.51; p &lt 0.05). Conclusions. Based on the results of the present study we conclude that thyroid autoimmunity is associated with female gender, the presence of anti-thyroid and TSH receptor antibodies, and low levels of TSH. Importantly, higher mean insulin concentrations were observed in hyperthyroid patients. The presence of TRAbs and high insulin concentrations in patients with TSH between 0.01-0.44 µU/mL and positive ATPO, may indicate a higher risk of developing type 2 Diabetes Mellitus in adults. We recommend evaluation of TRAbs and insulin levels in at-risk populations.

A-168

Homeostasis model assessment of insulin resistance in a general adult population in Korea: Additive association of sarcopenia and obesity with insulin resistance

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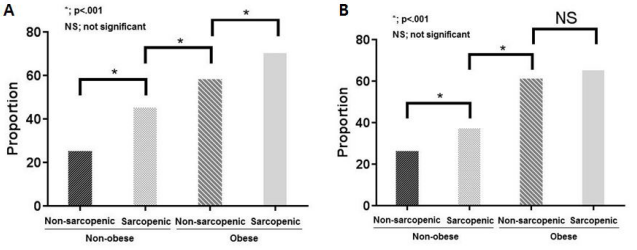
Background: Insulin resistance (IR) is a major factor associated with type 2 diabetes. The homeostasis model assessment of insulin resistance (HOMA-IR) is a useful method to assess IR in large populations. We aimed to elucidate the factors associated with IR risk, especially the cumulative effect of obesity and sarcopenia on IR. In addition, the appropriate cutoff of HOMA-IR for assessing IR was calculated.

Methods: This is a retrospective, cross sectional study. A total of 8,707 adults (4,192 men and 4,515 women) from the 4th and 5th Korean National Health and Examination Surveys were studied. Laboratory, anthropometric, and lifestyle factors were analyzed to reveal their association with HOMA-IR and IR risk. Subjects were divided into four groups according to the presence of obesity and sarcopenia to identify their effect on IR risk. For assessing the optimal cutoff of HOMA-IR for IR, the HOMA-IR of a healthy subgroup was used.

Results: We found that high triglycerides and alanine aminotransferase, low high-density lipoprotein cholesterol, obesity, and sarcopenia were independent risk factors for IR in both sexes. Obese men with sarcopenia had a significantly higher risk of IR than men who were obese or sarcopenic (but not both, figure 1A). The additive effect of sarcopenia with obesity on IR risk was not observed in women (figure 1B). Cutoffs of HOMA-IR for determining IR were calculated as 75 percentile value of young healthy subpopulation, 2.19 in men and 2.18 in women. These cutoffs could distinguish individuals with impaired fasting glucose from normal ones, with a sensitivity of 65.4% (men) and 73.3% (women), and a specificity of 68.8% (men) and 69.4% (women).

Conclusion: These data showed that obese men with sarcopenia exhibited a significantly higher IR risk than non-sarcopenic obese men. In women, body composition did not affect IR if they were already obese.



A-169

Serum adiponectin levels in overweight and obese women; Discrimination between insulin resistance and abdominal obesity

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Introduction

Insulin resistance and abdominal obesity are both associated with lower serum adiponectin concentrations. Since insulin resistance and abdominal obesity are related, the extent to which the association of adiponectin with insulin resistance is dependent on its relationship with abdominal obesity is not clear. The present study investigated the association between insulin resistance and abdominal obesity in its relationship with serum adiponectin.

Methods

Eighty-eight overweight or obese women (BMI>23) in the age group 35-65 years were enrolled. Anthropometric measurements, blood pressure were recorded and a fasting blood sample was obtained for biochemical parameters. Insulin resistance (IR) was quantified by homeostasis model assessment of insulin resistance (HOMA-IR). Abdominal obesity was assessed by waist circumference (WC). Subjects were divided according to WC quartiles: Q1) WC < 89cm (n = 21); Q2) WC 89-96cm (n = 21); Q3) WC 97-102cm (n = 25); and Q4) WC > 102cm (n = 21) and on the basis of insulin resistance. Data were analysed by SPSS 16.0.

Results

The mean serum concentration of adiponectin in women were 5.93±1.9 µg/mL. In linear regression analysis, significant correlates of serum adiponectin were serum insulin (r = -0.439, p=0.000) and insulin resistance (r = -0.415, p<0.001). BMI, waist circumference, systolic and diastolic blood pressure, serum triacylglycerides and low-density lipoprotein (LDL) had negative correlations with adiponectin but statistically not significant (p>0.05). High-density lipoprotein (HDL) correlated positively with adiponectin level (p<0.05). Across quartiles of WC, insulin-resistant (HOMA-IR > 2.5) subjects had significantly lower (p<0.05) adiponectin levels when compared with insulin-sensitive (HOMA-IR < 2.5) subjects irrespective of the level of abdominal adiposity.

Conclusion

High adiponectin levels are associated with insulin sensitivity and a favourable lipid profile. Serum adiponectin levels are more tightly linked with insulin resistance than with abdominal obesity.

	WC<89 cm		WC 89-96 cm		WC 97-102 cm		WC >102 cm	
	IR< 2.5	IR>2.5	IR< 2.5	IR>2.5	IR< 2.5	IR>2.5	IR< 2.5	IR>2.5
Mean Adiponectin ±SD	6.1 ±1.49	5.6 ±1.94	7.27 ±1.36	5.40 ±1.72	6.50 ±2.95	5.18 ±1.69	7.05 ±2.82	5.57 ±1.79
p value	0.046*		0.03*		0.045*		0.03*	

Difference among WC quartiles by one-way ANOVA: IR<2.5 groups, p = 0.65, IR>2.5 groups, p = 0.32

A-170

Validation of a new glycated serum protein assay on Siemens Vista analyzer

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Introduction: Glycated Serum Protein (GSP) or fructosamine, estimates the average blood glucose over a 2-3 week period versus over a 3-4 months period for HbA1c. GSP may be used to monitor diabetics with hemoglobinopathies or have conditions that affect RBC (red blood cell) lifespan. HbA1c is falsely decreased when the RBC lifespan is less than 120 days, while GSP is not affected. Fructosamine assay is widely used as an alternate test for certain diabetes patients with hemoglobinopathies and for pregnant woman. However, most of the fructosamine assays that are currently in the market are nitro blue tetrazolium (NBT) based colorimetric assays and they suffer from a variety of interferences like vit-c, bilirubin, glutathione which lead to inaccurate results. These analytical issues led us to investigate for an alternate assay that could be adapted to our existing Siemens Vista analyzer.

Study Objectives: The objective of this study is to evaluate and validate a user-defined application protocol for glycated serum protein (GSP) assay from Stanbio Laboratory - an EKF Diagnostics company on Siemens Vista chemistry analyzer. In addition to the method validation, we also established the specimen stability and adult reference ranges for GSP. **Materials and Methods:** GSP from Stanbio Laboratory - an EKF Diagnostics Company is a new FDA cleared three step enzymatic colorimetric assay based on trinder endpoint reaction measured at 546-600 nm for quantifying GSP in serum. The assay was evaluated on Vista chemistry analyzer using open channel user defined method. Performance of the assay was evaluated for inter and intra assay precision, accuracy, linearity, reference ranges and specimen stability.

Results and Discussion: With-in-run imprecision was 6.5 % for control 1 (mean=264 µmol/L) and 3.7% for control 2 (mean=715 µmol/L). Between-run precision with 17 days were 4.2% (mean= 267 µmol/L) and 2.5% (mean = 728umol/L). Analytical measurement range was verified using 5 level calibrators and acceptable across the range (40-1185 µmol/L). Accuracy and recovery of the assay was acceptable with a mean recovery of 100±5% across the analytical measurement range (AMR). All values were considered acceptable. Comparisons between laboratory assay and vendor predicted assay on Stanbio Sirrus clinical chemistry analyzer compared well (r-square=0.996, slope=1.0 and intercept=-1.49). Stability studies proved that samples stored at 2-4 °C are stable up to 7 days with no significant variations. Lab also verified the reference interval as 151-300 µmol/L using adult patient population (18-65 yrs).

Conclusion: The user defined application for GSP assay enhances the versatility of the Vista system for specialized glycemic monitoring for a specific diabetic subpopulations where the patient has either a genetic variant of hemoglobin (hemoglobinopathy) or a condition or treatment that affects RBC turnover. Furthermore, this application provides laboratories with a simple, sensitive, fast, and convenient alternative glycemic monitoring test with no endogenous substance interference that are typically observed in NBT based colorimetric fructosamine assays.

A-171

Free thyroid hormone measurements in pregnancy: Comparisons of immunoassays and mass spectrometry

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Background: Second trimester maternal thyroid deficiency has been associated with adverse neurological development in children and a high rate of subsequent permanent hypothyroidism in the mother (1). Accurate assessment of thyroid hormone concentrations during pregnancy is therefore essential. In pregnancy, measurement of free thyroxine (FT4) and free triiodothyronine (FT3) is complicated by increased binding protein levels. Ultrafiltration or equilibrium dialysis followed by tandem mass spectrometry (MS) is a recommended method for improved sensitivity of FT4 concentrations; however, these techniques are expensive and laborious. The present study compares multiple immunoassay methods for FT4 and FT3 with MS to determine suitability of automated assays for large population-based studies in pregnancy. Previously, MS results for FT4 and FT3 have been compared to a limited number of immunoassay methods.

Methods: Residual sera (n=60) for the comparative study were collected, aliquoted, and distributed by the Women and Infants (WIH) laboratory; TSH concentrations were within the reference interval (0.3-5.0 µIU/mL) in 50 samples, elevated in 8 samples, and low in 2. Ultrafiltration followed by liquid chromatography-tandem mass spectrometry was performed as previously described (2). Immunoassay platforms for FT4 and FT3 testing included the Abbott Architect $i2000_{SR}$, Roche cobas e602, Beckman Coulter Dxl, and Siemens Immulite 2000. Formal pairwise method comparisons were performed, after logarithmic transformation. This study was approved by the WIH IRB.

Results: Of the 60 samples, one failed MS quality control for FT4 (hypothyroid) and 18 for FT3 (14 euthyroid and 4 hypothyroid); 41 samples remained. FT4 correlations between the four immunoassays ranged between 0.82 and 0.93; correlations between MS and the four immunoassays, however, were lower (r values: 0.74, 0.74, 0.66, and 0.71 for Architect, cobas, DxI, and Immulite, respectively). Among the three samples with TSH elevations, all four immunoassays ordered the FT4 results the same as MS. FT3 correlations between the four immunoassays ranged between 0.46 and 0.89; correlations between MS and the immunoassays were low (r values: 0.27, 0.40, 0.37, and 0.18, respectively).

Conclusions: FT4 immunoassay measurements appear to be a reasonable surrogate for MS in pregnant euthyroid patients. Agreements between immunoassays for FT4 are high. MS was unable to reliably determine FT3 in 18 pregnancy samples, and agreement between the remaining 41 FT3 MS results with immunoassays was poor. Agreement was also poor between FT3 immunoassays. These results generate concern regarding the reliability and usefulness of FT3 assays in samples from pregnancy. The measurement of total T3 as an alternative to fT3 is currently under investigation.

A-172

Inappropriate Inpatient HbA1c Repeat Testing

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Background: There is presently much interest in reducing waste in health care. In laboratory medicine, unnecessary repeat testing is such a focus and HbA1c measurement with its known biological half-life and monitoring requirements is a good model test. This study examined the pattern of repeat HbA1c testing in inpatients at a 1400 bed general hospital in Singapore (note that HbA1c is not used for diagnosis of diabetes mellitus in Singapore). **Methods:** Anonymised details of all HbA1c testing (Beckman-Coulter DxC-800 immunoturbidometric assay) for 2014 were extracted from the laboratory information system for analysis in Excel. Inappropriate repeat testing was defined as a retest interval < 60 days (Association of Clinical Biochemistry UK Minimum Retesting Interval guidelines). Logistic regression analysis was performed using age, sex, HbA1c, race and hospital discipline to predict repeat testing within different time frames. **Results:** There were 13875 tests (38 per day). 1152 (9%) were repeat samples (1012 duplicates, 127 triplicates, 13 quadruplicates). The cumulative distribution of the repeat tests was: 8.5% within 3 days of the initial test, 11.1% within 7 days, 13.7% within 14 days, 15.6% within 21 days, 18.3% within 30 days, 29% within 60 days and 42.9% within 90 days. The significant predictors

of repeat testing < 60 d were: increasing age, surgical (vs. medical) discipline and higher HbA1c. For repeats < 7 days, surgical discipline and higher HbA1c were the only significant predictors while for < 3 days, higher HbA1c was the sole predictor. **Conclusion:** Inappropriate repeats earlier than 60 days represent 2.4% of all HbA1c measurements on inpatients or 332 tests per year. The strongest predictors of early repeat testing are increased HbA1c and surgical patients. Poor clinician understanding of the timeframe for HbA1c change may contribute to this practice - better education and/or introduction of computerized minimum retest interval guidelines should reduce such over-requesting.

A-175

The development of a method for detecting IGF-I misuse in elite athletes

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Background: Growth hormone (GH) and insulin-like growth factor-I (IGF-I) have anabolic and metabolic effects that make them attractive as performance-enhancing drugs. Both substances are included in the World Anti-Doping Agency (WADA) list of prohibited substances because of their potential to improve performance and the risks of harm to the athlete's health. The GH-2000 and GH-2004 research teams developed a method to detect GH misuse based on changes in GH-sensitive serum peptides - the GH-2000 biomarker method. As GH misuse detection methods have improved, athletes may have turned to IGF-I as an additional or alternative doping agent. Recombinant human IGF-I (rhIGF-I) is structurally identical to endogenous IGF-I and the main challenge in detecting rhIGF-I misuse is to distinguish exogenous from endogenous IGF-I. We previously showed that serum IGF-I increases in recreational athletes after administration of rhIGF-I/rhIGF binding protein-3 (rhIGFBP-3) complex for 28 days.

Objective: To assess whether measuring other GH-sensitive serum markers, in addition to IGF-I, could improve the sensitivity and specificity of a test for detecting IGF-I misuse.

Methods: Serum samples had been stored from a randomised, double-blind, placebo-controlled rhIGF-I/rhIGFBP-3 administration study. 56 recreational athletes (30 men, 26 women, age 18-30 years) were randomly assigned to receive placebo, low dose (30 mg/day) or high dose (60 mg/day) rhIGF-I/rhIGFBP-3 complex. Treatment was self-administered by subcutaneous injection for 28 days. The following serum peptides were measured during the four-week treatment and eight-week washout period, using commercial immunoassays: IGF-II, IGFBP-2, IGFBP-3, acid-labile subunit (ALS), osteocalcin, procollagen type I carboxyterminal propeptide (PICP) and type 1 collagen cross-linked carboxy-terminal telopeptide (ICTP).

On each visit day, marker concentrations were compared between treatment groups using one-way ANOVA. Logistic regression was used to determine which combinations of markers could discriminate between treatment and placebo groups, creating four "IGF score" formulae. Useful markers were then measured in serum samples collected from 250 elite athletes (161 men, 89 women) and IGF scores were calculated. Decision limits for each score were estimated using the mean and standard deviation of scores in elite athletes. Values above the decision limit suggest an athlete has misused rhIGF-I/rhIGFBP-3. An age-correction factor was incorporated into the score formulae because all scores decreased significantly with age.

Results: IGFBP-2 increased and IGF-II decreased in both women and men in response to rhIGF-I/rhIGFBP-3 administration. When these markers were combined with IGF-I results in IGF score formulae, all scores increased rapidly during the first week of drug administration, remained elevated throughout the administration period and then declined. On Day 21 of the administration study, the IGF scores had an estimated sensitivity of 80-94% with specificity of 99.99% (equivalent to the WADA-required false-positive rate of 1 in 10,000).

Conclusions: Serum IGF-I, IGF-II and IGFBP-2 concentrations change in response to rhIGF-I/rhIGFBP-3 administration. These markers have been combined to create IGF score formulae. We have proposed decision limits for the IGF scores, based on marker concentrations in 250 elite athletes, which could be used to detect doping with IGF-I.

A-176

Thyroid autoantibodies in pregnancy: changes across trimesters and association with intrauterine growth restriction in a multi-ethnic population

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Background

Subclinical thyroid diseases are relatively common in women but the significance of detectable thyroid autoantibodies in pregnant women with no clinical thyroid disease is currently unclear. This study aimed to determine the serum levels of thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) across all three trimesters in pregnant women in a multi-ethnic cohort. An association between thyroid autoantibodies in maternal blood and intrauterine growth restriction (IUGR) was also investigated.

Methods

926 women with singleton pregnancies confirmed by ultrasonography at less than 14 weeks of amenorrhoea were recruited to this study conducted at a maternity hospital. Exclusion criteria included chronic medical conditions, aneuploidy, fetal anomalies and pregnancies ending in termination, miscarriage or fetal death. Women who were on any thyroid medication or had a history of thyroid disease were also excluded from subsequent analyses.

IUGR was defined by estimated fetal weight or abdominal circumference less than the 5th percentile (adjusted for gender and ethnicity) in the presence of either oligohydramnios or abnormal umbilical artery flow or both after 22 weeks of gestation.

Participants attended four separate study visits during pregnancy. Venous blood samples were taken at 9-14 weeks (visit 1), 18-22 weeks (visit 2), 28-32 weeks (visit 3), and 34-39 weeks (visit 4). Serum TPO-Ab and Tg-Ab concentrations were measured using Abbott i2000 immunoassays according to the manufacturer's protocols.

Statistical analyses (chi-squared and Kruskal-Wallis tests) were performed using the Analyse-it software.

Results

Median serum TPO-Ab levels demonstrated a mild decreasing trend with increasing gestational age, and were 0.49, 0.49, 0.48 and 0.45 IU/ml at study visits 1, 2, 3 and 4, respectively ($p<0.05$). In comparison, median serum Tg-Ab levels decreased from 1.25 IU/ml at visit 1, to 1.09 IU/ml at visit 2, 1.02 IU/ml at visit 3, and 1.00 IU/ml at visit 4 ($p<0.0001$). The proportion of women tested positive for Tg-Ab (4.11 IU/ml or above) also decreased from 20.2% to 15.3%, 14.4% and 13.6% at study visits 1, 2, 3 and 4, respectively ($p<0.05$).

Women tested positive for both TPO-Ab and Tg-Ab during study visit 2, 3 or 4 were found to be at increased risk of having babies with IUGR; the odds ratios for IUGR in this study group were 2.1 (95% CI, 1.1-4.0) at visit 2, 2.2 (95% CI, 1.1-4.2) at visit 3, and 2.4 (95% CI, 1.2-4.9) at visit 4, compared to controls.

Conclusions

Maternal serum concentrations of TPO-Ab and Tg-Ab decreased with increasing gestational age in this study on women with uncomplicated singleton pregnancies. Positivity for both autoantibodies in the mother during the second or third trimester was associated with an increased risk of IUGR of the fetus.

A-177

Evaluation of TSH, FT4 and FT3 assay using a novel automated analyzer for chemiluminescent enzyme immunoassay (AIA-CL2400).

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Background: The measurement for free thyroxine (FT4), free triiodothyronine (FT3), and thyroid stimulating hormone (TSH) requires high sensitivity, accuracy and rapidness in clinical laboratories. AIA-CL2400 is a newly developed analyzer with chemiluminescent enzyme immunoassay technique, which automatically measures TSH with a two-step sandwich enzyme immunoassay and FT4 and FT3 with one-step delayed competitive enzyme immunoassays. The aim of the present study is to perform an analytical validation of TSH, FT4 and FT3 assays using the AIA-CL2400 analyzer.

Methods: We investigated the within-run and between-day precision, the analytical sensitivity, and the influences of interfering substances for TSH, FT4 and FT3 assays. We also validated linearity and recovery and determined effective sensitivity for the

TSH assay. We performed a correlation analysis with an AIA-2000 analyzer based on fluorescent enzyme immunoassay technique. We measured thyroid hormone concentrations in subjects with various thyroid diseases and investigated the correlation between serum TSH, FT4 and FT3 levels. The study was performed in collaboration with the Tosoh Corporation.

Results: The coefficients of variation (CV) of within-run and between-day precision on the AIA-CL2400 were less than 3.0% and 3.4% (TSH), 3.9% and 5.8% (FT4) and 3.1% and 5.0% (FT3), respectively. The minimal detectable concentration defined as two standard deviations of the blank was 0.0007 µIU/mL (TSH), 0.008 ng/dL (FT4) and 0.10 pg/mL (FT3). Hemoglobin (up to 445 mg/dL), free bilirubin (up to 18.5 mg/dL), and conjugated bilirubin (up to 17.6 mg/dL) had no effects on these assays. Turbidity did not influence TSH and FT4 assays; however it decreased the values of FT3 in high concentration range. The dilution linearity was validated up to a dilution factor of 625 for the TSH assay. The recovery rate was above 90.1% and the effective sensitivity was 0.0027 µIU/mL. Correlation with the AIA-2000 analyzer revealed that the slopes and correlation coefficients were 0.950 and 0.994 (TSH), 1.069 and 0.991 (FT4), and 0.935 and 0.993 (FT3), respectively. The distribution of thyroid hormone concentrations in various thyroid diseases was concordant with the clinical characteristics of each disease and the time course of treatment. The correlation of each hormone supports the existence of the negative feedback between thyroid hormones and TSH.

Conclusion: These results indicated that TSH, FT4 and FT3 assays with the AIA-CL2400 analyzer have excellent sensitivity, precision, and correlation with the AIA-2000 analyzer. Considering that these assays on the AIA-CL2400 analyzer measures more rapidly and require a smaller amount of sample compared to the AIA-2000 analyzer, the TSH, FT4 and FT3 assays on the AIA-CL2400 analyzer will be useful for clinical diagnosis and provide more efficient routine analytical performance in clinical laboratories. Disclaimer: The performance characteristics presented for TSH, FT4 and FT3 are based on an independent, third party study. They do not represent performance claims made by the manufacturer of these assays. The TSH, FT4 and FT3 assays have not yet been reviewed or cleared to be legally marketed in the United States.

A-178

Development and Validation of a Dried Blood Spot Method for Leptin

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Background: Leptin is a hormone produced by adipocytes to provide a satiety (fullness) signal to the hypothalamus. In individuals with obesity, leptin levels may remain elevated in circulation due adipose tissue abundance, and leptin resistance may ensue. Recent interest in the analysis of blood leptin has increased among the medical community related to its association with the development of the metabolic syndrome and pre-diabetes. With increasing awareness of the need to detect metabolic syndrome before progression to adult-onset diabetes, a convenient, precise, and accurate method for leptin testing was sought. Our aim was to develop and validate leptin testing in dried blood spot (DBS) samples. Our laboratory has previously validated blood spot methods for other metabolic markers, namely hemoglobin A1c, high-sensitivity C-reactive protein, and insulin. The addition of leptin to this menu of blood spot analytes is designed to provide additional insight into the contributing factors of compromised metabolic control. Single use and self-retracting bloodletting devices enable the self-collection of capillary blood by lay users. Highly-standardized filter paper collection and transport media are increasingly used for clinical laboratory testing. The employment of sensitive enzyme-linked immunosorbent assays (ELISA) that require sample pre-dilution facilitates the expansion of DBS offerings in clinical laboratories for the reliable analysis of emerging metabolic markers, including leptin.

Methods: Capillary blood samples are collected using SurgiLance™ sterile lancets, and spotted onto PerkinElmer® 226 Spot Saver Cards and permitted to dry. Cards are desiccant packaged and shipped via standard postage to the laboratory. From a 50 µL blood drop, ten 3-millimeter spots are punched using a calibrated hole punch and extracted into 280 µL buffered solution, equivalent to the specified serum dilution of the ELISA kit. Extracts are analyzed via an ELISA microplate assay from Alpco Diagnostics. Results are read from 5-point DBS calibration curves, derived from Alpco-provided calibration standards. Analytical precision, linearity, recovery, trueness, reference interval and stability of DBS leptin were assessed.

Results: The intra-assay and total imprecision coefficients of variation (CV) (n=20) at 8.9 ng/mL were 3.7% and 5.4%, and at 12.4 ng/mL were 3.2% and 6.3%, respectively. Leptin in DBS was confirmed linear between 1.0 - 71.0 ng/mL, with recovery between 94.3% - 117.9% (n=8). Volunteer donors provided samples to permit sample matrix comparison. Least-squares regression analysis comparing leptin values in serum to DBS (n=45, range 1.1 - 50.0 ng/mL) yielded a correlation coefficient of 0.986, $y = 0.932x + 0.365$; standard error of estimate ($S_{y/x}$) = 1.76. Reference intervals of 1.8 - 20.0 ng/mL and 4.7 - 39.0 ng/mL were confirmed in adult males and females, respectively. Leptin in DBS demonstrated stability for 19 days with collection cards desiccated in sealed Ziploc™ bags at 25°C or lower.

Conclusion: This analytical method for determining DBS leptin using the Alpco ELISA kit has been validated to be precise and accurate. The collection system for this method has proven to be well-accepted by lay users, with 99% of submitted samples of adequate quantity and quality. The transportation system provides extended stability, enabling shipping from remote locations to a central laboratory for analysis.

A-179

Comparison of the Associations of Circulating Total Adiponectin and Adiponectin Multimeric Complexes According to Metabolic and Glycemic Status

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Introduction and Objectives: Adiponectin, a protective adipokine that increases insulin sensitivity (IS) and regulates glucose metabolism circulates in plasma as high (HMW), medium (MMW) and low molecular weight forms (LMW). Studies show the HMW form as the best indicator of IS. This study compares Total Adiponectin (TA) and the multimeric complexes according to their associations with metabolic and glycemic status.

Methods: Fasting TA, HMW, MMW, LMW, insulin, glucose, lipid profile and HbA1c were measured in 66 patients with Type 2 diabetes (T2DM) and 59 non-diabetic first degree relatives. Clinical and anthropometric data were recorded. Subjects were classified by adiposity, insulin resistance (IR - homeostasis model assessment) and the number of the criteria of the Metabolic Syndrome (MetS) (International Diabetes Federation).

Results: TA (r = -0.21 & -0.24), HMW (r = -0.37 & -0.38) were significantly ($p<0.05$) inversely correlated with BMI and waist circumference respectively but MMW and LMW were not. TA (r = 0.23 & -0.24), HMW (r = 0.40 & -0.39) were significantly correlated with IS and IR respectively but MMW and LMW were not. Compared to HMW, Receiver Operating Characteristic (ROC) analysis showed that TA had the higher area under the curve for diagnoses of MetS (0.749 vs 0.712) and T2DM (0.644 vs 0.612) whereas HMW had the higher area under the ROC curve for diagnosis of IR (0.629 vs 0.689)

Conclusions:

Circulating TA and multimeric complexes show variable associations with metabolic indices and glycemic status. Our results suggest that MMW and LMW forms of adiponectin are not a major determinants of the metabolic perturbations of the adipokine. HMW is a better predictor of IR but TA is a better predictor of T2DM and MetS. As multimerisation is genetically determined, the predominant form of adiponectin could be the main determinant of the metabolic phenotype and disease associations. Multimeric forms should always be considered in the interpretation of the associations of circulating adiponectin.

A-180

Higher FT4 or TSH Below the Normal Range are Associated with an Increased Risk of Dementia: a Meta-analysis of 10 Studies

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Background: Observational studies of thyroid function and dementia have reported conflicting results. This study is to address the conflicting results of the contribution of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) in participants with dementia.

Methods: We reviewed cohort and case-control studies from MEDLINE, EMBASE and Web of Science that focused on the association between serum TSH, FT4 and dementia. Studies were initially included in the detailed assessment if they met the following criteria: 1) sufficient information on the study population; 2) cohort or case-control studies; 3) clear criteria for outcome (dementia or Alzheimer's disease); 4) sufficient data to calculate risk estimates of the association between FT4 and TSH levels and dementia or AD: relative risk (RR), hazard ratio (HR), or odds ratio (OR) with 95% confidence intervals (CIs) or the number of outcome events; 5) adjustments for potential confounders; and 6) valid measurements of FT4 and TSH. We excluded studies that were cross-sectional, lacked usable data, or focused on vascular dementia

or dementia secondary to other diseases. If multiple reports used the same population, the study with the longest follow-up was included. Reviews, case reports, abstracts and conference proceedings were excluded. Two reviewers independently collected the data and assessed the study quality by the Newcastle Ottawa Scale (NOS). Any disagreement between the two investigators was resolved by consensus or by discussion with a third reviewer.

Results: Two case-control and eight cohort studies published from 2003 to 2015 that evaluated a total of 24721 participants, including 1372 patients with dementia, were finally included. Most studies controlled for some conventional risk factors, including age (n=10), gender (n=10) and thyroid medication (n=7). All the included studies were of high quality by NOS and scored from 7 to 9. The average score was 7.4, and the follow-up duration ranged from 4 to 17 years. The relationships between dementia and the per standard deviation (SD) increment of FT4 (random RR=1.08, 95% CI 1.00-1.17) and TSH (fixed RR=0.91, 95% CI 0.84-0.99) were well established. TSH levels in the low category were associated with an increased risk of dementia (fixed RR=1.55, 95% CI 1.24-1.94). However, the positive association was confined to TSH levels below the normal range (fixed RR=1.68, 95% CI 1.25-2.24), not those in the lower tertile of the normal range (fixed RR=1.39, 95% CI 0.98-1.97). Additionally, dementia was not significantly associated with high TSH levels. In the analysis of three studies focused on men, no positive association between dementia and the low or high categories of TSH were found. Furthermore, all the models showed stability in the sensitivity analysis performed by omitting each study and no significant publication bias were detected by Egger's test.

Conclusion: This meta-analysis suggests that patients with higher FT4 levels and those with TSH levels below the normal range have an increased risk of dementia. However, a relationship between lower tertile of TSH within the normal range and dementia was not well established, and more studies are urgently needed.

A-181

Novel assay for oxytocin using bioluminescence enzyme immunoassay

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Background:

The neurohypophyseal peptide hormone oxytocin acts in the central nervous system and plays an important role in various complex social behaviors, including affiliation, sexual behavior, social recognition, stress buffering, aggression, and trust. There is increased interest in measuring peripheral oxytocin levels to better understand the role of this peptide in human social behavior. The levels of oxytocin in plasma have been measured by radioimmunoassays and enzyme immunoassays (EIA), but the many reported methods lack sufficient sensitivity and specificity for oxytocin. We have already developed an EIA using colorimetric with a high affinity and high specificity antibody towards oxytocin, and published in 2015 AACC Annual Meeting. In this study, we report a highly sensitive EIA by bioluminescence to increase the sensitivity of detection for oxytocin.

Methods:

Antibody: Anti-oxytocin antiserum was obtained by immunizing rabbits with oxytocin-bovine thyroglobulin conjugate prepared by the glutaraldehyde method.

Assay method: Oxytocin sample was added to a second-antibody-immobilized magnetic particles and reacted overnight at 4 °C. Biotinylated oxytocin was then added for 1 hour at 4 °C, followed by the addition of streptavidin-biotinylated-luciferase complex and incubation for 30 min at room temperature. The sample was then washed three times with buffer to separate bound/free and the activity of luciferase bound to antibody was measured by bioluminescent assay with luciferin, ATP, Mg^{2+} and O_2

Examination of cross-reactivity:

The cross-reactivities of three oxytocin-like peptides, [Arg^8]-vasopressin (AVP), [Lys^8]-vasopressin (LVP), and [Arg^8]-vasotocin (VT) with oxytocin were examined by cross-reactivity tests.

Results:

The proposed method is based on the principle of competitive EIA using anti-oxytocin antibody from rabbit and biotinylated oxytocin as the labeled antigen. First, the detection limit of biotinylated luciferase was examined using luciferin-luciferase reaction and measured up to 1×10^{-21} mol/assay. Next, seven labeled antigens were prepared and used to develop a bioluminescence EIA. The labeled antigen comprised biotin chemically bound to oxytocin containing 0 to 6 lysines, providing bridge-link heterology. Rabbits were immunized with oxytocin bound through the N-terminus to the carrier protein bovine thyroglobulin. The produced antibody and the seven biotinylated oxytocins were used in various combinations. The sensitivity of the EIA improved as the number of lysine residues increased; consequently, biotinylated oxytocin bridged with 5 lysines was used thereafter. A standard curve range for oxytocin was 1.0 to 1000 pg /assay. The detection limit of the assay was 1.0 pg and the reproducibility of each point in the standard curve had an average coefficient of variation value (n = 5) of 5.3 %. The specificity of the assay was tested using three compounds with structures similar to that of oxytocin: AVP, LVP, and VT. Cross-reactivity with all three compounds was less than 0.01%, indicating that this antiserum is very highly specific for oxytocin. In addition, there was a good correlation of oxytocin measured values between colorimetric assay and bioluminescent assay (r = 0.9665, n = 48).

Conclusions:

The bioluminescent method is sensitive and more specific than conventional immunoassays for oxytocin and can be applied to the determination of plasma oxytocin levels.

A-182

Age and gender related differences in concentrations of parathyroid hormone-related protein measured by LC-MS/MS

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Background: Measurement of parathyroid hormone related protein (PTHrP) is diagnostically useful in patients suspected of hypercalcemia of malignancy. PTHrP and its gene are also known to be expressed in number of normal cells and tissues. We previously developed a method for the measurement of PTHrP in plasma using LC-MS/MS and established reference intervals in healthy adults. Measurable concentrations of PTHrP were observed in plasma samples of all participants, suggesting that PTHrP is present in circulation in health. The aim of this study was to evaluate associations between PTHrP concentrations and age in men and women and to evaluate between-gender differences. **Methods:** The LC-MS/MS method was fully validated according to CLSI guidelines and is in routine use in a clinical laboratory. The analysis was performed as follows, stable isotope-labeled internal standard was added to samples and PTHrP was enriched using anti-PTHrP antibody conjugated to magnetic beads, digested with trypsin and samples were analyzed by LC-MS/MS. The lower limit of quantification and upper limit of linearity of the assay were 0.3 and 1100 pmol/L, respectively. Total imprecision of the method was < 10%. Specificity of the measurements was confirmed by monitoring two mass transitions of PTHrP and the internal standard. Using this method we analyzed 284 plasma samples collected from adults: 132 men (age 18-81 y, mean 40 y) and 152 women (age 18-84 y, mean 41 y). Differences between groups were evaluated using nonparametric statistics; p-values ≤0.05 were considered as statistically significant. **Results:** Overall, significantly higher PTHrP concentrations were observed in women compared to men ($p<0.0001$). In women, the highest concentrations were observed in the 21-30 y group; concentrations were the lowest in the 41-50 y group and were progressively higher in the groups of older women. Statistically significant differences in concentration were observed between the age groups of women 18-30 y and 31-40 y ($p<0.0078$); 18-40 y and 41-50 y ($p<0.0405$); and 41-50 y and 51-84 y ($p=0.0001$). Statistically significant higher concentrations were observed in women of post-menopausal age (>50 y vs. <50 y, $p=0.0009$). In men, the lowest concentrations were observed between the ages of 21 and 50 y. In men, significantly lower concentrations were observed in the age group 51-60 y, as compared to 61-81 y ($p=0.019$). Statistically significantly higher concentrations were observed in women than in men in the age groups of 21-30 y ($p=0.0017$), and 51-84 y ($p=0.0054$). **Conclusion:** Concentrations of PTHrP were higher in women than in men and were statistically significantly higher in older individuals in both sexes. The highest concentrations of PTHrP were observed in women 21-30 y and >51 y, and in men >71 y. This sex and age distribution overlaps with some age-related diseases, including osteoporosis; these observations raise questions regarding PTHrP's involvement in disorders of calcium regulation associated with age.

A-183

Development of an Anti-Müllerian Hormone Assay on the ADVIA Centaur XP Immunoassay System*

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Background: Anti-Müllerian hormone (AMH), or Müllerian-inhibiting substance, is a transforming growth factor beta protein that controls proliferation and differentiation

in various cell types, including embryonic and adult tissues. AMH levels vary with gender and age but are also influenced by other biological fluctuations. In published clinical studies, in-vitro AMH levels are known to correlate with the antral follicle count for assessment of the ovarian reserve and the onset of menopause. Abnormal AMH values are also known to be associated with polycystic ovarian syndrome and existence of specific tumors. The objective of this study was to evaluate the initial analytical characteristics of an AMH assay from Siemens Healthcare Diagnostics on the ADVIA Centaur® XP Immunoassay System*. **Methods:** A single-pass sandwich immunoassay for the detection of AMH has been developed using direct chemiluminescent technology, which uses two antibodies to AMH. The first antibody in the lite reagent is a mouse monoclonal anti-AMH antibody labeled with acridinium ester. The second antibody in the solid phase is a biotinylated mouse monoclonal anti-AMH antibody, which is coupled to streptavidin coated magnetic particles. A direct relationship exists between the amount of AMH present in the patient sample and the amount of relative light units (RLUs) detected by the system. Method comparison (using 45 female and 5 male samples; age: 1 month to 53 years), precision (three levels with means of 0.55, 5.5, 16.4 ng/mL), and linearity studies were performed using the ADVIA Centaur XP system. Stability of the native purified AMH was also evaluated up to 51 days at 2-8°C. **Results:** Method comparison between the ADVIA Centaur XP AMH assay and the predicate assay showed a Passing-Bablok regression slope of 1.00 (dose range from 0.10 ng/mL to 22.4 ng/mL; n = 44) and slope of 1.02 (dose range from 0.10 ng/mL to 112 ng/mL; n = 47; three samples were excluded due to the dose being below detection limit in both assays). Repeatability coefficients of variance (CV) for three samples at the concentrations stated above were 3.7%, 2.1%, and 2.5%, while within-lab precision CVs were 8.0%, 3.8%, and 4.9%. Assay linearity was demonstrated from 0.10 ng/mL to 67 ng/mL (linear regression slope: 0.95; 95% confidence interval: 0.92-0.99). The stability study for the purified AMH after storage in protein buffer at 2-8°C showed average dose recovery of 99% as compared to the AMH concentrations measured on Day 0 (0.30-5.68 ng/mL). **Conclusion:** The results from the studies have demonstrated reproducible and scalable performance that is also comparable to a widely used method. Stability of the native purified AMH will improve the performance of the assay with enhanced in-use and storage life of the calibrator and quality control materials. *Disclaimer: Under feasibility evaluation. Not available for sale and its future availability cannot be guaranteed.

A-184

Analytical Performance Characteristics of the New Beckman Coulter Access TSH (3rd IS) Assay

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Background:

Beckman Coulter has developed a new highly sensitive 3rd generation TSH assay for use on the Access Immunoassay Systems called the Access TSH (3rd IS) assay. TSH (thyroid-stimulating hormone) is the principal regulator of thyroid function, stimulating the synthesis and release of thyroid hormones thyroxine (T4) and triiodothyronine (T3). The principal clinical use for TSH measurement is for the assessment of thyroid status.

Methods:

The new Beckman Coulter Access TSH (3rd IS) assay is a paramagnetic particle, chemiluminescent sandwich immunoassay for the quantitative determination of thyroid-stimulating hormone in human serum and lithium heparin plasma. Two mouse monoclonal antibodies are utilized in the sandwich assay, one as an anti-hTSH alkaline phosphatase conjugate and the other is immobilized on paramagnetic particles. The Access TSH (3rd IS) is standardized to the third WHO International Standard NIBSC code: 81/565 and achieves 3rd generation TSH sensitivity (0.01 µIU/mL with 10% CV).

Results:

The Access TSH (3rd IS) assay demonstrated acceptable linearity throughout the analytical measuring range of 0.005 to approximately 50.0 µIU/mL and is capable of measuring samples above the range using sample dilution with recovery of ≥ 93%. The LoB for the TSH (3rd IS) assay was measured at 0.0004 µIU/mL, the LoD was measured at 0.0008 µIU/mL, and the LoQ was measured at 0.0013 µIU/mL. Four samples ranging from concentrations of 0.02 - 38.76 µIU/mL were used to test for assay imprecision over 20 days. Within-run imprecision was 2 - 4% and total imprecision was 3 - 6% for the samples. No cross reactivity was observed to hCG at 1,000,000 mIU/mL (< 0.010%), to hFSH at 1,000 mIU/mL (< 0.10%), or to hLH at 3,000 mIU/mL (< 0.10%). No interference was detected from endogenous interferences bilirubin (450 µg/mL), hemoglobin (10 mg/mL), or triglycerides/Intra Lipid (33 mg/mL). No hook effect was observed for samples up to 1,000 µIU/mL TSH concentration.

Conclusions:

The new Beckman Coulter Access TSH (3rd IS) assay is a 3rd generation TSH assay capable of TSH measurement in hyperthyroid patient samples down to 0.001 µIU/mL with the accuracy and precision expected from a 3rd generation TSH assay.

A-185

A longitudinal Evaluation of Thyroid hormones by MassSpectrometry During Normal Pregnancy

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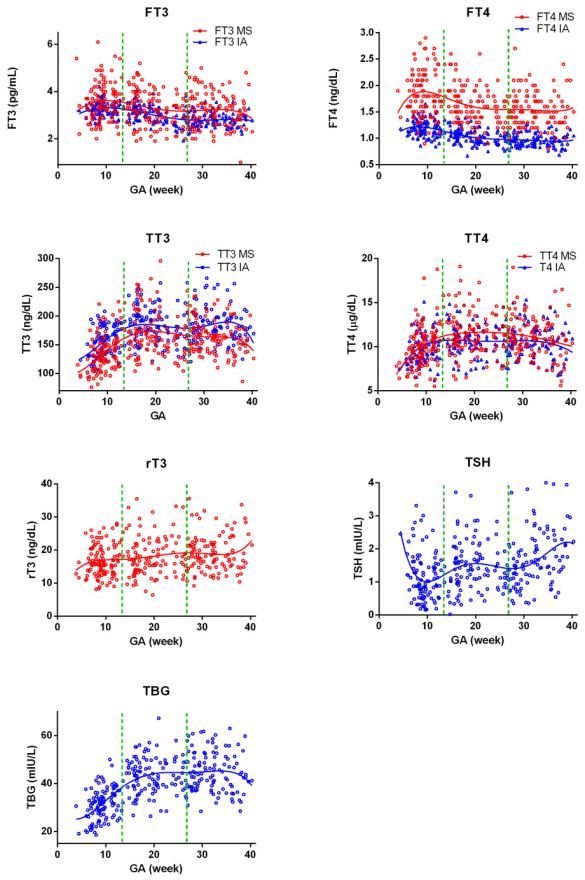
Background: Maternal thyroid dysfunction can be associated with adverse outcomes for both mother and child. The validity of immunoassays (IAs) for thyroid hormones has long been questioned due to lack of specificity, sensitivity to alterations in protein concentrations, poor correlation with gold standard equilibrium dialysis methods and poor inverse linear log relationship to TSH. Thyroid hormone measurements by ultrafiltration LC-MS/MS have been shown to be more accurate than IAs. We performed a comparison of thyroid hormone measurements by LC-MS/MS and common IA methods on longitudinal samples from a normal pregnant population. Methods: 107 subjects over 18 years-old with singleton/viable pregnancies were recruited through the Washington University Women and Infant's Health Specimen Consortium (WIHSC). Three sequential serum samples, one in each trimester were obtained from each subject. Exclusion criteria include: history of thyroid illness, medication for thyroid disease, and positive anti-TPO antibody. Total T3 (TT3), total T4 (TT4), and reverse T3 (rT3) were measured by LC-MS/MS on an Agilent 6460-Triple-Quadrupole-LC/MS system; free T3 (FT3) and free T4 (FT4) were measured by ultrafiltration-LC-MS/MS on a SCIEX Triple-Quad-6500 System; IA TT3, TT4, FT3, FT4, TSH and TBG were measured on Roche Cobas 6000 analyzer; and Anti-TPO antibody was measured on Siemens Immulite XPI 2000. Results: Figure 1 demonstrates that TT3, TT4, TSH and TBG were significantly lower and FT3 and FT4 were significantly higher in the first-trimester (<13 weeks) than those in the second (13-26 6/7 weeks)- and third (≥ 27 weeks)-trimesters. rT3 was significantly higher in the third-trimester than the first- and second-trimesters. The coefficient of correlation between MS and IA was poor with R2 values of 0.1754, 0.3585, 0.6065 and 0.5967 for FT3, FT4, TT3 and TT4, respectively. Conclusion: Gestational-age-specific measurements of thyroid hormones by the methods separating binding proteins are critical to evaluate thyroid function during pregnancy.



A-186

Assessment of Gender-Related Differences in Vitamin D levels, Cardiovascular Risk factors in Saudi Patients with Diabetes Mellitus

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Diabetes is a major risk factor for cardiovascular disease (CVD) including coronary heart disease, stroke and peripheral artery disease. CVD remains a leading cause of mortality throughout the world, affecting both women and men. This study aimed to assess gender based differences in cardiovascular risk factors among adults with diabetes mellitus (T2DM). This hospital-based cross-sectional study involving subjects was divided into two gender based groups; male diabetic (n=800) and non-diabetic (n=800); female diabetic (n=800) and non-diabetic (n=800) for each comparison. Blood samples were analyzed for fasting glucose (fg), HbA1c, total cho-lesterol (Tc), triglycerides (Tg), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and serum levels of 25(OH)-vitamin D in all groups. All the glycemic control parameters, lipid profile parameters were found significantly different in diabetic vs non-diabetic group ($p<0.0001$) in both genders. Triglyceride (Tg) level was borderline high in T2DM patients in both genders. However, HDL-C levels were significantly lower in diabetic group as compared to non diabetic group in both genders. The results also show that vitamin D concentration was lower in diabetic patients than the healthy individuals. Although the mean concentration of vitamin D in males in both groups was equal but in the women with diabetes was lower than the healthy women. Routine screening of these parameters in T2DM patients may assist early detection of these parameters and prevent risk of CVD. Women with diabetes mellitus are at higher risk of cardiovascular disease compared to men and this may be a result of greater clustering of risk factors among women. This indicates overall poor risk factor control but worse among women, emphasizing the need for better implementation of guideline recommendations for management of diabetes to reduce future cardiovascular diseases.

A-187

Testing for TSH receptor stimulating immunoglobulins: performances of a novel fully automated assay with improved specificity.

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Background: Measurement of TSH receptor autoantibodies (TRAb) is important for the diagnosis and monitoring of Graves' disease (GD). Several automated methods for testing are available but are not yet standardized and not specific of TSH receptor stimulating immunoglobulin (TSI). Our objective was to determine the performances of a novel automated assay with improved specificity for TSI. **Methods:** We evaluated the IMMULITE® TSI assay (Siemens), a fully automated immunoassay based on the chimeric receptor that specifically binds TSI but not blocking autoantibodies. Assay's imprecision was assessed with five pools of serum samples and with two levels of control materials. The linearity of the assay was tested a eight points dilution test of high titer samples. Reference values were determined with samples from 90 male healthy volunteers free of thyroid diseases and medications. Method comparison was performed with a second-generation TRAb enzyme immunoassay Medizym® as well as with the Cobas® 8000 and Kryptor® TRAb automated immunoassays. **Results:** Between run coefficients of variation were 6.5 and 4.7% for concentrations of 1.0 and 22.6 IU/L, respectively. The limit of quantification of the IMMULITE assay, determined with the precision profile built with the 5 pools of serum, was below 0.1 IU/L. The dilution test covered a range of concentrations ranging from 40 to 0.32 IU/L and the mean recovery was 108 %, confirming assay's linearity. The concordance correlation coefficients between the TSI and TRAb assays were 0.82, 0.68 and 0.82 with Medizym®, Cobas® and Kryptor® methods, respectively. The TSI levels measured with the IMMULITE assay in healthy volunteers were below 0.10 IU/L. The receiving operator curve analysis of patients with active Graves disease patients with other thyroid disorders and healthy controls revealed an AUC of 0.99 resulting in a sensitivity of 100% and a specificity of 99% at a TRAb level of 0.40 IU/L. **Conclusions:** Our data showed excellent analytical and clinical performances for this novel fully automated assay with an improved specificity for stimulating antibodies.

A-188

Evaluation of HbA1c turbidimetric immunoassay on the new high-throughput analyzer cobas c513 against ion exchange HPLC method and assessment of HbF interference on HbA1c measurement

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Background: diabetes mellitus is a serious lifelong condition characterized by hyperglycemia as a consequence of defective insulin activity. Its prevalence is rapidly increasing worldwide, which turns this disease into a major public health priority. Diabetic patients, and especially those with a poor control of blood glucose levels, are at high risk for subsequent microvascular and macrovascular complications. Glycated hemoglobin (HbA1c) is a useful diagnostic test for those at risk of suffering diabetes, and the preferred laboratory test to monitor glycemic control in patients treated for diabetes and other glucose-metabolism disorders. There are different methods available for HbA1c measurement, differing in accuracy, specificity and/or processing speed, as well as in the presence/absence of interferences by hemoglobin structural variants, chemically modified hemoglobins or high fetal hemoglobin (HbF) concentrations. More specifically, it has been previously described that anomalous HbF levels (>2%) cause negative interference in HbA1c measurement by turbidimetric immunoassay, but not by ion exchange HPLC, thus obtaining falsely decreased HbA1c results by the first method.

Objective: the first objective of this study was to compare Tina-quant HbA1c Generation 3 turbidimetric inhibition immunoassay (TINIA) on the new high-throughput analyzer cobas c513, with an ion exchange HPLC method, widely stablished in clinical laboratories for measuring HbA1c. The second objective was to determine the interference in HbA1c measurement caused by anomalous HbF concentrations, when using turbidimetric immunoassay on the new cobas c513 analyzer.

Methods: in order to compare both methods, HbA1c concentrations were analyzed in parallel in 143 whole blood samples on a cobas c513 analyzer and on a Menarini HA-8180V analyzer. Statistical analysis was performed using Pearson's coefficient and Passing-Bablok regression (MedCalc 12.5 software).

To determine the effect of HbF on HbA1c measurement, HbA1c concentrations were determined in parallel in 50 whole blood samples containing anomalous HbF levels (range 4.2 to 13.5%), again using the turbidimetric immunoassay and the ion exchange HPLC. HbF levels were measured by ion exchange HPLC on a Bio-Rad Variant II analyzer.

Results: a strong positive correlation was observed between Tina-quant HbA1c Generation 3 TINIA and the ion exchange HPLC (Spearman r: 0,9964; P<0,0001; 95% CI r: 0,9949-0,9974). Besides, method comparison demonstrated that both methods are equivalent (Passing-Bablok regression equation: y=0,0116667+1,005556x; 95% CI intercept: -0,08000-0,09308; 95% CI slope: 0,9923-1,0200).

Measurement of HbA1c on samples with anomalous HbF concentrations yielded lower values by the turbidimetric immunoassay on the cobas c513 than by the ion exchange HPLC method. This difference was directly proportional to the HbF concentration in the samples. NGSP criterion stablishes that a difference in HbA1c greater than 7% implies clinical significance; following this criterion it was observed that HbF concentrations greater than 10% produce a significant interference on HbA1c results on the new cobas c513 analyzer.

Conclusion: Tina-quant HbA1c Generation 3 TINIA implemented on the new analyzer cobas c513 shows excellent analytical performance, representing an optimal choice for high-throughput laboratories. HbF concentrations greater than 10% produce a clinical significant negative interference on HbA1c measurements by this method. Further research, including more samples with high HbF levels, should be conducted to confirm these results.

A-189

Utilization review of vitamin 1,25 $(OH)_2$D testing in a large teaching hospital

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Background: Consensus opinion supported by recent guidelines [1] recommend against measuring vitamin 1,25$(OH)_2$D unless the patient has an acquired vitamin 25(OH)D metabolic disorder such as chronic kidney disease, sarcoidosis, and lymphomas or an inherited disorder including congenital rickets and hereditary phosphate-losing conditions. For most other patients, vitamin 25(OH)D should be measured to screen for vitamin D deficiency as vitamin 1,25$(OH)_2$D has a short half-life and does not accurately represent vitamin D status. Despite available knowledge, requests for vitamin 1,25$(OH)_2$D as a screening test for vitamin D deficiency continue to be received into the laboratory. The purpose of this study is to determine the extent of inappropriate vitamin 1,25$(OH)_2$D orders, the level of training among ordering caregivers, and if significant, identify a potential solution to reduce unwarranted testing.

Methods: The electronic medical records of all patients with vitamin 1,25$(OH)_2$D orders over a 3 months period (7/1/2015 - 9/30/2015) at Parkland Hospital were reviewed to assess whether they were diagnosed with or suspected of having chronic kidney disease, primary hyperparathyroidism, hypoparathyroidism, lymphoma associated with hypercalcemia, a chronic granuloma-forming disorder, or an inherited vitamin D metabolic disorder. The ordering caregiver as well as their training level was also assessed.

Results: There were 341 vitamin 1,25$(OH)_2$D orders over the study period. Two orders were cancelled due to insufficient sample volume. Of the remaining 339 results, there were 330 unique patients with a total of 9 results being duplicate orders. One hundred forty-eight of the 330 patients (45%) did not have an appropriate indication for vitamin 1,25$(OH)_2$D testing. Those 330 patients had testing ordered by 164 different caregivers. Thirty three of 62 attending physicians (53%), 17 of 53 residents and fellows (32%), 12 of 30 interns (40%), and 9 of 19 (47%) physician assistants or nurse practitioners ordered vitamin 1,25$(OH)_2$D inappropriately. There was no significant difference in the ordering practices of caregivers at different training levels (p-value 0.14).

Conclusions: At a reimbursable healthcare cost of $52 per test, there was an approximate wastage of $31,000 in excessive annualized vitamin 1,25$(OH)_2$D tests. Vitamin 1,25$(OH)_2$D tests were inappropriately ordered regardless of caregiver training level. More efforts are needed to educate ordering providers of all training levels on appropriate vitamin D testing as well as implementing further restrictions in the electronic medical record to prevent unnecessary orders. These restrictions may include best practice advisories or hard stops. By preventing unwarranted testing, health care costs will be reduced without a negative impact on patient care.

1. Holick M, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*. 2011; 96: 1911-30.

A-190

Comparison of thyroid function test between two automated immunoassay analyzers

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Background: Thyroid function test (TFT) provides a starting point for differential diagnosis of disease associated with thyroid. While the accuracy and precision of the test have to be guaranteed, difference between assay methods or platforms is inevitable. The aim of this study is to evaluate and compare two automated immunoassay analyzers of their thyroid stimulating hormone (TSH), free thyroxine (FT4), and triiodothyronine (T3) results along with the distribution of functional thyroid status corresponding to TFT result.**Methods:** During the period from October to November 2015, 121 residual samples with results of TSH, FT4 and T3 using ADVIA Centaur XP analyzer (Siemens Healthcare Diagnostics, Munich, Germany) were retrospectively selected. The specimens were stored at -70℃ and analyzed using Architect i2000 analyzer (Abbott Laboratories, Singapore) at two months interval. Statistical analyses were done using correlation analysis and Bland-Altman plot. The distribution of TFT patterns based on FT4 and TSH was compared using kappa statistics. The reference range of ADVIA Centaur XP and Architect was adopted from the manufacturer (Centaur: 0.550-4.780 mIU/L (TSH), 11.46-22.65 pmol/L (FT4), and 0.01-0.03 nmol/L (T3), Architect: 0.350-4.940 mIU/L (TSH), 9.01-19.05 pmol/L (FT4), and 0.01-0.02 nmol/L (T3)).

Results: The TSH, FT4, and T3 values of Centaur statistically correlated with Architect (rho Spearman's: 0.997, 0.907, and 0.880; P<0.001). The percent biases of TSH, FT4, and T3 on the Bland-Altman plots were 10.3% (95% CI; -83.5-104.0), 5.1% (-26.7-37.0), and -12.9% (-41.4-15.69), respectively. Two analyzers represented moderate agreement (Cohen's weighted kappa coefficient= 0.415). An apparent difference of distribution of functional thyroid status based on FT4 and TSH was observed. The number (%) of cases with low FT4 and normal TSH in Centaur and Architect were 39 (32.3%) and 3 (2.5%), respectively. The cases with low FT4 and low TSH were 12 (9.9%) and 2 (1.7%), respectively. Underlying causes of patterns with low FT4 and normal or low TSH included rare condition such as central hypothyroidism, although other laboratory data and clinical information were not part of the investigation in this study. On the other hand, the number of cases with normal FT4 and TSH in Centaur and Architect were 4 (3.3%) and 57 (47.1%), respectively. Most of those discrepancies were observed at lower limit of reference ranges of FT4 or TSH.

Conclusion: Centaur assay demonstrated moderate agreement with Architect regarding TFT result. When stratified according to defined reference range by manufacturers, there were considerable discrepancies in interpretation of TFT results between two instruments. The standardization of thyroid hormone measurements is not yet. Therefore, the specialists of laboratory medicine have to know about the differences between methods or instruments and communicate with clinicians in interpreting the TFT results.

A-191

Development of an Enhanced Chemiluminescent C-peptide Assay* on VITROS® 5600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems

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Background: C-peptide is a useful biomarker to assess beta-cell function in the pancreas. Additionally, c-peptide measurements are used as an aid in the diagnosis of hypoglycemia, diabetes mellitus and insulinoma. We have developed a prototype enhanced chemiluminescent assay for the quantitative measurement of c-peptide in serum and plasma for use on the VITROS® 5600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems. **Methods:** Precision was evaluated per CLSI EP05-A3 by testing a 5 member panel in duplicate 2 times per day for 20 days. Cross reactivity with proinsulin was assessed up to 1000ng/ml; and cross reactivity with insulin was assessed up to 26,396µIU/mL. A total of 110 samples that spanned the assay range were tested in the prototype assay and an aliquot was sent out for

testing on a commercially available automated c-peptide test. The sample set included random samples, fasting samples, and post meal samples collected from in house volunteer participants as well as archived samples purchased from a vendor. LoB, LoD, LoQ were evaluated per CLSI EP17-A2 by testing 100 replicates of 1 LoB fluid and 5 LoD fluids over 5 days. High dose hook was assessed up to 200ng/mL. Testing was conducted across two reagent lots and three VITROS® systems. **Results:** The within lab %CVs ranged from 2.8% to 3.8% on the VITROS® 3600 and 2.1% to 3.6% on the VITROS® ECi for samples ranging in concentration from 0.28 to 12.4ng/mL. At 1000ng/mL, the observed % cross reactivity for proinsulin was 0.5%. At 26,396µIU/mL of insulin, no cross reactivity was detected. For the method comparison, Deming regression analysis yielded slopes ranging from 0.98 to 1.04, intercepts ranging from -0.02 to 0.07, and Pearson Correlation Coefficients ranging from 0.98 to 0.99 among the VITROS® 5600, VITROS® 3600 and VITROS® ECi systems. The overall mean % bias for the prototype method ranged from 0.56% to 4.25% among the VITROS® 5600, VITROS® 3600 and VITROS® ECi compared to the commercially available automated comparator method. The LoB was 0.009ng/mL, The LoD was 0.027ng/mL, and the LoQ at 20% CV was 0.045ng/mL. No high dose hook was observed for the assay up to 200ng/mL. **Conclusion:** Preliminary performance data demonstrate that the prototype assay has excellent precision, minimal to no cross reactivity with proinsulin and insulin, excellent correlation with a commercially available method, an LoQ consistent with other commercially available methods, and shows no high dose hook up to 200ng/mL.*Under development

A-192

IMMULITE 2000 IGF-I Assay Restandardization to 1st WHO IS for Insulin-like Growth Factor-I, NIBSC Code 02/254*

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Background: The IMMULITE® 2000 IGF-I assay from Siemens Healthcare is a solid-phase, enzyme-labeled, chemiluminescent immunometric method for in vitro diagnostic use with the IMMULITE 2000 Systems Analyzers - for the quantitative measurement of insulin-like growth factor I (IGF-I) in serum or heparinized plasma, as an aid in the evaluation of growth disorders. The IMMULITE 2000 IGF-I assay has been restandardized to the 1st WHO International Standard for IGF-I, NIBSC code 02/254, with full performance verification studies carried out to demonstrate its appropriate analytical performance. **Methods:** Alignment of the restandardized IMMULITE 2000 IGF-I assay to the WHO standard was demonstrated by accurate recovery of various gravimetrically prepared WHO 1st IS 02/254 spiking solutions in native patient samples. A method comparison of 164 native patient sera with IGF-I concentrations ranging from 23.0 to 900.0 ng/mL was conducted between the restandardized IMMULITE 2000 IGF-I assay and the IDS-iSYS IGF-I assay (which claims alignment to WHO 1st IS 02/254). Reference intervals are presented for N = 1321 individuals aged 29 days to 90 years. The data is stratified by age and gender per the recommendations for normative data made in the *Consensus Statement on the Standardization and Evaluation of Growth Hormone and Insulin-like Growth Factor Assays* (Clemmons, 2011). Gender-specific reference intervals are also presented for Tanner Stages 1 to 5. Repeatability and within lab precision was determined per CLSI EP-5A3. Six patient serum pools were assayed in duplicate in two runs per day over 20 days. Additional performance studies included diagnostic sensitivity (CLSI EP17-A2), linearity (CLSI EP6-A) and cross-reactivity (CLSI EP7-A2). **Results:** Mean recovery of the WHO 1st IS 02/254 spiking solutions was 3%, with individual sample recoveries ranging from -1 to 9%.The Passing-Bablok linear regression slope is 0.84 (95% confidence interval 0.83 to 0.86) and intercept 1.9 ng/mL (95% confidence interval -2.3 to 5.1 ng/mL). The restandardized IMMULITE 2000 IGF-I assay shows acceptable repeatability and within-lab precision. Samples at 60 ng/mL IGF-1 were seen to have repeatability of 7% CV or better and within-lab precision less than 8% CV. Samples with dose recoveries of 100 ng/mL or greater show repeatability %CV less than 5% and within-lab precision less than 6%. The restandardized IMMULITE 2000 assay demonstrated an LOD of ≤15 ng/mL and an LOQ of ≤25 ng/mL. Linearity studies demonstrate that the assay is linear across the measuring interval from the limit of detection to 1000 ng/mL. The restandardized assay shows no significant interference from hemolysis, lipemia, or icterus and less than 1% cross-reactivity to IGF-II, insulin, proinsulin, TSH, and LH. Lot-to-lot variability estimated by method comparison studies showed less than 3% variation between three unique verification reagent lots. **Conclusion:** The restandardized IMMULITE 2000 IGF-I assay is closely aligned to the WHO 1st IS 02/254 and compares well with the IDS-iSYS IGF-I assay. The restandardized IMMULITE 2000 IGF-I assay also demonstrates appropriate precision and sensitivity, is linear and shows no significant interference in the presence of hemolysis, lipemia, or icterus. *Disclaimer: Product availability may vary from country to country and is subject to varying regulatory requirements.

A-193

Performance Evaluation of an IGFBP-3 Assay on the ADVIA Centaur Systems

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Objective: Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) has been shown to be the major carrier of circulating IGF-I and IGF-II, and used as an adjunct to HGH and IGF-I testing in the diagnosis and follow-up of growth disorders. Siemens Healthcare Laboratory Diagnostics is developing a fully automated ADVIA Centaur® IGFBP-3 assay* in a chemiluminescent immunoassay format for the quantitative detection of IGFBP-3 in serum or plasma using monoclonal antibodies specific for human IGFBP-3 and ISODIZAE-NHS, a charge-neutral double-zwitterionic acridinium ester label (Natrajan A & Sharpe D (2013) Org. Biomol. Chem., 11, 1026-1039).

Methods: The diagnostic sensitivity of the assay was evaluated and the results were reported in ng/mL (CLSI EP17-A2). Method comparison against the IDS-iSYS and IMMULITE 2000/XPi IGFBP-3 assays, and between ADVIA Centaur XP and CP Systems, were evaluated using 50 apparently healthy patient samples (CLSI EP9-A3). Additional performance studies included linearity (CLSI EP17-A2), cross-reactivity to IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-5 and IGFBP-6 (CLSI EP7-A2), and precision using a 20-day protocol on two systems, two runs/day (CLSI EP5-A3). Finally, the stability of the reagents was assessed by measuring onboard stability and the calibration interval of the assay (CLSI EP25-A).

Results: The ADVIA Centaur IGFBP-3 assay in development had a LoB/LoD/LoQ of 25/40/80 ng/mL, respectively, on ADVIA Centaur XP with a working range up to 16,000 ng/mL. ADVIA Centaur XP and Centaur CP agreed with a slope of 1.000 and R^2 = 0.990, whereas comparisons between ADVIA Centaur XP IGFBP-3 and IDS-iSYS and IMMULITE 2000/XPi IGFBP-3 had slopes and R squared values of 1.026 and 0.966, and 1.003 and 0.966, respectively. Between run and between day precision of the ADVIA Centaur IGFBP-3 assay showed <5% CV on both ADVIA Centaur XP and ADVIA Centaur CP Systems for patient samples covering IGFBP-3 concentrations of 800-7,000 ng/mL. The assay demonstrated no cross-reactivity to IGF-I, IGF-II or any of the IGF Binding Proteins, and exhibited excellent linearity from LOQ to 16,000 ng/mL having a weighted linear fit slope of 1.05 and intercept of -1.60. The assay had 35 days onboard stability (OBS) and a 35 day calibration interval.

Conclusion: The results of these studies show good performance of the fully automated ADVIA Centaur IGFBP-3 assay and good agreement with the IDS-iSYS and IMMULITE 2000/XPi assays.

* For investigational use only. The performance characteristics of this product have not been established. Not available for sale.

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A-194

OPTIMIZING CORTISOL AFTER INSULIN STIMULUS IN CHILDREN: WHEN AND HOW MANY TIMES SHOULD WE COLLECT BLOOD SAMPLES TO ACCURATELY EXCLUDE HYPOCORTISOLISM

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Background: The insulin tolerance test (ITT) was developed in the late 1960s, and remains as the 'gold standard' test to determine the need for cortisol replacement in patients with hypothalamic-pituitary-adrenal disease. It assesses the integrated central and peripheral responses to a stressful event caused by hypoglycemia. However, this test is unpleasant for patients, is resource-consuming, and is not without risk. It has to be performed in a specialized unit with adequate supervision, and patients with documented ischemic heart disease or seizure disorders should not undergo ITT and require alternative diagnostic tests. Despite wide experience, uncertainty remains regarding the optimal duration of the test.Objectives:In order to optimize the specimen collection for the ITT we studied timing of peak cortisol value. The aim of our study was to examine whether the cortisol stimulation test could be performed with fewer samples without compromising its diagnostic value. **Methods:** We performed a cross-sectional retrospective examination of 297 consecutive children submitted to cortisol stimulation test with insulin who showed a positive response (cortisol ≥ 18 mcg/dL) to the stimulus. Regular insulin was applied intravenously at a dose of 0.075 units/kg body weight. If hypoglycemia was not reached 40 minutes after insulin administration, an additional dose, sufficient to achieve blood

glucose levels below 40 mg/dL, was applied. Blood samples for cortisol and glucose determination were taken at time 0, during hypoglycemia and 30, 60 and 90 minutes after hypoglycemia. Serum cortisol concentration was tested with the Cobas analyzer electrochemiluminescence immunoassay and glucose was tested by an enzymatic method. A test was considered responsive when peak cortisol at any time ≥ 18 mcg/dL. **Results:** Adequate hypoglycaemia (glucose < 40 mg/dL) was achieved in all patients. A second insulin dose to achieve hypoglycemia was necessary in 3% of our series. No significant side effects were recorded. The mean age of our patients was 9.9 ± 3, range 5-16 years, with male:female ratio 2:1. Median cortisol values at time 0, during hypoglycemia and 30, 60 and 90 minutes after hypoglycemia were respectively 11.2, 13.9, 21, and 14.2 mcg/dL.. The majority, 178 (60 %) of our patients showed peak cortisol 30 minutes after hypoglycemia, which usually corresponds to 60 minutes after insulin administration. Seventy six (25.6 %) showed peak cortisol 60 minutes after hypoglycemia (which usually corresponds to 90 minutes after insulin administration), however 74% of this patients showed cortisol ≥ 18 mcg/dL at any other collection time before. Twenty (6.7%) of our 297 patients showed cortisol ≥ 18 mcg/dL at this time (60 minutes after hypoglycemia) with no cortisol positive answer at any other time. However half of these patients showed a cortisol level ≥ 17 mcg/dL at any other time before the collection 60 minutes after hypoglycemia. **Conclusion:** As cortisol release happens after hypoglycemia, it seems rational to collect samples in accordance with the documentation of the event and not in pre-established times. Collection of cortisol after ITT can be optimized, without sacrifying sensitivity, by collecting only three specimens for cortisol and glucose determination: time 0, during hypoglycemia and 30 minutes after hypoglycemia.

A-195

A Laboratory Comparison Study of the Roche cobas and Siemens IMMULITE 2000 Adrenocorticotropic Hormone (ACTH) Assays

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Background: The measurement of plasma adrenocorticotropic hormone (ACTH) concentration is integral to the diagnosis of ACTH-dependent and ACTH-independent Cushing's Disease and primary, secondary and tertiary adrenal insufficiency. The objective of our study was to validate the Roche cobas ACTH assay including a split-sample comparison to the Siemens IMMULITE 2000 assay.

Methods: Precision, linearity and carryover studies on the Roche cobas ACTH assay were performed using a cobas e601 analyzer. A method comparison to the Siemens IMMULITE 2000 ACTH assay included 79 patient samples spanning the linear range of the Roche assay. Data was analyzed using EP Evaluator.

Results: The Roche cobas ACTH assay demonstrated acceptable within-day imprecision using Roche PreciControl QC material with coefficients of variation (CV) of 0.8% and 1.0% at mean ACTH concentrations of 49.9 pg/mL and 755.1 pg/mL, respectively. The day-to- day imprecision study demonstrated CVs of 2.1% and 1.5% at ACTH concentrations of 37.3 pg/mL and 895.7 pg/mL, respectively. The Roche ACTH assay was verified as linear from 1 to 1500 pg/mL and did not display significant carryover. In an attempt to independently verify the Roche cobas ACTH assay performance and to supplement the two concentrations of Roche PreciControl QC material we assayed the Siemens ACTH QC material on the Roche assay. The Siemens QC material demonstrated day-to-day imprecision of 1.2% and 1.4% with observed means of 10.4 pg/mL and 130.0 pg/mL, respectively. Interestingly, the observed concentrations of the Siemens QC material on the Roche assay was between two and four-fold lower than the Siemens expected range, implying non-commutability of the Siemens QC material on the Roche platform. We also tested the opposite combination; analysis of the Roche QC material with the Siemens assay demonstrated within-day imprecision of 2.6% and 1.7% with ACTH concentrations of 56.4 pg/mL and 970.6 pg/mL, values similar to those obtained on the Roche platform. A patient sample comparison study between the Roche cobas and Siemens IMMULITE platforms using Deming regression analysis yielded the following equation (95% confidence intervals): Roche cobas ACTH = 0.708(0.662-0.754)x Siemens IMMULITE ACTH + 0.689(0.644-0.735) , r=0.9606.

Conclusion: The Roche cobas ACTH assay demonstrated acceptable precision over a twenty-day period; however, the Roche cobas assay demonstrated a negative proportional bias as compared to the Siemens IMMULITE 2000 ACTH assay. We hypothesize that the inter-assay bias is related to differences in assay design. The Roche assay uses two monoclonal antibodies targeted to epitopes between ACTH amino acids (AA) 9-12 and 36-39, whereas the Siemens assay uses one monoclonal antibody targeted to ACTH AA 18-39 and one polyclonal antibody targeted to ACTH AA 1-24. Due to differences in assay design the Siemens assay may recognize the ACTH degradation product corticotrophin-like intermediary lobe peptide (CLIP) (ACTH AA 18-39) possibly contributing to positive bias relative to the Roche assay. Similarly the Roche assay likely demonstrates negative bias relative to the Siemens assay due to competition between full-length ACTH and CLIP for one of the two antibodies on the Roche assay. We are currently further investigating the cause of this inter-assay bias.

A-198

Linear Regression of NHANES Fasting Glucose to Hemoglobin A!c Equivalent to Nathan's Average Glucose Derivation from Hemoglobin A1c: Fasting Glucose is the Poor Man's A1c

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Introduction: Nathan's linear regression equation for relating average glucose to hemoglobin A1c [HbA1c] (data obtained from 3 months of 507 subjects' continuous glucose monitoring data) is now used by many clinical laboratories to transform patient HbA1c into estimated glucose (eAG). In our investigations of hematocrit's influence on Nathan's equation, we discovered almost the same equation relating fasting glucose and HbA1c.

Methods: All of the HbA1c, related fasting glucose, age, gender, ethnicity, waist circumference, hemoglobin and hematocrit data were extracted from the US National Health and Examination Survey (NHANES) for the years 1999 to 2012. All subjects with incomplete data were excluded. Linear regression was used to determine the relationship between fasting glucose and HbA1c for the entire population, and then separately for all men, all women, for Mexican Americans (MA), NonHispanic Blacks (NHB), NonHispanic Whites (NHW) and MA, NHB and NHW with hematocrits between 30 and 50%.

Results: A total of 18694 subjects were identified who had the requisite examinations. The Table compares our regression statistics to Nathan's.

Glucose vs HbA1c	N	Slope	Y Intercept	RxR
Nathan et al	507	28.7	-46.7	0.84
All subjects	18694	28.1	-52.6	0.68
Male	9239	27.8	-52.5	0.68
Female	9455	28.0	-51.0	0.68
MA (Code 1)	4816	28.7	-54.8	0.78
NHB (Code 4)	4792	27.0	-50.2	0.64
NHW (Code 3)	9086	29.0	-55.0	0.63
MA (30%<Hct<50%)	4621	28.7	-55.1	0.78
NHB (30%<Hct<50%)	4671	27.0	-50.4	0.65
NHW (30%<Hct<50%)	8779	28.8	-54.7	0.63

Conclusions: Hba1c is usually thought of as average blood glucose but correlates very well with the fasting glucose which is usually the daily nadir of glucose concentrations. HbA1c is highly correlated to both long term glucose and to a slightly lesser extent to fasting glucose and with surprisingly the same regression coefficients. Fasting glucose thus can be used to calculate HbA1c using Nathan's formula and can be used for quality assurance purposes in cases of discrepant measured fasting glucose and HbA1c values.

A-199

Access Estradiol Sensitive immunoassay, performance of a new sensitive and accurate automated assay

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Background: Commercially estradiol immunoassays are efficient for the assessment of female reproductive function i.e. infertility, oligo-amenorrhea, menopausal status and monitoring of ovulation induction during in vitro fertilization. However they generally have insufficient sensitivity and/or accuracy for assessment of inborn errors of sex-steroid metabolism, disorders of puberty, estrogen deficiency in men, therapeutic drug monitoring during low-dose female hormone replacement therapy and antiestrogen treatment.

Methods: Within-run and within-laboratory were calculated based on five serum samples tested over 20 days according to CLSI EP05-A3. Method comparison was assessed according to CLSI EP09-A3 by measuring eighty-nine samples covering the

physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (≈2-4000 pg/mL) on ID-GC/MS and Beckman Coulter Access Estradiol Sensitive immunoassay. Correlation was assessed using Pearson correlation. Calibration curve and open vial calibrator stability were assessed according to CLSI EP25-A.

Results: The Access Sensitive Estradiol assay is traceable to JCTLM-approved methods according to ISO 17511, covering a measuring range from 15 to 5000 pg/mL. Within-run and within-laboratory are below 6% and 8%, respectively, for a sample at 30 pg/mL and below 3% and 4%, respectively, for a sample at 200 pg/mL. Limit of Detection (LoD) was 10 pg/mL. Limit of Quantitation (LoQ) was 15 pg/mL. Compared to the ID-GCMS method, the Pearson correlation was above 0.98, the method comparison exhibited a slope within 1.00+/-0.05 with an intercept below 5 pg/mL. The calibration curve and open vial calibrator stability are 31 and 90 days, respectively.

Conclusion: The Beckman Coulter Access Sensitive Estradiol assay is accurate and precise down to 15 pg/mL, making it an efficient tool for assessing estradiol status of children, males and monitoring females under antiestrogen or hormonal treatment.

A-200

Performance evaluation of the Lifotronic H9,a new HbA1c analyser

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Background: Hemoglobin A1c(HbA1c) is a powerful tool for both monitoring long-term glycemic control, and to diagnostic diabetes. Ion-exchange HPLC (IE-HPLC) assay has been consolidated as a gold standard to measure HbA1c, This study was aimed to evaluate the analytical performance of a new IE-HPLC analyser to measure HbA1c (Lifotronic H9) . and evaluate Lifotronic H9 in comparison to two other widely used HPLC systems(Primus Ultra2 ®boronate affinity HPCL and Bio-Rad Variant II turbo2.0® ion exchange HPLC) in patients with normal and abnormal Hb.**Methods:** The current study was conducted at the *Sun Yat-sen University affiliated Zhongshan hospital* laboratory. All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity and interference were evaluated according to CLSI protocols EP5-A2, EP9-A3, EP6-A and EP7-A2 respectively. Measurements of HbA1c by the three methods were made in blood from 124 patients with normal Hb(HbA)and 34 patients with abnormal Hb(Hb E).Primus Ultra2 was used as comparative system, and the other 2 systems were test systems. Comparative analysis and bias evaluation were conducted on the results from three detection systems, Appropriateness of data for linear regression analysis was checked regards CLSI EP9-A3 document, then performed both linear regression and difference plot analyses.

Results: The within-run imprecision values(CV%) were less than 1.75% and the total imprecision values(CV%) were less than 2.02%**.** Bias using reference samples from NGSP ranged from -2.78 to 2.63%. The linearity of was excellent in the range between 3.0% and 18.0%.Comparison of both methods against Primus Ultra2 demonstrated significant correlation (Lifotronic H9: r = 0.997; slope= 0.96; intercept =0.20; Bio-Rad Variant II turbo2.0: r = 0.998; slope = 0.98; intercept =0.13). The differences of the 95% confidence interval (95%CI) between the test systems and the comparative system in normal HbA samples and HbE samples,were within ±0.70% HbA1c, bias% were less than 6%, (P >0.05).The results showed that the Lifotronic H9 and Bio-Rad Variant II turbo2.0 were not affected by HbE.

Conclusion: The Lifotronic H9 demonstrated high analytical performance similar to previous systems such as Primus Ultra2 and Bio-Rad Variant II turbo2.0widely used HPLC systems. were not affected by HbE. and is therefore suitable for its utilization in modern clinical laboratories.

A-201

Standardization and Harmonization* of Ortho-Clinical Diagnostics Thyroid Function Tests - VITROS® Immunodiagnostic Products TSH and Free T4 Assays (* In development)

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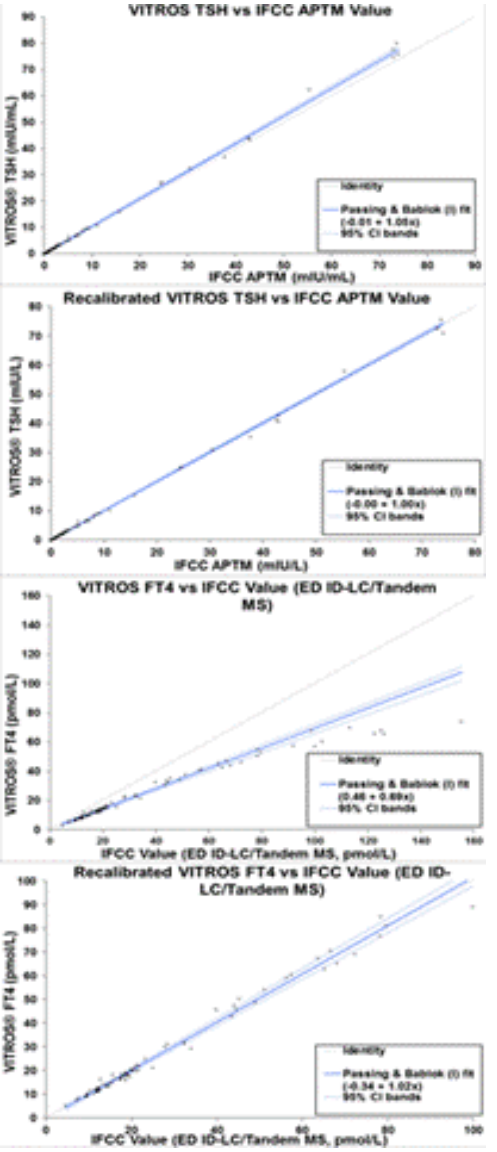
Background: The International Federation of Clinical Chemists (IFCC) has an ongoing program of standardization and harmonization for Thyroid function tests (C-STFT). The project aims to develop reference measurement systems (reference materials/reference methods) to establish traceability of free thyroid hormone and TSH assays. The IFCC intends that; FT4 assays will become traceable to the conventional reference measurement procedure based on equilibrium dialysis isotope dilution-liquid chromatography/tandem mass spectrometry (ED ID-LC/MS/MS), TSH assays to the statistically inferred all-procedure trimmed mean (APTM).

Methods: Ortho generated data as part of the IFCC Phase IV Harmonization and Standardization study by testing two panels of samples (90 FT4 & 102 TSH samples), results returned to the C-STFT. The committee provided Ortho with panel member results as determined by ED ID-LC/MS/MS for FT4 and the statistically derived APTM values for the TSH panels. Ortho then adjusted the values of their master reference calibrators to achieve closer agreement to these values. To achieve the best possible agreement of the VITROS® TSH assay to the APTM values at doses <0.3mIU/mL Ortho introduced two additional reference standards to their master reference calibrator set.

Results: Prior to the recalibration exercise slopes of 0.69 and 1.05 were obtained for the VITROS® Free T4 and TSH assays respectively. After adjustment of Ortho's internal reference standards, slopes of 1.02 and 1.00 were obtained for the VITROS® Free T4 and TSH assays.

Conclusion: Agreement of the VITROS® TSH assay (at doses below 0.3mIU/mL) against the IFCC APTM panel was improved by introduction of two additional master reference calibrator levels, and manipulation of the assigned doses. Manipulation of the master reference calibrator values for the VITROS® Free T4 assay can improve the correlation to ED ID-LC/MS/MS. However the VITROS® Free T4 assay is a true free hormone assay which is currently well aligned with other immunoassays.



A-202

What's your favorite color? A comparison of two albumin dye methods and their influence on calculated free testosterone

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Background: Over the past several decades, measurement of serum albumin has been dominated by two dye methods: bromcresol green (BCG) and bromcresol purple (BCP). Both methods have reported advantages and disadvantages. Even though specificity of BCG has improved since its inception, BCP continues to be more specific. However, BCP methods underestimate serum albumin covalently bound to bilirubin and in dialysis patients. Undoubtedly, the clinical utility of serum albumin and the patient population of the laboratory should be considered. Here we investigated the differences between serum albumin measured using BCG and BCP dye methods by two chemistry analyzers. Furthermore, the influence of albumin on the determination of free testosterone (FT) was assessed.

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 by liquid chromatography tandem mass spectrometry (LC-MS/MS). FT was determined in men using equilibrium dialysis (ED)-LC-MS/MS (mean FT concentration, 73.4 pg/mL). All samples were further tested for albumin with both the BCG and BCP dye methods using the Abbott ARCHITECT *ci*8200 and Roche cobas c702. FT was calculated using the Vermeulen equation, where total testosterone determined by LC-MS/MS and sex hormone binding globulin measured by Roche Modular E170 were used (mean calculated FT concentrations ranged from 71.3-73.7 pg/mL). The following comparisons were made: 1) albumin comparisons between the two dyes (BCG and BCP) for each analyzer, along with comparing the same dye between the ARCHTECT and c702, 2) comparisons of calculated FT using different albumins, and 3) comparison of calculated FT with directly measured FT in men; including calculated FT using a generic albumin value of 4.3 g/dL. Significance was characterized as *p*-values <0.05, determined by paired t-tests.

Results: For all populations combined, serum albumin mean concentrations were 4.4, 4.3, 4.2, and 4.1 g/dL for the ARCHITECT BCG, ARCHITECT BCP, c702 BCG and c702 BCP, respectively. When comparing these 4 datasets against each other, the associations were all significantly different (*p*-values ≤0.0001). To evaluate whether or not these significant differences in albumin influenced calculation of FT, each albumin dataset was entered into the Vermeulen equation, and all other variables remained the same. Comparisons of calculated FT were all significantly different (*p*-values ≤0.0001), except for c702 BCG vs. c702 BCP (*p*-value 0.818); indicating that calculated FT would not be influenced by albumin dye method used on the c702. Calculated FT was compared to FT by ED-LC-MS/MS in men and was found to be significant only for the ARCHITECT BCG method (*p*-value 0.034).

Conclusions: Comparisons of the albumin methods show that they are statistically different. However, when albumin is used for determination of FT, most do not show a significant impact, as indicated by the comparison of calculated and directly measured FT in men. Due to the potential significant differences that could occur, careful evaluation is recommended when selecting and switching albumin dye methods; the clinical use of serum albumin testing and the patient populations served by the laboratory should be considered.

A-203

Comparison of iPTH Assays on the ADVIA Centaur and Roche Modular E170 Analyzers and Biotin Interference Evaluation

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Background: The intact parathyroid hormone (iPTH) consists of 84 amino acids secreted from the parathyroid glands. iPTH is the major circulating factor regulating extracellular calcium concentration. The measurement of intact PTH provides a more-accurate assessment of parathyroid tissue secretory status, especially in patients with renal impairment. Several commercial immunoassays are available for testing iPTH. However, the presence of high endogenous levels of biotin may interfere with immunoassays that use biotin-streptavidin mechanisms in their assay designs. The objective of this study was to compare the ADVIA Centaur® iPTH assay from Siemens Healthcare and the Roche Modular E170 iPTH assay and to determine the effect of biotin on iPTH concentration. Both immunoassays use the biotin-streptavidin interaction in their assay designs. The ADVIA Centaur iPTH assay was designed to eliminate interference from high endogenous levels of biotin.

Methods: The method comparison was performed for the ADVIA Centaur iPTH and Roche Modular E170 iPTH assays using a total of 1089 EDTA plasma samples from predialysis patients with end-stage renal disease (ESRD). The effect of exogenous biotin was studied on the ADVIA Centaur iPTH and Roche Modular E170 iPTH assays to evaluate the interfering role of biotin. Various amounts of free biotin were added (0, 10, 25, 50, 100, 150, and 200 µg/L) to three plasma pools with known concentrations of iPTH (50, 200, and 620 pg/mL, respectively).

Results: The comparison between the two methods showed good correlation, but there were significant between-method differences in iPTH concentrations. Linear regression analysis yielded the following: ADVIA Centaur = 1.41(Roche Modular E170) − 16.2; R = 0.982. The addition of exogenous biotin (0, 10, 25, 50, 100, 150, and 200 µg/L) to the three plasma pools resulted in a decrease of iPTH determined on the Roche Modular E170 analyzer (percent recoveries: 100, 99, 95, 91, 76, 51, and 40%, respectively); however, the percent recoveries were unchanged for iPTH measured on the ADVIA Centaur analyzer (100, 97, 98, 100, 102, 103, and 102%, respectively).

Conclusion: The ADVIA Centaur iPTH and Roche Modular E170 iPTH assays showed good correlation in predialysis patients with ESRD despite between-method differences in iPTH concentrations. An in vitro interference study showed that the ADVIA Centaur iPTH assay was unaffected by high concentration of biotin, whereas the Roche Modular E170 iPTH assay was affected, especially at higher biotin concentrations.

A-204

Assessment of full thyroid profile in pediatric primary care focusing on the use of serum thyrotropin and free thyroxine only. Less is more?

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Background: It has been widely accepted that the major changes in thyroid function in adult subjects may initially be studied with the assessment of serum thyrotropin (TSH) and free thyroxine (fT4) only; however, there is little evidence whether the same criteria can be used in the pediatric population. Objective: The aim of this study was to describe the distribution of results obtained and subsequent medical management in a pediatric population assessing serum full thyroid profile focusing on serum TSH and fT4 only.

Methods: We retrospectively analyzed consecutive patients in whom thyroid function was studied with a full thyroid test seen between November 2014 and September 2015. Samples were tested on the day at the same laboratory. We excluded those subjects who were not seen by a physician after the studies, those who were treated with levothyroxine, methimazole, or antiepileptic drugs, and seriously ill patients. The remaining 5739 subjects were categorized according to age from one week to 12 years. TSH, T3, and fT4 were measured with Architect i4000 (Abbott) and total T4 IMMULITE 2000 (Siemens).

Results: Subjects with serum TSH and fT4 within the reference interval (RI) according to age were selected (n = 4008, 69.83% of total). From this group, children who had T3 and/or T4 outside the RI were selected (n = 391, 9.75%). For both analytes, 9.75% of healthy subjects is statistically expected to be found outside the RI ($(0.95)^2$). In the clinical chart (CC) of 381 patients (97.4%) no comment was made on thyroid function or the thyroid profile was reported to be normal, in spite of T3 and/or T4 being outside the RI; maybe because in all cases T3 and/or T4 were within the reference change value (RCV) on the limits of the RI. In 10 patients (2.6%) a comment was made in the CC; five patients were sent home without further interventions, in one patient with high T3 and T4 high serum levels of Thyroxine-Binding Globulin were found, and four were finally treated with levothyroxine. All of them had T4 and fT4 at the 10th percentile of the RI and two additionally had TSH above the 90th percentile of the RI.

Conclusion: Of the study population, 69.83% presented with normal TSH and fT4 levels. Of these patients, 9.75% had T3 and/or T4 outside the RI, which is statistically acceptable for a normal population. As to the 10 patients that had some comment in their CC regarding test results, only four were put on treatment, and all had fT4 below the 10th percentile and two had TSH above the 90th percentile as well. Screening of serum TSH and fT4 only may be useful in children tested to rule out thyroid disease. It is recommended to assess fT4 and TSH results with a stricter RI with a range between the 10th and 90th percentile considered acceptable. A prospective study in a pediatric population would be necessary to clinically confirm these results as well as the subsequent medical management.

A-205

Plasma calcitonin: maintaining assay harmonisation following method transfer

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Background: Plasma calcitonin is known to be an unstable analyte and vulnerable to repeated freeze-thaw cycles, incurring risk of sample rejection through thawing in transit when sent to another centre. We therefore undertook a method comparison between the Siemens Immulite method employed at the referral centre and the Roche platform, with a view to enable processing within our own laboratory. Roche Elecsys/Cobas and Siemens Immulite are both two site immunometric assays that measure the mature 32 amino acid monomer of calcitonin. Both methods are calibrated to WHO 89/620 and with similar sample requirements. **Objective:** To compare calcitonin measurement by the Roche and Siemens assays and examine the clinical concordance of results. To examine the effect of freeze-thaw cycles on calcitonin measurement. **Methods:** Clinical specimens (n=47) were exchanged between the two laboratories (June 2013 to December 2014). All samples were stored and transported frozen. Three patient samples with different calcitonin levels were run 10 times (9 times for one sample as it was insufficient for the tenth run) on the same day to assess within run imprecision. Another 3 samples with different levels of plasma calcitonin were analysed over 15 days to test for between run imprecision. Three different samples with high calcitonin levels were diluted serially to test for assay linearity. To justify the validity of including twice thawed samples in the method comparison, 9 samples that had not been thawed previously were subjected to repeated freeze/thaw cycles (n=4) and calcitonin measured. **Results:** 27 out of 47 samples had detectable calcitonin, and the remaining samples were below detection limit (<0.5 ng/L) by the Roche Calcitonin method. 5 out of 47 samples were paired (2 aliquots from the original patient samples were made before freezing) and the remaining 42 samples had undergone 2 freeze/thaw cycles before measurement on the Roche analyser. The Roche calcitonin assay agreed well with the Siemens Immulite method statistically [Slope= 1.03 (0.98 to 1.09), Intercept=0.05 (-0.65 to 0.75) by Weighted Deming Regression]. There was good clinical concordance between results from the two laboratories (n=47). Within run imprecisions (CV_w) were 3.4%, 9.2% and 10.4% for the 3 samples, with between run imprecisions (CV_b) being 9.9%, 10.3% and 12.9%. The Roche assay showed satisfactory dilution linearity, with mean +/- SD of diluted calcitonin = 94+/- 2% of the original calcitonin level. The mean recovery of twice thawed samples was 94% +/- 10.2% using the Roche assay. **Conclusion:** It was valid to have included twice thawed samples in the calcitonin method comparison. The Roche calcitonin assay agreed well with the Siemens Immulite method both clinically and statistically. Harmonisation can therefore be maintained by transferring from the Siemens Immulite to the Roche Elecsys/Cobas calcitonin assay.

A-206

Is one-minute microcentrifugation of samples ensuring rapid and reliable results for intraoperative PTH measurement in a routine lab?

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Background Intraoperative parathormone (ioPTH) monitoring is now a common practice and a guide for surgical decisions during parathyroidectomy. With a half-life of only few minutes, PTH levels directly reflect in vivo activity of parathyroid tissue and provide assurance that all hyperfunctioning tissues have been removed. According to the Miami criteria, PTH levels should be assessed just before excision as a baseline value and success of surgery is defined as a fall of ≥ 50% from the baseline at 10 minutes post-excision. If the criterion is not met additional measurement at 20 minutes or extended neck exploration is necessary. The goal of this study is to improve turnaround time of ioPTH laboratory testing by shortening preanalytical phase.

Methods Whole blood specimens from 29 parathyroid surgeries were collected on EDTA tube at different time intervals. For each intervention, a pre-skin incision and/or pre-gland-excision sample was obtained and represented baseline value. The number of intraoperative specimen varied from 2 to 10, with a total of 163 analyzed samples. Each sample underwent a one-minute microcentrifugation at 13000 rpm before performing PTH STAT assay on the Roche Cobas-6000 analyzer. At the same time all specimens were tested after a 10 minutes centrifugation step as initially done in our laboratory.

Results Statistical analysis showed an extremely significant and positive correlation coefficient (r=0.997, p< 0.0001) between the two methods. The mean difference observed on Bland and Altman plot was 9,9 ng/L (95% CI: − 16.6 to 36.4). The Passing and Bablock regression analysis provided a slope of 0.91 (95% confident interval: 0.90 to 0.92) and an intercept of 0.51 (95% confident interval: − 0.18 to 1.24), meaning that no systematical but a slightly proportional difference was observed.

Conclusion This study shows that reliable results of ioPTH are obtained with minimal pre-analytical phase. Implementation of this new procedure in our laboratory along with close collaboration with surgical teams will have direct benefits on turn-around time and patient care.

A-207

Development of a High-Sensitivity Prototype Assay for Thyroid Stimulating Hormone (TSH) on the VITROS® 3600 and ECi/ECiQ Immunodiagnostic and 5600 Integrated Systems

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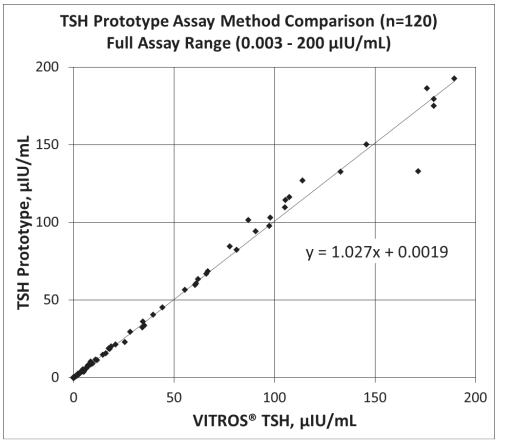
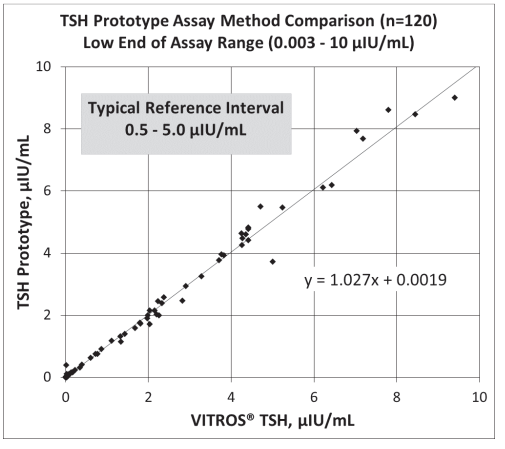
Background: A new prototype assay for thyroid stimulating hormone (TSH) is under development for the VITROS 5600, 3600 and ECi/ECiQ Systems. The prototype assay targets a faster time-to-result with reduced sample volume, expanded measuring range and improved sensitivity.

Methods: All testing was conducted on the VITROS 3600 and ECiQ Immunodiagnostic Systems. LoB/LoD/LoQ and precision were evaluated using modified CLSI protocols. Five precision pools were evaluated, with TSH values ranging from 0.105 to 71 µIU/mL. Precision testing occurred in ten runs on two instruments over a period of five days, with two replicates per run (n=40). Accuracy of the prototype assay was evaluated in a method comparison study by testing 120 serum samples ranging from 0.002 to 189 µIU/mL TSH. Samples were tested on the prototype assay and the current VITROS Immunodiagnostic Products TSH assay with an extended calibration range. Passing-Bablok regression was used to analyze the results.

Results: The prototype assay was determined to have LoB = 0.0005 µIU/mL, LoD = 0.0025 µIU/mL and LoQ (20% CV) = 0.0031 µIU/mL with a time-to-result of 24 min. Testing of the five precision pools (n=40) produced total imprecision ranging from 5.0 to 6.4 %CV. Analysis of the method comparison study (n=120) generated a slope of 1.027 and y-intercept of 0.0019.

Conclusion: The results demonstrate that the prototype TSH assay is accurate, precise and sensitive, while delivering assay results 40% faster than the current TSH assay.





A-208

Identification hemoglobin variants during glycosylated hemoglobin measurement

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Background: Glycated hemoglobin, measured as hemoglobin A1c (HbA1c), is the most reliable marker for monitoring long term glucose control in patients with diabetes mellitus (DM). Hemoglobinophaties do not usually have a clinical impact buy they can interfere with the analytical determination of some parameters such as the HbA1c. Many hemoglobin variants (HbV) are detected incidentally during the measurement of HbA1c.

The aims of this study were to describe HbV detected during HbA1c measurement since the introduction of the Hb-Advisor™ application (instrument Manager v.8.09, Data Innovations LLC) and to check if affect the accuracy of the HbA1c measurement.

Methods: Descriptive study, from 2012 to April 2014. HbA1c quantification was performed by HPLC assay on Bio-Rad VARIANT II™ TURBO HbA1c Kit-2.0 (Hercules, CS, USA) system with the 1.5 min. Screening abnormally chromatograms was done with the computer application Bio-Rad Hb-Advisor. Predefined rules warning were: anomalous peaks; P3 or P4> 7%; HbA1a> 2.6%; HbAqb> 3.5%; HbA1c< 4% or > 11.5%; Labile HbA1c> 4% and fetal hemoglobin (HbF)>10%. In those samples with anomalous chromatograms, HbA1c was measured again using a method based on a different analytical principle: boronate affinity chromatography (Afinion™ AS100; Axis-Shield, Oslo, Norway), HPLC with Bio Rad VARIANT II™ thalassemia Short Program and immunoagglutination method DCA 2000 (Bayer, Vienna, Austria)

We reviewed laboratory parameters: routine biochemistry and hemogram parameters. Since the abnormal chromatography indicated a suspected HbV, blood samples were sent to a thalassemia and hemoglobinopathy reference laboratory for: Hemoglobin was assessed by capillary zone electrophoresis on Sebia Capillarys Flex system using the Capillary Hemoglobin kit (Sebia, Norcross, GA, USA). Globin chains were studied by reverse-phase HPLC using a Vydac large-pore C4 column (The Separations Group, Hesperia, CA, USA). Functional studies were carried out with a Hemox-Analyzer TCS Medical Products (Huntingdon Valley, PA, USA). Hemoglobin stability test with isopropanol. For molecular characterization of the gene, genomic DNA from isolated leukocytes was extracted using an automatic extractor (BioRobot EZ1; Quiagen).

Results: We analyzed 121424 samples, median 4562.6 [3162-5765] samples/month. We detected 84 HbV, 62 were identified. Median age 51.5 (10-86) years, 51.2% women. 22.7% non-Caucasian. Hemoglobinopathies identified (all heterozygous): 38.7% HbS; HbC 27.4%; 17.7% HbF persistence; 8.1% Hb-Valme; 3.2% HbA2 increased; 1.6% Hb-Sevilla, Hb-South Florida and Hb-J-Paris I. We found the interference of HbV is variable and the extent of the interference is method dependent. HbS (heterozygous), HbC (heterozygous) and HbF≤25% not affect the accuracy of HbA1c measurement in HPLC Variant II Turbo, already described in literature.

Conclusion: The use of applications such as Hb-Advisor™ allows us to discriminate extreme values of HbA1c or anomalous chromatograms. In such cases, a visual inspection of the HPLC chromatogram may give valuable information regarding HbV, allowing the detection of inaccurate results . When the laboratories suspect inaccurate results that may lead to over- or under-treatment of diabetic patients, choosing an alternative non-Hb-based methods for assessing long-term glycemic control (such as fructosamine assay) may be useful. It is important to know those HbV that interfere with HbA1c measurement method used.

A-209

Characterization of 1.5-Anhydroglucitol levels in Brazilian adult subjects without diabetes

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Background: 1.5-Anhydroglucitol or 1.5-AG is a glucose-like monosaccharide contained in food and it is a validated marker of short-term glycemic control. During periods of hyperglycemia, glucose blocks reabsorption of 1.5-AG in the renal tubules. Thus, low blood levels of 1.5-AG are associated with hyperglycemia. 1.5-AG may predict more rapid changes in glycemia than glycated hemoglobin -A1C or fructosamine. Since 1.5-AG levels can be influenced by racial or ethinic groups, reference values should be specific for each population. Aim**:** to establish the 1.5-AG levels in Brazilian adult patients without diabetes.

Methods: a group of population without clinical and laboratory evidence of diabetes was evaluated. The study was submitted and approved by our Internal Review Board. The blood samples of 966 subjects, 506 women and 460 men, aging 40 ± 10 years, were analyzed. The blood was collected by venipuncture after 12 hours fast. The samples were collected in serum gel evacuated tubes for 1.5AG and glucose tests and EDTA tubes for A1C -Vacuette, Greiner Bio One International GmbH, Germany. Serum 1.5AG was measured using the GlycoMark assay - GlycoMark, Inc., Japan and glucose using enzymatic hexokinase method by Roche. The analysis were carried out on *cobas 8000 modular analyzer series,* C702 module -Roche Diagnostics GmbH, Germany. The HPLC method certified by NGSP was used to measure the A1C level on Tosoh automated glycohemoglobin analyzer HLC-723G8 -Tosoh Corporation, Japan.

Results: the mean results in the whole population were 1.5 AG: 15.89±6.47 µg/mL, glucose: 89±6 mg/dL and A1C: 5.2± 0.3%. Sex-specific mean results were 1.5 AG: 16.71±6.59 µg/mL, glucose: 90±5 mg/dL and A1C: 5.3± 0.3% for men and 1.5 AG: 15.14±6.26 µg/mL, glucose: 88±6 mg/dL and A1C: 5.2±0.3% for women.

Conclusion: in Brazilian patients without diabetes the reference intervals, 2.5th 97.5th percentiles were 11.68-29.18 µg/mL for males and 10.54-28.94 µg/mL for females. Since we were able to establish reference values in a Brazilian population, the use of 1.5AG assay would be suitable an alternative marker for glucose monitoring in the short-term glycemic control allowing rapid practitioner intervention in uncontrolled diabetic subjects.

A-210

Free T3 and Total T3 Assays with Six Point Calibrators on the ARCHITECT Analyzer (In Development).

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BACKGROUND: In a clinical setting, thyroid function is often evaluated through testing for thyroid stimulating hormone (TSH) and other thyroid hormones such as thryroxine (T4) and triiodothyronine (T3). In the particular situation where the TSH result does not agree with the T4 result and thyroid disease is suspected, additional testing for T3 (FT3 and TT3) may be needed to confirm diagnosis. T3 is bound to endogenous proteins such as thyroxine binding globulin, pre-albumin and albumin; and as a result, a small portion of T3 is free in the bloodstream. This fraction represents the active form of the hormones. The ARCHITECT Free T3 and Total T3 assays were developed to aid in the diagnosis and monitoring of hyperthyroidism. METHODS: The ARCHITECT Free T3 and Total T3 assays are two-step immunoassays to determine the presence of free (unbound) T3 and Total T3, respectively, in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology. In the first step, sample and anti-T3 coated paramagnetic microparticles are combined. Free T3 (unbound) or T3 present in the sample binds to the antiT3 coated microparticles. After washing, T3 acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of Free T3 or T3 in the sample

and the RLUs detected by the ARCHITECT optical system. RESULTS: Competitive Free T3 and Total T3 immunoassay formats were optimized for robust analytical performance. For the Free T3 Assay, twenty-day precision results were <5.3 %CV across three controls and serum based panels and <8.7% CV across the measuring interval (1.45 to 22 pg/mL). Limit of quantitation was less than 0.96 pg/mL. The assay demonstrated linearity from 1.4 to 23.8 pg/mL. For the Total T3 Assay, twenty-day precision results were <5.4 %CV across three controls and <8.0% CV across the measuring interval (0.5 to 6 ng/mL). Limit of quantitation was less than 0.21 ng/mL. The assay showed linearity from 0.38 to 6.58 ng/mL. No endogenous and common drug interferences were observed for either of the assays. Both Free T3 and Total T3 assays have consistent results across the measuring interval between i1000SR and i2000SR instrument platforms. CONCLUSIONS: The ARCHITECT® Free T3 and Total T3 immunoassays are reliable and robust tests to measure serum T3 levels. The assays are currently in development.

A-211

Elecsys® AMH immunoassay: Evaluation of the novel assay's precision under routine conditions

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Background: Measurement of ovarian reserve levels plays a key role in predicting treatment response to controlled ovarian stimulation (COS) in assisted reproductive therapy. Anti-Müllerian Hormone (AMH), released from ovarian granulosa cells leading to serum levels proportional to the number of developing follicles in the ovaries, is a promising marker for assessing the ovarian reserve and optimizing *in vitro* fertilization treatments. Currently available manual AMH assays exhibit limitations with respect to run time and reliability of results. The Elecsys® AMH assay is a fully automated sandwich electrochemiluminescence immunoassay for the *in vitro* quantitative determination of AMH in human serum and lithium heparin plasma. The aim of this study was to evaluate the technical performance of this immunoassay in terms of precision under routine conditions.

Methods: Three laboratories in the United States evaluated the reproducibility performance of the assay according to CLSI EP-15-A2 guidelines using a **cobas e** 411 analyzer. Precision experiments were performed with Human Serum Pools (HSP) generated using human serum from postmenopausal women (BIOMEX GmbH), known to contain very low levels of AMH. Tested AMH concentrations, covering major parts of the measuring range (0.2-20 ng/ml [1.4-143 pmol/L]) were obtained by adding fetal bovine serum with high levels of AMH to these pools. Subsequently, the pools were aliquoted and stored frozen until measurement at the respective sites. In total, the analyzed imprecision pool consisted of five HSPs (HSP 01-05) and two PreciControl AMH samples (PC01=1.0 ng/ml [7.14 pmol/L] and PC02=5.0 ng/ml [35.7 pmol/L]). The sample pools were tested in replicates of three in one run per day for five days.

Variance component analysis was calculated for the reproducibility experiment according to CLSI-EP15-A2 using ANOVA Type 1 approach for unbalanced data. The two-sided 95% confidence intervals (CI) for reproducibility coefficients of variance (CVs) were calculated using a chi-square-based approach to construct CIs which relied on the original Satterthwaite approximation for degrees of freedom. Measurements were captured using the WinCAEv software and the statistical analysis conducted using R Version 3.0.1. Validation of the reproducibility dataset and results were performed using SAS Version 9.3.

Results: For the reproducibility experiments, the CVs (95% CI) were shown to be <5.5% across all three sites (HSP01 3.99% [2.68-7.77], HSP02 4.05% [2.79-7.42], HSP03 3.45% [2.33-6.66], HSP04 4.62% [3.01-9.80], HSP05 4.38% [2.94-8.48], PC01 5.24% [3.46-10.69] and PC02 4.70% [3.18-8.91]. The CVs for repeatability were all <2% (HSP01 1.58% [1.34-1.92], HSP02 1.74% [1.48-2.12], HSP03 1.41% [1.19-1.71], HSP04 1.69% [1.44-2.06], HSP05 1.69% [1.43-2.06], PC01 1.42% [1.21-1.73] and PC02 1.89% [1.60-2.30]).

Conclusion: The fully automated Elecsys AMH immunoassay run on the **cobas e 411** under routine conditions demonstrated very good precision performance in the concentration range of 0.2-20 ng/mL (1.4-143 pmol/L). Consequently, the availability of this immunoassay will represent a fast and reliable alternative to conventionally used manual methods for AMH testing.

Disclaimer: This product is not cleared or approved for use in the USA.

A-212

Monitoring of the standardization and harmonization status of FT4 and TSH assays by use of patient medians.

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Background: For the diagnosis of thyroid dysfunction clinicians rely in first instance on TSH and FT4 testing. However, the lack of comparability between assays is an issue for patient safety. Therefore, the IFCC Committee for Standardization of Thyroid Function Tests (C-STFT) is involved in standardization/ harmonization efforts. Once assay recalibration to generate comparable results will be accomplished, the C-STFT will be challenged to monitor sustainability of this status. We believe this will be possible by applying our recently developed Percentiler and Flagger. They are intended to serve as quality indicator for stability of performance (Percentiler) and flagging rate (Flagger). Although the recalibration of FT4 and TSH assays is not yet completed, we considered it worth to start applying the tools in the pre-implementation phase, because it would allow us to timely recruit participants and build experience.

Methods: For the Percentiler, laboratories calculate and electronically send us instrument-specific daily medians from outpatient results. For the Flagger, daily flagging rates (% hypo and hyper) are reported. We developed software and user interfaces to plot the course of the instrument-specific moving medians (Percentiler) and flagging rates (Flagger) in comparison to instrument-based peer group medians. Laboratories have access via a lab-specific password. They see their own plots and data, including their long term median(s) in comparison to the peer group. Laboratories can visually infer whether the performance of their instruments and of their peer is stable, i.e., when the moving medians remain between desirable bias limits proposed by us. For the Percentiler, these are guided by biological variation and state-of-the-art performance, i.e., ±3.3% for FT4 and ±7.7% for TSH, respectively. For the Flagger, the limits are preliminary set at ±30% of the long-term flagging rate.

Results: In the Percentiler, we currently have 77 participants with 147 instruments, from which we distinguish 4 peer groups (n >15); in the Flagger, only 29 laboratories with 38 instruments participate. From our short time experience we see already that for FT4 and TSH the moving medians are more variable than for clinical chemistry analytes; in spite of this, most laboratories have satisfactory stability of performance. Nevertheless, we sometimes observe significant shifts due to lot changes, differences between instruments, drifts or saw-tooth patterns due to reagent instability. Since we group laboratories according to instrument peer, we can also monitor/compare the stability of instruments. We also see the interplaying effect between the Percentiler and Flagger, i.e., an increase of the median values in the Percentiler results in a decrease of the %-hypo and increase of the %-hyper in the Flagger, and vice versa.

Conclusion: By starting to use the Percentiler and Flagger applications for the C-STFT, we showed their utility for monitoring the stability of FT4 and TSH performance in laboratories and instruments and the effect of instability on the flagging rate. This gives evidence that the tools will also be useful to document the sustainability of the post-standardization/harmonization status. Nonetheless, we need to recruit more participants, so that all instruments participating in the C-STFT activities are represented.

A-213

Establishing reference intervals for hCG in postmenopausal women

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Background: Human chorionic gonadotropin (hCG) screening tests are performed in nearly all female patients prior to any medical intervention regardless of age. Plasma hCG concentrations have been shown to increase with age due to pituitary secretion, resulting in positive hCG in the absence of pregnancy. We previously recommended that an hCG cutoff of 14.0 IU/L be used for women >55 years of age. However, it remains unknown whether concentrations greater than 14.0 IU/L can be expected in women with advanced age. Additionally, the relationship between FSH and hCG has not been examined in postmenopausal women. Our objectives were to establish serum hCG reference intervals and correlate FSH and hCG concentrations in non-pregnant postmenopausal females age >55 years.

METHODS: A total of 796 residual plasma samples from women >55 years were collected with 303, 269, and 224 samples belonging to the age groups 55-69, 70-84, and >85 years, respectively. FSH and hCG were measured using the Abbott Architect.

Patients with FSH <8.0 IU/L were assumed to be on hormone replacement therapy and were excluded. All positive hCG samples were analyzed for heterophile antibody interference and 3 were excluded. Patient chart review was performed for each positive hCG sample. 4 were excluded due to malignancy.

RESULTS: 10% (67/676) of women age >55 years had plasma hCG >5 IU/L. There were 22, 21, and 24 patients with hCG >5 IU/L in the age groups 55-69, 70-84, and >85 years, respectively. The hCG concentrations observed in each age group were as follows: 55-69 years maximum =12.4 IU/L and 97th percentile = 11.7 IU/L; 70-84 years maximum = 18.09 IU/L, 97th percentile = 9.12 IU/L, and >85 years maximum = 11.1 IU/L and 97th percentile = 10.9 IU/L. We found no correlation between hCG and FSH concentrations. Additionally, hCG concentrations did not continue to increase with age over 55 years.

CONCLUSIONS: In women>55 years of age, plasma FSH concentrations do not predict hCG concentrations and age-specific reference intervals are not needed beyond 55 years. This study confirms that our previously recommended cutoff of 14 IU/L should be used for women >55 years of age.

A-215

Use of liquid chromatography/tandem mass spectrometry to assess diurnal effects of steroids and neurosteroids

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Background: Alterations of the hypothalamic-pituitary-adrenal system are reported in a number of conditions. Diurnal variation has been demonstrated for cortisol and a number of steroids using liquid chromatography-tandem mass spectrometry (LC-MSMS) with significantly higher circulating levels in the morning, indicating the need to develop time-specific reference intervals. Neurosteroids are a class of steroids with central nervous system modulation activity. The neurosteroids allopregnanolone, dehydroepiandrosterone (DHEA), tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone are key mediators of the stress response and are linked to several neurologic, endocrine, and psychiatric disorders; however, diurnal variation of these neurosteroids and reference intervals have not been previously evaluated. Our aim is to develop reference intervals for LC-MSMS measurement of a steroid profile and neurosteroids, and assess if diurnal variation could also be observed in neurosteroids.

Methods: Early morning serum samples were collected between 6:00 am to 8:00 am, and evening serum samples between 6:00 pm to 8:00 pm from 24 healthy volunteers. Study volunteers were generally healthy with no current or past major illnesses. LC-MSMS was used to measure a steroid profile that includes androstenedione, cortisol, corticosterone, cortisone, 11-deoxycortisol, 17-α-hydroxyprogesterone, and testosterone. The neurosteroids allopregnanolone, DHEA, tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone were also measured. The paired sample Wilcoxon test was used to compare differences between morning and evening values.

Results: The median values and 2.5th - 97.5th percentiles for morning and evening steroid levels are shown in Table 1. Statistically significant differences were observed between morning and evening values for all steroids and the neurosteroid DHEA. The neurosteroids allopregnanolone, tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone were undetectable in most of the normal volunteers.

Conclusions: Time-specific reference intervals are essential to evaluate the clinical relevance of steroid profiles and the neurosteroid DHEA.

Table 1. Morning and evening steroid profiles measured by LC-MSMS. Data shown are the median (2.5th - 97.5th percentile).

	Morning	Evening	p-value
Androstenedione, ng/dL	66.5 (33.8 – 188.0)	46.5 (28.2 – 186.1)	0.0005
Cortisol, ng/dL	12.0 (6.5 – 20.8)	3.95 (1.8 – 11.1)	<0.0001
Corticosterone, ng/mL	1.41 (0.46 – 5.0)	0.45 (0.1 – 1.6)	0.0001
Cortisone, ng/mL	20.9 (11.9 – 29.9)	8.1 (3.9 – 23.9)	<0.0001
DHEA, ng/dL	239.0 (61.3 – 693.0)	191.0 (43.0 – 438.4)	0.002
11-deoxycortisol, ng/dL	39.0 (0.6 – 112.3)	11.6 (0.0 – 59.4)	0.0012
17-α-hydroxyprogesterone, ng/dL	29.8 (1.1 – 158.2)	11.5 (0.0 – 154.9)	0.0006

A-216

Comparison of HbA1c values from the Alere Afinion and Tosoh G8 HbA1c Analyzers Before and After Tosoh Assay Recalibration

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Background: Analysis of hemoglobin A1c (HbA1c) is a cornerstone in the management of patients with diabetes. Although Point-of-Care (POC) HbA1c devices are not recommended for use in the diagnosis of diabetes, they are used frequently in physician offices and clinics to monitor the glycemic control of patients. An added benefit of POC analyzers is the rapid analysis time, which allows clinicians to give a result to the patient during the office visit and counsel the patient based on that result. Our institution uses two methodologies for measuring HbA1c: a HPLC method (Tosoh G8) in the laboratory and a POC boronate affinity method (Alere Afinion) at satellite locations. It is not uncommon for diabetic patients in our hospital system to be seen at different clinic locations and have HbA1c testing done by both methods. All methods for measuring HbA1c undergo an accuracy based evaluation with comparison to National Glycohemoglobin Standardization Program (NGSP) reference methods. Historically, when the results of the Tosoh peer group had been compared to the reference method in College of American Pathologists (CAP) surveys, the results had been consistently higher than the reference method. In response to customer concerns, in 2015 the manufacturer recalibrated the Tosoh assay to bring HbA1c values closer to the reference method. In contrast, the Afinion assay has been shown to have a negative bias compared to reference methods when measuring HbA1c values greater than 8%. As many of our patients have HbA1c values above 8% and could have measurements performed by both instruments, we wanted to compare values from both methods before and after the Tosoh recalibration. **Methods:** Analysis of data from comparison studies of HbA1c results assayed by the Afinion and the Tosoh before and after recalibration in 2015. Pre-recalibration data included initial validation and method comparison studies between the Tosoh G8 and the Alere Afinion HbA1c analyzers. Post-recalibration data was gathered from method comparison studies performed on new lots of Afinion cartridges. **Results:** Initial method comparison studies showed that results from the Afinion had a negative bias when compared to the Tosoh (y=0.9531x-0.2164). This was especially pronounced at values above 8%, where the majority of specimens from the Tosoh were higher than the Afinion. After a preliminary evaluation of the data comparing the Afinion to the Tosoh after manufacturer recalibration of the assay, this bias has decreased, but is still present (y=1.0197x-0.3396). Further analysis will be done to focus on HbA1c values greater than 8%. **Conclusions:** After recalibration of the Tosoh assay, the agreement between the two assays, particularly at values above 8%, is better.

A-218

Derivation of the biologic variation of data-mined hCG

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Introduction: Most estimates of biologic variation (s_b) are based on acquiring and storing specimens from reference subjects, followed by analysis within a tightly controlled analytic run. The s_b of certain analytes, such as hCG cannot be easily derived in this manner as hCG is produced in select subjects over a relatively short period. We propose that data-mined intra-patient hCG results can be statistically analyzed to provide s_b.

Methods: A data repository provided all outpatient and inpatient hCG measured over 5 years at a large referral laboratory in Edmonton and at 5 Edmonton hospitals. The hCG measurements were made with Roche (4thWHO IS) and Beckman (3rdWHO IS) instrumentation for the referral and hospital patients, respectively. Patient hCG results were analyzed if at least two intrapatient results were obtained within 84 hours. Three different hCG ranges were studied: under 1,000 IU/L, 1,000 to 10,000 IU/L and 10,000 to 100,000 IU/L. We tabulated the pairs of intra-patient hCG that were separated by 0-6, 6-12, 12-18, . . 72-78 and 78-84 hours. We calculated the standard deviations of duplicates (SDD) of the paired data. The SDD were regressed against the midpoints of the time intervals. While the y intercept represents the sum of s_b and short term analytic variation (s_a): $y_0 = (s_a^2 + s_b^2)^{1/2}$, we assumed that short term imprecision was negligible compared to s_b.

Results: The Table summarizes the results; CV_b is derived from the ratio of s_b to the median hCG. While the CV_bs are plausible for the two lower hCG concentrations, the Roche and Beckman s_b are highly divergent for the high concentrations. These differences may be attributable to the Roche system's wider linear range.

Conclusions: Complex intra-patient data such as hCGs in pregnancy can be mined to provide useful estimates of biologic variation.

hCG Range, IU/L	Assay	Mean, IU/L	Median, IU/L	sb, IU/L	CVb
0 to 1,000	Roche (outpatient)	237	118	117	99.2%
1 to 1,000	Beckman (inpatient)	252	137	77	56.2%
1,000 to 10,000	Roche (outpatient)	4087	3422	660	19.3%
1,000 to 10,000	Beckman (inpatient)	4166	3588	785	21.9%
10,000 to 100,000	Roche (outpatient)	42510	36940	416	1.1%
10,000 to 100,000	Beckman (inpatient)	42700	36470	5360	14.7%

A-219

Evaluation of a new automated method for glycosylated hemoglobin on the Abbott Architect C8000

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Background We verified the analytical performance of an enzymatic HbA1c assay on the Abbott Architect C8000 Chemistry System (Abbott) and compared it to a contemporary immuno-turbidimetric assay on the Cobas 502 (Roche).

Methods In the Abbott HbA1c assay glycosylated N-terminal dipeptide (fructosyl-VH) of the hemoglobin beta chain is cleaved by the addition of protease. Fructosyl-VH then reacts with peroxidase and fructosyl peptide oxidase. HbA1c concentration is measured by determining the resultant hydrogen peroxide. The Abbott HbA1c was verified for imprecision, linearity and accuracy. Imprecision was carried out by measuring 2 levels of QC material (BioRad) in triplicate over five days in accordance to CLSI EP5-A2 guidelines. Linearity and accuracy were assessed by analysing 5 HbA1c specimens ranging between 4.0-15.0% in triplicate. All data are evaluated using EP Evaluator Software.

Consecutive patient samples (n=168) with a range of HbA1c values - below 5.6% (non-diabetes=50), 5.7-6.4% (prediabetes=50), above 6.5% (diabetes=68) - were analysed on both the Abbott and Roche platforms and compared. Statistical analyses were performed using MedCalc v16.2 software (Ostend, Belgium).

Results Assay imprecision (CV) for control materials at levels of 5.4% and 10.0% HbA1c were 1.1% and 1.5% respectively. The Abbott assay is linear across the manufacturer's claimed measuring range of 4.0-15.0% (slope=1.002, intercept= -0.11) and sufficiently accurate (maximum deviation for a mean recovery from 100% was 3.1%).

For the assay comparisons samples from 79 women and 89 men were used (age: range 17-99, mean 57.8, SD 19.0 years respectively). Passing-Bablok Regression: Abbott = 1.025641 Roche - 0.235897; Cusum test for linearity - No significant deviation from linearity (P=0.06); r = 0.99 (95% CI 0.9865 to 0.9926). Bland-Altman plots revealed a mean absolute difference (Abbott-Roche) in HbA1c values of 0.01% (range -0.75 to +0.77%). For HbA1c samples below 6.5% (n=100), the mean difference was 0.08% (range: - 0.33 to +0.16) and for HbA1c samples over 6.5% (n=68), the mean difference was 0.16% (range: -0.95 to +1.26).

Conclusion The imprecision of the Abbott HbA1c assay is well within the NGSP targets of <2.0% CV for samples targeted to 6.5% HbA1c and ≤3.5% CV for samples over 7.0% HbA1c. There was close agreement between the Abbott and Roche A1c assays. The new Abbott HbA1c assay offers clinical laboratories an additional choice for HbA1c measurement.

A-220

Clinical Utility of Aldosterone, Renin Mass and the Aldosterone/ Renin Mass Ratio for the work up of suspected Primary Aldosteronism

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Background: Primary aldosteronism (PA) is a group of disorders characterized by inappropriate aldosterone production. PA is caused by adrenal adenoma or hyperplasia. The Endocrine Society recommends a multi-tiered approach for the diagnosis of PA in high risk patients (i.e. drug resistant hypertension, hypokalemia and/or hypertension with adrenal incidentaloma) by: (1)Screening, via calculation of the aldosterone/ renin ratio (ARR), and (2)Abnormal ARRs should be confirmed with provocative testing and adrenal vein sampling. The accepted method for renin quantification is by plasma renin activity (PRA). New assays are available that quantitate renin mass by immunoassay (Direct Renin Concentration or DRC).

Objective: To compare the clinical utilities of renin measured by the activity or mass assay to predict primary aldosteronism.

Methods: A retrospective cohort study was performed utilizing residual plasma-EDTA specimens of 200 hypertensive adults (134 female and 66 male) being worked up for PA. Leftover specimens sent to the Vanderbilt University Medical Center Esoteric Chemistry laboratory with orders of PRA and plasma aldosterone concentrations (PAC) were utilized. Specimens were included from patients ≥18 years with hypertension and an order for PRA and/or PAC. Plasma-EDTA specimens were frozen until analysis. Review of the electronic medical records was conducted to determine patient history, diagnosis, and outcomes. The gold standard for PA diagnosis was based on ES guidelines of abnormal ARR (PRA cutoff >30 or DRC >5.7), PAC (>15ng/dL) and provocative confirmatory testing. PAC and DRC were measured by immunoassay on the DiaSorin Liaison. PRA was measured with DiaSorin's radioimmunoassay kit. Stability studies were performed at different storage conditions for PAC and DRC. The clinical utility of the ARR calculated with either PRA or DRC (ARR-PRA or ARR-DRC) to predict PA was assessed by Receiver Operating Characteristic (ROC) analysis, using GraphPad software.

Results: PAC and DRC were stable for at least two months at -80°C. PRA and DRC results correlated well, r = 0.95. Of the 200 patients, 8 had likely diagnosis of PA (2 confirmed by gold standard, 3 with an elevated ARR, aldosterone and adrenal nodule, and 3 with physician documented diagnosis). ROC analysis demonstrated that ARR-PRA and ARR-DRC showed similar ability to predict PA; areas under the ROC curves were 0.98 and 0.95 respectively. At the ES recommended cutoffs, sensitivity, specificity and positive-likelihood ratios (LR+) were 100% (16 - 100[95% CI]), 78%(72 - 84), and 4.6 respectively for ARR-PRA and 62%(25 - 91 [95% CI]), 96%(92 - 98), and 15.2 respectively for ARR-DRC. Specificity and LR+s improved with the use of both the elevated ARR and aldosterone to 95.5%(92 - 98) and 19.4 for ARR-PRA and 97%(93 - 99) and 20.3 respectively for ARR-DRC. False positive ARRs were seen with kidney disease, no follow up testing, and/or interfering medications.

Conclusion: The combination of elevated aldosterone and ARR (calculated with PRA or DRC) showed superior clinical utility to predict PA in high-risk patients. Because of false positives, interfering PA must be confirmed with provocative testing. Performance characteristics were similar for the DiaSorin DRC and PRA assays. Furthermore, DRC testing is rapid, stable overtime and eliminates the need for radioisotopes.

A-221

Age specific TSH reference ranges generated by electronic medical record database mining: data from over 33,000 healthy patients

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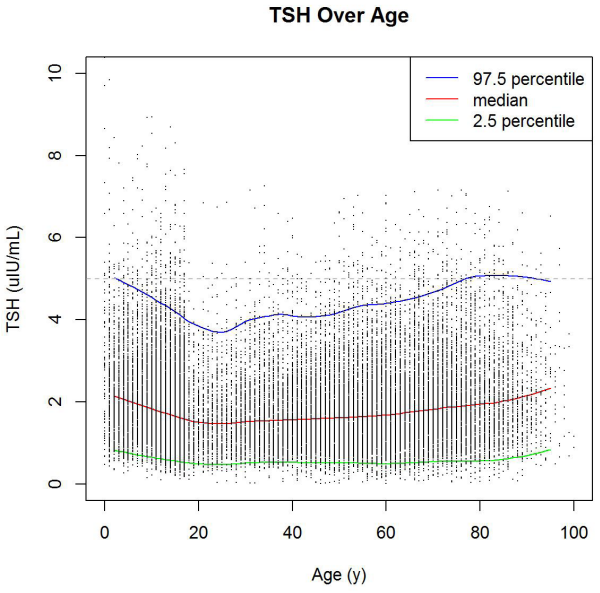
Background: Serum TSH reference ranges are dependent on population characteristics, including prevalent thyroid disease and iodine status. Studies in the United States have demonstrated increasing TSH levels with age, and the American Thyroid Association recommends higher TSH goals for older patients on thyroid supplementation. Our objective was to establish TSH reference ranges in our racially diverse population in Northern California.

Methods: To gather TSH data from a healthy population, we used a retrospective strategy of database mining of our electronic medical records. We generated a report of all TSH results run in our laboratory from two weeks one year in the past. Only results from patients who were under our care for at least one year before the TSH test and continued care for the following year were included. Results were excluded from the reference population if the record of the patient noted thyroid-related disease or thyroid-related medication use at any time before or after the TSH test. Additional exclusion criteria included inpatient status at the time of collection and pregnancy during the two year period surrounding the TSH test. To obtain more data from our pediatric population, additional results from a 6-month period were added for patients age 6-17 years and from a 1-year period for patients <6 years. The final cohort numbered greater than 33,000. The population identified as 47% white, 18% Asian, 17% Hispanic/Latino, 8% black, and 8% other or unknown. We prospectively analyzed TSH on a separate cohort of 388 healthy patients with fresh serum samples and no TSH ordered to validate reference ranges established with patients from the retrospective report.

Results & Conclusions: These data demonstrated an increase in the median and 97.5 percentile of TSH levels with increasing age in adults (Fig. 1). No clinically significant difference was seen between females and males or between the self-identified races.



A-223

Evaluation of the Multi-site Anti-müllerian Hormone (AMH) Age-related Reference Intervals on Women with Proven Natural Fertility using the Beckman Coulter Access Immunoassay Systems

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Background: Anti-müllerian hormone (AMH)* is a naturally occurring hormone found in both males and females. Published literature suggests AMH has potential for evaluating the ovarian reserve in women of reproductive age and is known to vary by age. Beckman Coulter has developed an automated version** of the AMH Gen II assay used on the Beckman Coulter Access 2 Immunoassay Analyzer. Age-specific reference intervals were evaluated.

Methods: 622 women with proven natural fertility were prospectively enrolled from three U.S. centers. All racial backgrounds were eligible. Subjects were ≥ 18 years of age, had regular menses (21-35 days) and both ovaries. Women with PCOS, previous ovarian surgery, exposure to cytotoxic drugs or pelvic radiation therapy, or recent contraceptive use were excluded. Serum samples were analyzed using the Beckman Coulter Access 2 Immunoassay Analyzer. Data were initially stratified to age ranges: 18-25, 26-30, 31-35, 36-40, 41-45, and ≥ 46 years. Outliers were removed using Tukey's method on Box-Cox transformed data. The robust method was used to estimate the 2.5th and 97.5th percentiles and their 90% confidence intervals.

Results: Reference intervals are reported in Table 1. Data groups 18-25 and 26-30 years were combined as the overlapping 90% confidence intervals suggested no difference between the two groups. AMH levels were age related, with values generally higher at younger ages, and decreasing with age. There was a wide range of AMH values observed within the reference intervals, especially in the younger groups.

Conclusion: This is the first report of AMH reference intervals using the Access 2 Immunoassay Analyzer. Results are consistent with published data and support that AMH concentrations in women generally decrease with age but with a wide range of values within the same age group.

*Not intended as off-label promotion of any Beckman Coulter Inc., product

**Access AMH is not available in the U.S.

		95th PercentileReference Interval	90% Confidence Interval of AMH (ng/mL)	
Group (years)	**Sample Size**	**AMH ng/mL**	**Lower 2.5%**	**Upper 97.5%**
18-30	229	**0.92 - 12.87**	0.77 - 1.09	11.61 - 14.07
31-35	109	**0.33 - 10.11**	0.22 - 0.51	8.84 - 11.23
36-40	119	**0.16 - 6.57**	0.11 - 0.23	5.65 - 7.46
41-45	107	**0.00 - 3.21**	0.00 - 0.01	2.51 - 4.04
≥46	48	**0.00 - 1.98**	Not calculated	Not calculated

A-224

Impact of alcohol consumption before thyroid evaluation

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Background: Patient related variables, such as physical exercise, stress and fasting status are important sources of variability in laboratory testing. However, clear instructions regarding alcohol consumption before thyroid evaluation is presently neglected by laboratory. This study aims to evaluate the impact of alcohol consumption on thyroid evaluation.

Methods: We studied 12 healthy volunteers at PNCQ (Brazilian National Program of Quality Control). A first blood sample was collected after an overnight fast (12 hours). Immediately after blood collection, the volunteers drank 50 mL (40% alcohol/volume) of Blended Scotch Whisky (Red Label, Johnnie Walker, Edinburgh, Scotland). Subsequent blood samples were collected at 1, 2, 4 and 6 hours after drank the Blended Scotch Whisky. Each phase of sample collection was carefully standardized, including the use of needles and vacuum tubes from the same lot. All thyroid evaluation was performed on the same analytical platform. The instrument was calibrated against appropriate proprietary reference standard material and verified with third-party control material (independent from calibrator material). Differences between samples were assessed by Wilcoxon ranked-pairs test. The level of statistical significance was set at $P < 0.05$.

Results: Main results are showed in Table 1.

Table 1. Impact of alcohol consumption on thyroid evaluation

Parameter	Basal	1h	2h	4h	6h
TSH µIU/mL	1.91 [1.32-2.26]	1.51 [1.08-2.12] P=0.071	1.36 [1.14-1.88] P=0.016	1.44 [1.26-1.68] P=0.005	1.59 [1.38-1.86] P=0.077
FT4 ng/dL	0.89 [0.79-0.96]	0.87 [0.82-0.98] P=0.102	0.90 [0.81-0.93] P=0.755	0.88 [0.81-0.97] P=0.307	0.89 [0.84-0.99] P=0.109
FT3 pg/mL	3.58 [3.38-4.09]	3.54 [3.29-4.19] P=0.286	3.56 [3.34-4.07] P=0.272	3.60 [3.47-4.17] P=0.875	3.77 [3.43-4.22] P=0.021

TSH, human thyroid-stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine;

Values expressed as median [interquartile range].
Bold P values represents significance by Wilcoxon ranked -pairs test.

Conclusion: Alcohol consumption could jeopardize thyroid evaluation. Laboratory professionals should inform their customers to avoid alcohol consumption six hours (as minimal) before thyroid evaluation.

A-225

Correlation and Performance of the SHBG, SDHEA and HCG assays between Chemiluminescence and Electrochemiluminescence Platforms

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Background: In reproductive assessment, monitoring of steroids are of uttermost importance to evaluate normalization of reproductive function. There are basic tests required for each procedure. To ensure the accuracy of the released results, it is important to ensure the correlation, precision and linearity of the tests used. This study

aims to compare the analytical performance of the Sex hormone-binding globulin (SHBG) between ADVIA Centaur XP® and IMMULITE 2000® (Siemens Healthcare Diagnostics) platforms, Dehydroepiandrosterone sulfate (SDHEA) and Human Chorionic Gonadotropin (HCG) between Cobas® E- 170 (Roche) and ADVIA Centaur XP, aiming the analytical validation of these assays in ADVIA® Centaur XP platform in a large laboratory in Barueri, Brazil.

Materials and Methods: Samples showing concentrations whitin linearity range for each assay were selected from laboratory routine. SHBG samples were tested in IMMULITE 2000® and ADVIA Centaur XP; DHEAS and HCG samples were tested on ADVIA Centaur XP and Cobas E- 170 (which uses the chemiluminescence and electrochemiluminescence methods, respectively). In order to test accuracy, linearity and dilution recovery, high and low analite concentration pools were used.

Results: For SHBG, the following data were obtained: Correlation, n = 82 (y = 1.365x + 0.947, R^2 = 0.973). Intra-assay precision CV = 4.48% and 4.88%, inter-assay CV% = 4.66% and 3.1% at concentrations of 85 and 160 nmol/L, respectively. Linearity (0,999x y = - 1.012, R^2 = 0.999). Dilution Recovery (1/2) = 13% (ETa = 20.42%) or 86.95% at a concentration of 154 nmol/L. For DHEAS the following data were obtained: Correlation, n = 91 (y = 0.865x + 0.051, R^2 = 0.979). Intra-assay precision CV = 5.08% and 4.65%, inter-assay CV% = 3.23%

and 3.87% at concentrations of 85 and 590 ug/dL, respectively. Linearity (y + 1x = 1.995, R^2 = 0.999). For HCG the following data were obtained: Correlation, n = 46 (y = 26.929 + 1.299x; R^2 = 0.995). The correlation results for the three tests showed that no divergences between methods were observed. Linearity test confirmed the values mentioned for the ADVIA Centaur IFU assays: SHBG (1.6 to 180 nmol/L) and DHEAS (3 to 1500 ug/dL).

Conclusion: Results obtained from correlation studies for SHBG comparing IMMULITE 2000 and ADVIA Centaur XP platforms, DHEAS and HCG assays on platforms ADVIA Centaur XP and Cobas E-170 shows statistical and clinical equivalence, corroborated by the analysis of the laboratory's physicians.

A-226

Vitamin D Trends in a South Central Wisconsin Healthcare Network: 2013 - 2015

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Background: Vitamin D testing has been consistently increasing over the previous 10 years. For many labs, the increase in volume justifies testing in-house. LC-MS-MS methods offer the advantage of accurately quantifying both the 25-hydroxy (OH) D2 and D3 levels, while antibody targeted methods such as those found on high throughput immunochemistry platforms do not delineate these two forms but rather provide a total 25(OH) level. To determine what the clinical impact would be when switching from our reference lab method (LC-MS-MS), patient data was collected during 2009 to evaluate D2, D3, and total vitamin D levels. These data helped influence a decision to bring the testing in-house. Patient data collection continued more recently to assess physician ordering and patient result trends.

Methods: An ad hoc report was generated for data analysis using Sunquest v. 7.1 and Ad Hoc Report Writer™. These data were originally collected in 2009. Yearly data collection continued through 2015. Data was processed for trends related to seasons, and frequency of result extremes indicating deficiency or possible toxicity. 25(OH) Total Vitamin D testing was performed on the Abbott ARCHITECT ci8200 system.

Results: Reference lab results indicated 86.7% of the patients tested in 2009, had undetectable vitamin D2 levels.

	Low-Norm-High		Possible Toxicity		Severely Deficient		Summary Statistics	
Season/Year	N	Percent-age	N	Percent-age	N	Percent-age	Mean	S.D.
WINTER/2013	2057	92.6%	49	2.2%	114	5.1%	36.27	18.009
SPRING/2014	2695	91.2%	104	3.5%	156	5.2%	39.30	20.051
SUMMER/2014	2574	90.0%	216	7.5%	67	2.3%	44.17	21.389
FALL/2014	2878	89.9%	153	4.7%	170	5.3%	41.14	20.465
WINTER/2014	2526	86.0%	114	3.8%	296	10.0%	37.39	20.717
SPRING/2015	3002	85.8%	192	5.4%	303	8.6%	39.13	21.720
SUMMER/2015	2906	92.4%	148	4.7%	91	2.8%	41.39	20.103

Conclusion: Immunoassay testing for Vitamin D has been scrutinized for its ability to measure 100% of 25(OH) D2 effectively, but the clinical implications of this are less clear. An ad hoc report of patients from southcentral Wisconsin, suggests that most patients do not have significant 25(OH) D2 levels, and that assays that can recover >90% of 25(OH) D2 should not misclassify patients clinically. Although there are ongoing discussions about how to define healthy or toxic vitamin D levels, this study suggests that the majority of patients are maintaining adequate vitamin D levels throughout the seasons.

A-227

Serum testosterone levels in statin therapy with diabetes and hypertension.

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Background: Hypogonadism in men is one of the conditions that need therapeutic attention and intervention. It is reported to be associated with aging, diabetes and statin therapy. Obesity, diabetes and statin therapy are common in Libyan subjects. There are few studies undertaken to study the prevalence, association with diabetes and statin therapy in Libyan subjects. Therefore the present study was carried out to evaluate and measure serum testosterone levels. Subjects on statin therapy were grouped further into two categories - those who are undergoing statin therapy less than one year and those more than one year. **Materials and Methods:** Libyan subjects who attended the Medicine Department, Faculty of Medicine, Benghazi University, Libya from 2013-2014 were screened and 160 subjects were taken for the study. Controls, Patients with high cholesterol on statin therapy,Diabetics,Diabetes on statin therapy for 1 year, Diabetes on statin therapy for more than one year, Diabetic hypertensives, Diabetic hypertensives on statin therapy for 1 year and Diabetic hypertensives on statin therapy for more than year .The age group of the cases studied were 45±8.5 years. Cases who had serum LDLcholesterol > 190mg/dL were included as hypercholesterolemic subjects. They were given statin therapy (atorvastatin,40mg/day). Diabetic patients included in the study were either diagnosed using the following criteria or those who are already under medications. **Serum total testosterone** was measured by Enzyme immune assay. (Testosterone enzyme immunoassay test kit Catalog Number: 1115, Oxis International, Inc 323 Vintage Park Dr. Foster City, CA 944040) Intra assay and inter-assay precision were 6.5% to 4.5% respectively. **Results:** The serum testosterone level was comparatively lower in patents on statin therapy compared to the controls ($p<0.05$). There was no correlation between serum testosterone level with age, weight and waist circumference. The serum testosterone levels were lower in diabetic patients compared to the controls ($p<0.05$). The serum testosterone level is comparatively lower in diabetic patients on statin therapy for less than year ($p<0.010$). The diabetic patients on statin therapy for more than a year showed marked reduction in Serum testosterone levels ($p<0.001$). **Conclusion:** There was significant reduction in serum testosterone levels in patients treated with statin therapy. The levels of serum testosterone were significantly reduced compared to control subjects. There was marked fall in serum testosterone levels in diabetic subjects which was further reduced by statin therapy. The presence of hypertension did not elicit greater fall in serum testosterone level in hypertensives or diabetics with hypertension.

A-228

Evaluation of Access TSH (3rd IS) Assay with Comparison to Multiple Platforms

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Objective: Standardization of TSH assays has been a concern for several years. Beckman Coulter has developed a new Access TSH (3rd IS) assay*. Method comparison was performed with the new Access TSH (3rd IS) assay and five commercially available TSH assays.

Method: 156 frozen serum samples with TSH values between 0.011 and 41.71µIU/mL were tested using Beckman Coulter Access TSH (3rd IS), Access HYPERsensitive hTSH and Access Fast hTSH, Roche cobas® e602 TSH, Siemens ADVIA Centaur® XP TSH3-Ultra, and Abbott ARCHITECT TSH assays. Aliquots were tested in singleton by two clinical laboratories. Method comparisons were performed following CLSI EP09-A3 guidelines. Passing-Bablok regression analysis was conducted using

Access TSH (3rd IS) as the comparison method. Two-sided 95% confidence intervals were reported on slopes, intercepts and predicted biases at clinically relevant TSH concentrations of 0.4 and 4 µIU/mL.

Results: Method Comparison results presented in Table 1.

Conclusion: Method comparison biases varied significantly between TSH assays and manufacturers. This is consistent with literature and supports efforts to harmonize TSH amongst manufacturers.

*Access TSH (3rd IS) assay was, as of the date of abstract submission, *i.e.,* Feb 15th, 2016, CE marked and pending FDA approval.

** Not intended as off-label promotion of any BCI product.

*** All trademarks are the property of their respective owners.

Access TSH (3rd IS)					
Method	**Slope [95% CI]**	**Intercept [95% CI] (µIU/mL)**	***r***	**Predicted bias at 0.4 µIU/mL**	**Predicted bias at 4.0 µIU/mL**
Access hTSH	0.97 [0.94 – 0.99]	-0.04 [-0.09 – (-0.02)]	0.97	-0.05 (13.6%) [-0.10 – (-0.03)]	-0.17 (4.2%) [-0.30 – (-0.08)]
Access Fast hTSH	1.03 [1.01 – 1.06]	-0.04 [-0.07 – (-0.02)]	0.98	-0.02 (6.2%) [-0.05 – 0.00]	0.08 (2.0%) [0.00 – 0.19]
Cobas TSH	0.89 [0.86 – 0.91]	-0.04 [-0.06 – (-0.02)]	0.99	-0.08 (20.9%) [-0.11 – (-0.06)]	-0.48 (12.0%) [-0.58 – (-0.39)]
Centaur TSH	0.95 [0.91 – 0.99]	-0.02 [-0.07 – 0.00]	0.99	-0.04 (10.0%) [-0.09 – (-0.01)]	-0.20 (5.1%) [-0.39 – (-0.08)]
ARCHITECT TSH	1.19 [1.17 – 1.21]	-0.04 [-0.06 – (-0.03)]	0.99	0.04 (9.7%) [0.02 – 0.05]	0.73 (18.3%) [0.65 – 0.81]

A-229

Impact of metformin in Thyroid Stimulating Hormone (TSH) in elderly persons with diabetes mellitus

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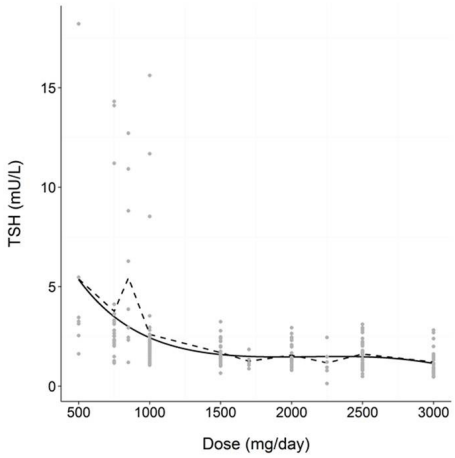
Background: Studies have reported higher prevalence of hypothyroidism in persons with diabetes mellitus (DM). Metformin (MTF), the most widely used drug to treat DM, reportedly interfere with TSH levels. The aim of this study was to evaluate the influence of MTF on serum TSH. Methods: 633 elderly were studied, 224 with DM in use of MTF, (MTF patients), compared to 409 non-DM (NDM) with undiagnosed hypothydoidism without levothyroxine use. A statistical model was used providing estimated values for TSH according to different MTF doses using the class of generalized linear models (GLM). The analysis were performed using R software (R Core Team, 2014). Results: Females were 125 MTF patients and 233 NDM, aged 70.4±7.4 and 69.9±7.6, respectively. In MTF patients fewer cases of hypothyroidism were observed (4.5%) *vs* NDM (5.1%), p = 0.0432. TSH was different between MTF patients and NDM in euthyroid patients: 2.8 mU/L *vs* 3.2 mU/L, (p = 0.0032), and also in hypothyroid: 11.8 mU/L *vs* 15.6 mU/L (p= 0.0046). With regard the relationship between TSH levels and MTF dose the selected model assumes an inverse distribution for the square root of TSH in the random component of the GLM (Figure). Discussions and Conclusions: DM patients who were administered MTF had significantly lower TSH compared to NDM, possible due to its direct action in pituitary/hypothalamus, suppressing AMP-activated protein kinase activity (AMPK). We observed an inverse correlation between doses of metformin and TSH, which could explain different results observed in different series. The overall conclusion is that there was a significant reduction of TSH as the metformin dosage increased. At this point, these results cannot be considered definitive, but are preliminary data that offer new perspectives regarding the relationship between metformin and TSH, requiring confirmation with a larger number of cases.



A-230

Evaluation of the Testosterone II Assay* on the ADVIA Centaur System

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Background: Testosterone (4 androsten 17ß-ol-3-one) is a C19 steroid hormone with a molecular weight of 288.4 daltons. Testosterone is the major androgen in males and is controlled by luteinizing hormone (LH). LH is released from the anterior pituitary exerting the primary control on testosterone production and acting directly on the Leydig cells in the testes. Testosterone stimulates adult maturation of external genitalia and secondary sex organs, and the growth of beard, axillary, and pubic hair. Siemens has developed an improved testosterone assay with acceptable sensitivity and precision to be able to measure both adults and pediatric males and females. This is an 18-minute competitive immunoassay with an assay range of 7.0 to 1500 ng/dL. The assay is aligned to the CDC HoSt standardization program.

Method: The alignment to the CDC HoST was achieved by running 40 serum samples with ID-LC/MS/MS Testosterone RMP assigned values on 3 lots of ADVIA Centaur® Testosterone II reagents and calibrators. Ten (10) standards were value assigned by fitting the raw (RLU relative light units) data obtained from the ADVIA Centaur instrument independently for each lot. The method comparison equation closest to an r value equal to 1.0 was selected. The equation was solved for the RLU associated with the 10 standards. The assay's performance was assessed by measuring 3 reagent lots for imprecision and functional sensitivity which were evaluated by assaying control materials and serum pools twice a day for 20 days, for a total of 80 replicates. A method comparison to the CDC HoSt Testosterone reference method was assessed using 1 lot of reagent and 128 serum samples. Reference ranges were also generated for adult and pediatric males and females.

Results: The data obtained with the ADVIA Centaur Testosterone II assay demonstrated good correlation to the ID LC/MS/MS Testosterone RMP yielding a Passing-Bablok slope of 0.97, intercept of + 1.94 ng/dL, and regression coefficient of 0.98. A 20 day precision study yielded a within lab precision CV's of between 3.9% and 8.4% for the 3 lots using samples between ~25.0 ng/dL to ~1120.0 ng/dL of testosterone for the assay. The functional sensitivity for all 3 lots was < 7.00 ng/dL. Reference ranges (median and 95% confidence intervals) for both male and female adults and pediatrics (ages and Tanner stages) have been generated.

Conclusion: The Siemens ADVIA Centaur Testosterone II assay alignment with the CDC HoSt should be a valuable tool in clinical laboratories for the accurate measurement of testosterone in human sera.

* Under FDA review. Not available for sale. Product availability will vary by country.

A-231

Population study of *TSH Variant* in Brazilian population

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Background: The Thyroid Stimulating Hormone (TSH) is a hormone formed by two chains: alpha and beta polypeptide. Released by the pituitary through hypothalamic TRH action TSH interacts with a specific cell receptors in the surface of the thyroid cell applying two main functions: To stimulate cells and hypertrophy reproduction, and to stimulate thyroid gland on synthesizing and secreting T3 and T4. Thus, TSH becomes the main regulator of thyroid function and best indicator of discrete changes in the production of thyroid hormones. First generation assays allow the diagnosis of hypothyroidism, second and third generation assays increase the diagnostic certainty and it is possible to be used also for the detection of hyperthyroidism since it has a sensitivity and specificity of 96% and 93% respectively. A study in California with 1.61 million patients identified a rare variant of TSH in which the monoclonal antibodies used in the 3rd generation tests failed and resulted in a falsely low value, this variant was found in a small percentage of the population with descendants of South Asia. This study aims to investigate the presence of this variant of TSH in the Brazilian population.

Materials and Methods: A total of 329.175 samples were tested according to the manufacturer's instructions for the TSH3-UL assay (Siemens Healthcare Diagnostics) in ADVIA Centaur System (Siemens Healthcare Diagnostics). Within those samples, 1508 had TSH <0.01 U/L of which 704 were evaluated. 804 samples were not evaluated because 793 had diagnosis of hyperthyroidism (decreased TSH and increased free T4) and 11 diagnosis of secondary hypothyroidism (decreased TSH and free T4).

Results and Conclusion: The 704 samples evaluated were measured in TSH-2 assay (Siemens Healthcare Diagnostics, 0:35 to 5:50 ref U/L) and TSH3-UL (Siemens Healthcare Diagnostics, ref 0:55 - 4.78 UIL), all presented results <0.01 showing complete agreement between the methods. This study shows that TSH variant was not found in the studied population. In addition, the California study showed that the variant was found in people of South Asian descendants, and according to data from IBGE 2010 Brazil does not have immigrants of this region but from East Asia, suggesting the potential non-existence of carriers of this mutation in the Brazilian population.

A-232

Investigtation of posture specific reference intervals for plasma metanephrine and normetanephrine

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Introduction: Metanephrines, the metabolites of catecholamines, are tested in the investigation and workup of pheochromocytoma and paraganglioma (PPGL). In the majority of cases of PPGL, plasma metanephrine (PM) and/or normetanephrine (PNM) are elevated at least 2 times the upper limit of normal. Like many other tests, PM and PNM are affected by many preanalytical variables such as diet, drugs, exercise and stress that may result in a slightly elevated result in the absence of PPGL. Also, several studies have recently shown that collection posture is a significant preanalytical interference as patients without PPGL have lower PM and PNM if they are supine for 30 min prior to phlebotomy. The 2014 PPGL guidelines from the American Endocrine Society recommend supine collection for plasma metanephrines to reduce the number of false positive results. However, most laboratories do not require supine collection for plasma metanephrines and do not provide a posture specific reference range. In this study, we investigated our laboratory population to determine the performance of our current reference intervals with and without collection posture specifications.

Materials & Methods: All PM and PNM results between May 2010 and September 2015 were requested from our laboratory information system. Patient information was deidentified and the final data set obtained included the numerical metanephrines result, order date, and collection location. Data was analyzed using Microsoft Excel 2007 into histograms displaying the distribution of patient results and was compared to the current reference intervals of <0.5 nmol/L for metanephrine and <0.9 nmol/L for normetanephrine.

Results: The data pull resulted in 5452 plasma metanephrines results from 5068 patients. All specimens were assumed to be collected in the seated position (as indicated in our SOP), with the exception of samples collected at our local endocrinology testing unit which is known to collect after 30 min in the supine position. This subgroup consisted of 313 specimens on 269 patients and was analyzed separately. No effect of posture was observed between PM collected in the supine and seated position; epinephrine, the precursor for PM, is an adrenal catecholamine and should not change with posture. However, the distribution of PNM collected in the seated position had a large tail that extended over the upper limit of normal (0.9 ng/mL) , resulting in 14% of all patients between 0.9 and 1.8 nmol/L. Only 7% of patients collected in the supine position fell into this range, which shows the clear effect of posture. Norepinephrine, the precursor for PNM, is secreted from both the CNS and is expected to change with posture.

Conclusion: Collection of plasma metanephrines in the seated position can lead to a significant number of slightly elevated PNM results in patients who do not have PPGL. Investigation of posture specific reference ranges should be considered.

A-233

Validity of Free and Bioavailable Testosterone Calculations Using Abbott Architect Total Testosterone and Sex Hormone Binding Globulin Immunoassay Results

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Objective: Comparison of directly-measured and calculated free (fT) and bioavailable testosterone (BioT) concentrations.

Background: In the diagnosis of testosterone deficiency in men and excess in women, clinicians often rely upon calculated fT and bioT derived from web-calculators. The accuracy of such calculations should be formally validated for the specific immunoassays employed.

Methodology: Sera from thirty seven patients were used, including a mixture of men and women. Measurement of fT and bioT was performed using tandem mass spectrometry after equilibrium dialysis or ammonium sulfate precipitation, respectively. Abbott Architect total testosterone (T) and sex hormone binding globulin (SHBG) immunoassay results were used in fT and BioT calculations applying the Vermeulen formulae with measured (bromcreosol green and purple) or fixed (4.3 g/dL) albumin concentrations. The Abbott SHBG assay was recalibrated after completion of our measurements, so fT and BioT were calculated using both SHBG results with the former calibration and after numeric adjustment for the new calibration: SHBG (recalibrated) = 0.94 *SHBG (old calibration) - 0.2

Results:

Concentration span: T (LC/MS) 7 - 1287 ng/dL; SHBG (Abbott) 18 - 89 nmol/L; fT (LC/MS) 0.5 - 284 pg/mL; BioT (LC/MS) 1 - 952 ng/dL.

Correlation studies:

T (Abbott) = 1.14 * T (LC/MS) -22.6; R2 = 0.989

fT (Calc) = 1.53 * fT (LC/MS) + 0.58 ; R2 = 0.952 [old SHBG calibration; Alb = 4.3 g/dL]

fT (Calc) = 1.30 * fT (LC/MS) + 0.25; R2 = 0.981 [SHBG recalibration; Alb = 4.3 g/dL]

BioT(Calc) = 1.11 * BioT (LC/MS) + 42.3; R2 = 0.932 [old SHBG calibration; Alb = 4.3 g/dL]

BioT(Calc) = 1.14 * BioT (LC/MS) + 47.2; R2 = 0.924 [SHBG recalibration; Alb = 4.3 g/dL]

Calculated fT and BioT with fixed or BCP or BCG measured albumin did not differ significantly.

Qualitatively, there was significant dispersion among BioT results in the crucial range of 150 - 450 ng/dL with or without SHBG recalibration.

Conclusions:

1. fT results calculated from Abbott Architect T and SHBG results and the Vermeulen formula with fixed albumin concentration correlate well, but do not correspond to directly measured results. Transformed reference ranges should be transferrable.

2. SHBG recalibration has improved the fT correlation

3. BioT results calculated from Abbott Architect T and SHBG results and the Vermeulen formula did not correlate well across the typical clinical decision range.

4. SHBG recalibration did not improve the BioT correlation.

5. Use of measured rather than fixed albumin concentration did not significantly alter the fT or BioT correlation.

A-235

A Sudden Manufacturer Discontinuation Makes Free Testosterone Testing Quite Testy

A. J. McShane, R. Kreller, M. Strizzi, S. Wang. *Cleveland Clinic, Cleveland, OH*

Introduction: Testosterone is a hormone that stimulates the development of male genital and secondary sex characteristics. In women, it has a role as an estrogen precursor. Elevated testosterone can lead to premature puberty in males or virilization in females. Conversely, decreased testosterone may lead to hypogonadism in males and decreased libido in females. Testosterone is primarily found tightly bound to sex hormone binding globulin (SHBG) and loosely bound to albumin in blood. Measurement of the free fraction is indicated in patients with abnormal SHBG. It can be obtained via equilibrium dialysis or ultrafiltration. In our laboratory, radioactive (tritium-labeled) testosterone is added to the patient's serum. After equilibration, the sample is filtered through a low molecular weight cut-off filter which allows the free testosterone to pass through. The activity of the retentate and filtrate is then measured to determine the ratio of free over total testosterone. This is then multiplied by the total testosterone to achieve the free testosterone levels. Our commercially purchased, tritiated testosterone is purified via column chromatography before use to remove potential radioactive impurities. However, the commercial provider of the column is discontinuing its production. Three alternatives to the current chromatography were purposed for this high-volume test: in-house column assembly, a new column manufacturer, and forgoing column purification. The first option was abandoned because of potential differences in column-to-column performance, without strict quality control parameters. The latter options were evaluated to determine an acceptable replacement for the discontinued columns. **Methods**: Twenty-six patients were compared between the current and potential columns. An additional 26 patients were compared between the current columns and a column-less sample preparation. We further compared the column-less approach with the current columns using 100 additional samples (50 males and 50 females). **Results**: In the current versus potential column study, Deming regression of the free testosterone levels revealed a slope of 0.926, a correlation coefficient of 0.9929, and a mean percent bias of -6.19%. The column-less comparison gave a slope of 1.405, a correlation coefficient of 0.9772, and a free testosterone mean bias of +33.76% affirming that a freely-filtered, radioactive impurity exists in our commercial radioactive testosterone. The regression formula from the current column versus column-less methodologies was used to correct this bias, and applied to the 100 sample study. The Deming regression comparison of the 100 patients, after the correction, gave a slope of 0.914, a correlation coefficient of 0.9872, and a mean percent bias of -4.27%. **Conclusion**: A new column-less technique gave similar results (bias <5%) for free testosterone levels, to the discontinued commercial columns. The column-less technique requires a correction; however, has the added benefit of requiring less radioactive material and significantly reducing technician time.

A-236

Determination of Reference Intervals in Apparently Healthy Pediatric Subjects for IMMULITE Thyroid and ADVIA Centaur TSH3UL Assays

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Background: Establishing age-specific reference intervals for thyroid hormones improves interpretation of laboratory measurements and facilitates diagnosis of endocrine diseases in pediatric practice. A challenge for establishing pediatric reference intervals has been the availability of samples from well-characterized healthy pediatric subjects. This study used methodology consistent with CLSI guidelines to pedigree and collect samples from apparently healthy pediatric subjects presenting for regular well-child care. It then determined reference intervals for IMMULITE® Thyroid and ADVIA Centaur® TSH3UL assays from Siemens Healthcare Diagnostics.

Methods: Eight U.S. 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 assessed by pediatricians and determined to be free of chronic and acute diseases, were not on medication, had no family history of thyroid dysfunction and no visible or palpable goiters, and were negative for anti-thyroglobulin anti-thyroid peroxidase antibodies. Three age strata were analyzed, each with approximately equal numbers of males and females. Samples were tested at a central laboratory in singleton using the IMMULITE 2000 and ADVIA Centaur Immunoassay Systems. For the two older subgroups, the respective lower and upper reference limits were defined as the 2.5th and 97.5th percentiles of test results. For the infant subgroup, a robust method (Horn and Pesce) was used to calculate the reference intervals.

Results:

System	Assay[a]	Infants (1-23 months)		Children (2-12 years)		Adolescents (13-20 years)	
		2.5-97.5th	n	2.5-97.5th	n	2.5-97.5th	n
IMMULITE	TSH 3G	0.83-6.5[b]	90	0.58-4.1	195	0.39-4.0	148
IMMULITE	FT3	3.6-7.5	90	3.7-6.6	195	3.1-5.9	148
IMMULITE	FT4	0.80-1.27	90	0.74-1.28	195	0.75-1.27	148
IMMULITE	T3	116-241	90	109-206	195	93-170	148
IMMULITE	T4	6.2-11.8	90	5.4-11.1	195	4.9-10.2	148
ADVIA Centaur	TSH3UL	0.87-6.15[b]	94	0.67-4.16	198	0.48-4.17	150

[a]Units: TSH 3G—µIU/mL; FT3—pg/mL; FT4—ng/dL; T3—ng/dL; T4—µg/dL; TSH3UL—µIU/mL. [b]Infant group Upper Reference Limit 90% CI is 5.58 to 7.65 µIU/mL for IMMULITE TSH 3G and 5.32 to 6.98 µIU/mL for ADVIA Centaur TSH3UL.

Conclusion: Pediatric reference intervals were established for the IMMULITE Thyroid and ADVIA Centaur TSH3UL assays using rigorously pedigreed samples. These data will assist with the appropriate interpretation of thyroid measurements in pediatric patients.

*Pediatric reference intervals for IMMULITE FT4 and TSH 3G assays are under FDA review. Product availability varies by country.

A-237

Establishing a clinical cutoff for aldosterone-direct renin concentration ratio using retrospective data

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Objective: To characterize aldosterone-direct renin concentration ratio (DRC-ARR) ranges appropriate for evaluation of primary aldosteronism using population data for validated plasma renin activity ARR (PRA-ARR) as a benchmark. **Relevance:** The ARR is the standard screening test for primary aldosteronism. Past methods used to derive the ARR mainly employed the PRA, but with increased workloads and automation capabilities, the DRC has become more widespread. However, studies focusing on appropriate ARR cutoffs for primary aldosteronism using DRC are lacking. **Methodology:** Data was obtained from Calgary Laboratory Services from January, 2010 to December, 2015. The renin method changed from PRA to DRC in February, 2014, thus allowing analysis of data for both methods within a single population. Indirect comparison of the performance of DRC-based ARR and PRA thresholds was assessed using ROC curve analysis analyses using PRA-ARR values as the gold standard. Renin levels from each method were also evaluated for classification of "low renin" status. **Results:** Results from 5864 patients were obtained (associated with 6074 PRA-ARRs and 1405 DRC-ARRs). The Canadian Hypertension Education Program PRA-ARR threshold of >550 pmol/L/ng/mL/h showed a prevalence of PA of 37.2%. Within the elevated PRA-ARR patients, "low renin" was defined as the 95th percentile of the renin values obtained (<2.05ng/mL/h). Using a DRC-ARR value of >25 pmol/LmIU/L the prevalence was 38.9% and "low renin" was <28.0 mIU/L. Employing these low renin values for prediction of elevated ARR demonstrated sensitivities and specificities of 95% and 49% for PRA <2.05 ng/mL/h and 95% and 56% for DRC <28.0 mIU/L. **Conclusions:** A DRC-ARR threshold of >25 pmol/L/mIU/L performed with similar high sensitivity to the conventional PRA-ARR threshold of >550 pmol/L/ng/mL/h for PA. Additionally, DRC levels <28.0 mIU/L should be considered compatible with low-renin status in a hypertension population. Retrospective assessment of laboratory data is a useful tool to establish reference intervals and cutoff for clinical management of patients.

A-238

Frequency of Pseudohyper- and Pseudohypo-calcemia in an Emergency Department Setting at a large Tertiary Care Hospital

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Introduction: The total serum/plasma calcium (Ca) concentration consists of three fractions with approximately 15% bound to organic & inorganic anions, 40% bound to albumin and 45% containing the physiologically active "ionized calcium" or more accurately the free & unbound calcium (CaIO). Total calcium (Ca) is part of the basic metabolic panel and is most often used as the primary indicator of calcium status because the assay is easily automated, rapid & cost effective. However, the CaIO (free or ionized calcium) concentration is considered to be a more accurate indicator of calcium status since it is the physiologically active fraction & is tightly regulated by parathyroid hormone & vitamin D. The concordance between total & ionized calcium measurements is moderate in healthy subjects and is poor in patients with severe acid-base disorders, hyperparathyroidism, hyperphosphatemia, or chronic kidney disease. Total calcium concentrations can change independently of ionized calcium concentrations and vice versa. If the Ca concentration is elevated in the setting of a normal CaIO, it is termed pseudohypercalcemia., and if Ca is low with a normal CaIO, it is termed pseudohypocalcemia.

Objective / Methods: This study was designed to determine the frequency of pseudohyper- and pseudohypo-calcemia and to calculate concordance of Ca & CaIO measurements from patients in an Emergency Department setting. Ca measurements were performed on the Roche Modular P800 system using 0-cresol-phthalein complexone and CaIO measurements performed on the Radiometer 827 blood gas system using ISE (ion selective electrode) methodology.

Results/Discussion: A computer search of the data base from the E. D. of a large tertiary care hospital in Long Island, NY identified 334 patients with paired Ca & CaIO results from specimens drawn within 1 hour on each patient. No clinical history or other lab results were considered for this study. Based on the Ca concentration (reference range 8.4 - 10.5 mg/dL), 7 out of 334 patients (2.1%) were classified hypercalcemic, 307 (91.9%) normocalcemic and 20 (6.0%) hypocalcemic. In the hypercalcemic group, 2 of 7 patients had normal CaIO results (reference range 1.12 - 1.30 mmol/L) and were classified as pseudohypercalcemic (0.6% of total group / 28.6% of hypercalcemic group). In the hypocalcemic group 14 of 20 patients had normal CaIO results and were classified as pseudohypocalcemic (4.2% of total group / 70% of hypocalcemic group). Additionally, in the 307 patients classified as normocalcemic by Ca, 36 had abnormal CaIO concentrations (29 low & 7 high CaIO) showing an 88.3% concordance between Ca & CaIO.

Conclusion: The frequency of pseudohypercalcemia is very low in the whole group (0.6%) but was 28.6% for the hypercalcemic group. The frequency of pseudohypocalcemia was higher in the whole group (4.2%) and much higher (70%) in the hypocalcemic group. Concordance of Ca & CaIO in the normocalcemic group was 88.3%. These results indicate that ionized calcium measurements should be considered, especially in hypo- and hyper-calcemia classified by Ca concentrations.

Tuesday, August 2, 2016

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

Factors Affecting Test Results

A-239

Nonlinearity of assay results within the presumed linear primary measurement range: examples from analysis of CAP linearity survey data

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Background: We recently described nonlinearity within the primary measurement range (PRM, undiluted specimen measurement range) of a lipase assay as the cause of inaccuracy in lipase results among samples undergoing automated repeat-on-dilution [PMID: 26474511]. Nonlinearity of this assay was also evident in interlaboratory data reported in the CAP linearity survey by the changing slope (Δresult/Δdilution) across sample dilution series results. As a follow-up to these observations, our objective in this study was to examine CAP linearity survey results for other assays in use at our laboratory (Roche Cobas c500 assays) to determine whether nonlinearity in the primary measurement range could be a factor affecting overall accuracy of assay results. Methods: Primary data were from the CAP June 2015 survey, LN2/LN2VB-A, Chemistry/Lipid/Enzyme Calibration Verification/Linearity, for examination of interlaboratory results for serial sample dilution measurements. Candidate assays for evaluation were those displaying a characteristic pattern in the bias plot for samples within the PRM, in which there was a concentration-dependent, non-random bias of sample dilution series results forming a smooth, inverted "U" shape relative to the central, zero-bias line (the line based on the assumption of linearity of results). Dilution series results data (y) within the PRM were then evaluated according to curve fitting of the form $y = F(x) = A(1\text{-}\exp(\text{-}x/x0))$, where x was the dilution factor, and A and $x0$ were fitted constants. Such curves $F(x)$ show a progressive variation in slope; assays were considered to be nonlinear if $F(x)$ was a better fit to y than that of linear correlation, and if the slopes ($F'(x)$) between limits of the PRM differed by more than 15%. Results: Three Roche Cobas c500 assays (albumin, creatinine, lactate dehydrogenase) were found to be nonlinear by the above criteria. Changes in slope across the PRM were -21% (albumin), -41% (creatinine), and -18% (lactate dehydrogenase). Revised bias plots based on $F(x)$ eliminated the inverted "U" pattern and its associated residuals. Despite curvature, however, linear correlation coefficients for dilution series data within the PRM were all high ($r > 0.99$), and all biases of data within the PRM were well within CAP-defined acceptable limits when linearity was assumed. However, in the CAP survey, each assay showed a disjunction in bias of results when comparing those for samples from within the PRM to those for samples from outside of the PRM (viz., from samples secondarily measured by automated repeat-on-dilution). Conclusions: Certain assays, for which calibrations are based on a linearization of the PRM response vs. concentration curve, exhibit instead functional non-linear characteristics within the PRM. The assumption of linearity leads to an inherent, predictable pattern in bias plots. Bias could be eliminated were calibration based instead on a nonlinear curve. Biases in results for these assays due to the assumption of linearity within the PRM are unlikely to be clinically significant, however. Nonetheless, as a technical note, laboratory directors should be aware that nonlinearity within the PRM for these assays can cause a disjunction of sample dilution series results across the upper limit of the PRM, as observed in CAP linearity surveys.

A-240

Effect of Intravenous Immunoglobulin on Hepatitis B Serology Testing

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Background: Intravenous immunoglobulin (Ig) is used in a variety of conditions, including immunodeficiency, ITP and Kawasaki disease. With a half-life of 40 days, its in vitro effect on serology tests may be overlooked, leading to false positive results and misdiagnosis. This study examined the effect of 3 different pharmacological concentrations of Intragam P (Singapore) on anti-HBe, anti-HBs, HBeAg, HBsAg, anti-HBc-IgM and anti-HBc-total assay results on 2 different immunoassay systems (Roche e601 and Abbott Architect). **Methods:** Three different concentrations of Intragam P (stock concentration 60 g/L) were prepared in saline to simulate the range of 0.6-2 g/kg used in clinical practice, and IgG were measured (Beckman Coulter DxC-800). Aliquots were then analysed for the following assays: anti-HBe, anti-HBs, anti-HBc-total (Roche e601 and Abbott Architect); HBeAg, HBsAg, anti-HBc-IgM (Roche e601 only). **Results:** The final samples had IgG concentrations of 7.7 g/L (sample A), 17g/L (B) and 26 g/L (C). The serology results were: HBeAg, HBsAg, anti-HBc-IgM all non-reactive (A,B,C); anti-HBc-total non reactive with (A) but reactive with (B,C) with both Roche and Abbott assays; anti-HBe non-reactive with both (A,B) but reactive with (C) with both Roche and Abbott assays. The anti-HBs (IU/L) concentrations with the Roche and Abbott assays were: (A) 71.51, 36.28, (B) 318.1, 210 and (C) 551.4, 369.6 respectively. **Conclusions:** Pharmacological concentrations of Intragam P can give false positive results for anti-HBc-total, anti-HBe and anti-HBs with the Roche e601 and Abbott Architect assays. HBeAg, HBsAg and anti-HBc-IgM are unaffected by the presence of Intragam P. Clinicians should ideally delay hepatitis serology testing 5-6 months following Intragam P administration to avoid false positive results.

A-241

Smartphones Can Monitor Medical Center Pneumatic Tube System Parameters

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Background:

The pneumatic tube system (PTS) has become a common means of patient sample transportation in medical centers. Although convenient and efficient, excessive acceleration force and time/distance traveled in the PTS have been correlated with increased sample hemolysis. As a result, regular monitoring and adjustment of pneumatic tube forces has been recommended to ensure sample integrity, but we have not found a fast and cost-effective way to do so. The purpose of this study was to assess the utility of smartphones to monitor acceleration and transport times in a hospital PTS.

Methods:

Two smartphones (iPhones) were sent through the PTS from two different hospital locations. Each smartphone used two apps as data-loggers; each app recorded force of acceleration vs time. To relate the data to sample integrity, blood was collected into Li-heparin-containing tubes from 5 volunteers in triplicate. One sample was transported by hand, and the others were transported through the two PTS routes. After transport, the hemolysis (H) index and plasma lactate dehydrogenase (LD) were measured in all samples (Abbott Architect). We then used a smartphone to illuminate a filled sample tube in a carrier and a second to make an audiovisual recording of the heparinized blood sample in the tube during transport through the PTS.

Results:

The smartphones showed a significant difference in duration of forceful acceleration during transport through the two PTS routes. Smartphones sent through both routes experienced acceleration forces exceeding 8 g (78 m/s^2), but route 1 generated forces in transit for roughly 150 seconds, versus 250 seconds for route 2. These data were consistent between the two smartphones and between the two apps used. The increased duration in Route 2 correlated with significant increases in the H index and LD. The mean H indexes were 13 (SD 10) and 36 (SD 18) and for route 1 and route 2 samples, respectively ($p<0.05$), and 4 (SD 3) for hand-delivered samples. Plasma LD was higher by a mean of 26% in samples from route 2 compared to route 1 or hand-delivered samples ($p<0.005$). Consistent with these data the video demonstrated extreme turbulence resulting in a foamy appearance of the blood sample, with large and small air pockets, during transit through the PTS.

Conclusion:

Our data demonstrate that smartphones can be used to quickly and economically monitor PTS parameters that affect integrity of patient samples. This method could be used to regularly evaluate PTSs to estimate the risk of sample hemolysis, particularly in new or altered routes or those servicing patient populations at higher risk of sample hemolysis.

A-242

The Preparation and Validation of Cystatin C Calibrators Traceable to ERM-DA471/IFCC

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BACKGROUND

Cystatin C has been an important biomarker to access kidney function for a few years. However, many calibrators in China market lacked the traceability document until recently. Here we produced recombinant cystatin C in E.coli, and the recombinant cystatin C was prepared and validated as calibrators in Gcell cystatin C assays, which were traceable to ERM-DA471.

METHODS

Gene cloning and protein purification: Recombinant human cystatin C was produced by expression in *Escherichia.coli.* and purified from cell extract.

Calibrators preparation: The recombinant cystatin C was diluted to approximate 8mg/L, 4mg/L, 2mg/L, 1mg/L ,0.5 mg/L and 0mg/L using 0.1mol/L KCl, 0.1% BSA, pH 7.0.

Value assignment: The values and uncertainty of the calibrators were assigned according to (1).

Commutability: the commutability of the calibrators was assessed by comparing the results of both Dako cystatin C assay kit and BSBE cystatin C assay kit to a set of 40 human serum samples.

RESULTS

DNA sequencing and N-terminal sequencing: The cystatin C gene sequence was validated by DNA sequencing and the N-terminal of the recombinant protein was identified as natural cystatin C. SDS-PAG showed that the molecular weight of the recombinant cystatin C is about 13kDa and that no other protein contaminants were observed.

Table 1. Value assignment and uncertainty of all levels calibrators

Commutability: The commutability of the six levels of calibrators was demonstrated by applying both the Dako cystatin C assay kits and the Gcell cystatin C assays kits to to a set of 40 serum samples, as there was no significant difference between those two assays.

CONCLUSIONS

We produced the recombinant cystatin C with amino acids sequences identical to natural cystatin C. This recombinant protein can be used as raw material for calibrators in clinical cystatin C assays, which could be traceable to international reference material ERM-DA471/IFCC.

REFERENCE

(1) S. Blirup-Jensen, Clin. Chem. Lab. Med. 46(2008)1470-9

Value assignment and uncertainty of all levels calibrators			
Levels	**Related Standard Uncertainty (%)**	**Extended Uncertainty (K=2)**	**Value (mg/L)**
level 1	490.3	-0.02	0.00±0.02
level 2	2.02	0.02	0.50±0.02
level 3	2.24	0.05	0.99±0.05
level 4	1.24	0.05	2.01±0.05
level 5	1.01	0.08	4.01±0.05
level 6	1.01	0.16	8.03±0.27

A-243

Assessment of Heterophilic Antibody Interference in the ADVIA Chemistry Hemoglobin A1c_3 Assay

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Background: The measurement of HbA1c, the glucose adduct of HbA1, in whole blood is used to monitor long-term care in diabetic patients. The ADVIA® Chemistry Hemoglobin A1c_3 assay, available on ADVIA Clinical Chemistry Systems (1200/1800/2400/XPT) from Siemens Healthcare, automatically pretreats a whole-blood sample and then measures HbA1c in the lysate immunoturbidimetrically. The assay also measures total hemoglobin (Hb) concentration in the same lysate and expresses the result as HbA1c% (or in HbA1c mmol/mol of Hb). Heterophilic antibodies (for example, human anti-mouse antibody [HAMA] and rheumatoid factors [RF]) are endogenous antibodies that could potentially interfere in the immunoreaction, resulting in erroneous results. Since the immunoassay component of this assay uses monoclonal murine antibodies in the reagent, it is important to know if HAMA or RF interferes with the assay.

Methods: The ADVIA Chemistry Hemoglobin A1c_3 assay pretreats a whole-blood specimen with a denaturant reagent that contains surfactants (to lyse the red blood cells) and porcine pepsin (to fragment resultant proteins). The lysate is then reacted with Reagent 1, which contains HbA1c haptens attached to a polymer as an agglutinator and a specific endopeptidase to cleave the HbA1c moiety containing five amino acid residues from the rest of the peptide. After the R1 reaction, Reagent 2, containing murine monoclonal anti-HbA1c antibody coupled to latex particles, is added. The resulting turbidity is measured at 694 nm. Sample HbA1c concentration is inversely proportional to the observed turbidity. To assess the interference of anti-mouse antibodies, we spiked serum with different levels of goat anti-mouse antibody, a quantifiable model for HAMA, and assayed the samples with and without denaturant treatment for the HbA1c portion of the Hemoglobin A1c_3 assay. In addition, we assayed 5 serum samples containing HAMA, 10 serum samples containing RF, and 5 normal serum samples with no RF present using the ADVIA Chemistry Hemoglobin A1c_3 assay. All samples were obtained from a commercial vendor.

Results: When goat anti-mouse antibodies were spiked in human serum and analyzed with the Hemoglobin A1c_3 assay following a denaturant pretreatment as specified in the assay instructions, the Hemoglobin A1c_3 results were undetectable as expected. However, if the samples were analyzed without the denaturant pretreatment, the Hemoglobin A1c_3 results (HbA1c) increased from 0-1.10 µmol/L as the goat anti-mouse antibody concentration increased from 0-750 µg/mL.

Conclusion: The data obtained in this study show that HAMA or RF (heterophilic interference) was not observed in the samples tested with the ADVIA Chemistry Hemoglobin A1c_3 assay. *(ADVIA and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates).

A-244

Updated Ammonia Handling and Storage Requirements Improve Specimen Rejection Rates and Reduce Risk to Patient Safety

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Background: Ammonia (NH_3) is routinely measured in plasma to assess liver function. NH_3 is considered a labile analyte and samples require special handling. Mishandling of the sample or delay in its reaching the laboratory can result in inaccurate NH_3 concentrations. Some studies report that plasma NH_3 is stable for up to four hours when stored at 4 °C (refrigerated) and up to four days when stored at -20 °C (frozen). The current analytical procedure at Henry Ford Hospital (HFH) states that plasma NH_3 is stable for three hours from sample collection if stored refrigerated. Between April 2014 and April 2015, 87% of plasma NH_3 specimens obtained from nursing homes did not meet the laboratory handling and storage requirements. **Objective:** The purpose of this study was to validate a longer stability limit for ammonia in plasma to improve specimen rejection rates and reduce risk to patient safety. **Methods:** To determine the ammonia stability limit, we used venous blood from patients with normal ("normal plasma") and compromised liver function ("abnormal plasma"). Blood was drawn into evacuated tubes with and without gel separator containing sodium heparin (NaHep), lithium heparin (LiHep), or ethylenediaminetetraacetic acid (EDTA) anticoagulants. For each sample type, blood was centrifuged immediately, plasma was removed from collection tube, aliquoted, and stored up to six hours refrigerated and up to 48 hours frozen. The first aliquot was tested immediately (t = 0) and the result obtained was used as a baseline. Subsequent aliquots were tested hourly. Ammonia concentrations were measured using a Beckman Coulter DxC 800 analyzer. **Results:** Linear regression analysis revealed no statistical difference between NH_3 concentrations in "normal plasma" collected into LiHep and EDTA tubes with gel separator. No clinically significant changes in NH_3 concentrations were observed after six hours of refrigerated and after 48 hours of the frozen specimen storage. Linear regression found a modestly increasing trend in HN_3 concentrations in frozen "normal plasma" collected into LiHep and NaHep tubes without gel separator over 48 hours in storage. More testing is required to investigate the cause. NH_3 in "abnormal plasma" (n=4) was also stable six hours refrigerated and 48 hours frozen. **Conclusion:** Special instructions for specimen handling and storage conditions for NH_3 in the HFH analytical procedure and the electronic laboratory user's guide (ELUG) were updated. According to the new procedure, plasma must be transported on ice and arrive at the laboratory within six hours of collection. Changes in the procedure were communicated to nursing homes and hospital staff. It was demonstrated, that by freezing plasma at the collection site, the specimen rejection criteria can be relaxed and the risk of obtaining inaccurate NH_3 concentrations is minimized. By using gel separator tubes, the need for manual separation of blood plasma from cells can be eliminated, which can significantly reduce time in the laboratory for specimen processing.

A-245

In vitro effect of two types of medical contrast media on routine chemistry results by three automated chemistry analyzers

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Background: Medical contrast has been elucidated as one of intefering analytical error sources which physicians face sometimes in daily practice. Various types of medical contrast media have been evaluated for their interference on laboratory results. Here we present the results of medical contrast media interference on the routine chemistry laboratory results performed by three most commonly used automated chemistry analyzers in Korea.

Methods: Ten levels of pooled serum were used in the study. Two types of medical contrast media [i.e. Iopamiro (iopamidol) and Omnipaque (iohexol)], which are infused most commonly in computed tomography and magnetic resonance imaging, were evaluated. To evaluate dose dependent effect of contrast media, Iopamiro and Omnipaque were spiked separately into aliquots of serum to make final concentrations of 1.8%, 3.6%, 5.5%, 7.3%, and 9.1%. To compensate the dilutional effects, negative controls with residual volume of distilled water were analyzed concomitantly. 28 analytes included in the routine chemistry panel were measured by Hitachi 7600 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan), AU5800 analyzer (Beckman Coulter Inc., CA, USA), and Cobas 6000/c501 analyzer (Roche Diagnostics, IN, USA). We calculated the percentage difference between the samples and the control, and examined dose-dependent trends to determine true interfering effects of contrast media.

Results: For the evaluation of dilutional recovery, the expected values and actual measurement of negative controls in each pair did not show a difference of more than 10%. Different levels of pooled serum specimens showed various trends by two kinds of medical contrast media in three analyzers. All percentage difference values in pair of contrasts and analyzers were less than 10% except serum iron in Hitachi 7600 analyzer and Cobas 6000/c501 analyzer.

Conclusion: Our study suggests that interference from organic iodine contrast media is minimal and does not affect overall routine chemistry lab results significantly except serum iron in specific analyzers. Based on these results, we can apply more flexible medical evaluation process for patients requiring both laboratory tests and imaging studies.

A-246

Pseudohypercreatinemia due to monoclonal IgM kappa

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Background: Plasma creatinine (PCr) is an important marker of glomerular filtration. However, misleading results can occur due to interfering substances. Enzymatic creatinine methods have been developed to replace the traditional picric acid method and are thought to be more specific, but these too are subject to interference. It has been suggested in previous reports that paraproteins can cause falsely elevated PCr by enzymatic methods, but this has not been thoroughly investigated. We present the case of a patient with a monoclonal gammopathy of undetermined significance (MGUS) who was found to have elevated PCr during evaluation for lung transplantation. We sought to conclusively demonstrate interference in enzymatic creatinine measurement by the patient's paraprotein. **Methods:** The Roche Creatinine Plus enzymatic assay method and the Roche picric acid (Jaffe) method were used to measure PCr. Glomerular filtration rate was measured by iothalamate clearance. Cystatin C was measured at the Mayo Clinic. Plasma proteins were removed by size exclusion filtration with molecular weight cutoff >30 kD. Protein electrophoresis and immunofixation were performed using the Helena SPIFE ImmunoFix method. Serum immunoglobulins were isolated by 40% saturated ammonium sulfate precipitation followed by dialysis with 0.9% saline. Non-IgM immunoglobulins were removed from plasma by adsorption with Protein G sepharose beads. **Results:** Comparison of results from the Roche enzymatic assay (1.51 mg/dL) in our laboratory and from the Jaffe method (0.84 mg/dL) using the same patient sample revealed a discrepancy. All other measures of renal function, including glomerular filtration rate (74 mL/min/1.73m^2) and Cystatin C (0.82 mg/L), were normal, suggesting interference in the enzymatic method. Serum immunofixation revealed an IgM kappa (IgMk) paraprotein. To determine if this was responsible for the interference, we filtered the patient's serum to remove large proteins. Creatinine concentration in the filtrate was 0.7 mg/dL by the enzymatic method. We then isolated the immunoglobulin fraction of the patient's serum and spiked it into four control patient plasma samples with normal PCr values. The patient's Ig fraction increased PCr in these samples by 0.58-0.62 mg/dL. Furthermore, removal of non-IgM immunoglobulins from the index patient's plasma did not reduce the interference, indicating it was not due to an endogenous IgG. Finally, comparison of enzymatic and Jaffe method results in samples from other patients with a monoclonal IgMk revealed that not all patients with an IgM paraprotein have falsely elevated PCr. **Conclusion:** These data show that this patient's PCr was falsely elevated in the Roche enzymatic method due to her IgMk paraprotein. The clinical team proceeded with lung transplantation. Altogether, these data definitively show that some paraproteins can interfere in enzymatic creatinine measurement.

A-247

Interference of multiple myeloma-targeted monoclonal antibody therapeutics with immunofixation electrophoresis: an emerging challenge

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Background: Since the first monoclonal antibody therapeutic was approved in the mid-1980s, there has been steady growth in this class of biopharmaceutical, with estimates projecting over 70 monoclonal antibody therapies in use by 2020. These drugs were first reported to interfere with serum protein electrophoresis tests in 2010, detectable as a monoclonal protein by both immunofixation and capillary electrophoresis. However none of the drugs at that time were specifically targeted treatments for multiple myeloma, and thus interference was not commonly encountered. Now, with the recent approval of two multiple myeloma-targeted monoclonal antibody therapies in November of 2015 (daratumumab and elotuzumab), laboratories should anticipate an increased rate of interference of these drugs with immunofixation and protein electrophoresis. Methods: Immunofixation was performed on the Sebia Hydrasis instrument, using reagents and materials from a Sebia Hydragel kit. Waste serum was obtained from a patient with a history of kappa light chain restricted multiple myeloma on daratumumab treatment, to determine the degree of potential interference with the Sebia immunofixation assay. Results: Immunofixation demonstrated bands in the IgG and kappa lanes, migrating at the cathodal end of the gamma region, as has been previously reported for daratumumab interference. This result confirms that a standard dosing regimen for daratumumab is sufficient to cause a clearly visible monoclonal protein by immunofixation electrophoresis.

Conclusion: Both daratumumab and elotuzumab are IgG-kappa monoclonal proteins. Therefore, in patients with IgG-kappa myeloma, co-migration of the therapeutic antibody with the patient's M-protein could lead to misdiagnosis of treatment-resistant or recurrent disease. In myeloma patients with non-IgG kappa M-proteins, this interference could lead to misdiagnosis of a new clone. With the recent FDA approval of two multiple myeloma-targeted monoclonal antibody therapeutics, laboratories need to develop proactive strategies to address the anticipated increase in interference with immunofixation and protein electrophoresis assays due to these drugs.

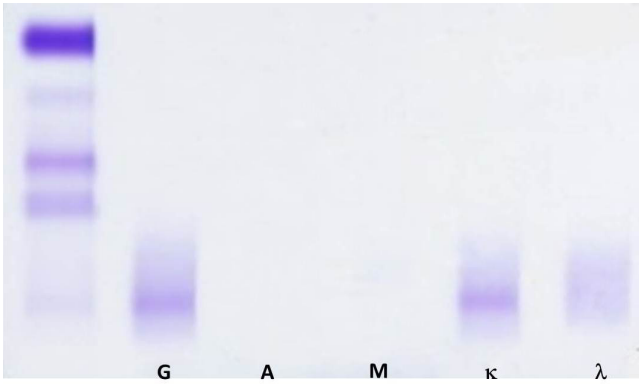

Figure 1: Detection of daratumumab by immunofixation electrophoresis in the serum of a multiple myeloma patient being treated with this IgG-kappa monoclonal antibody therapeutic.

A-248

Evaluation of Automating pH Verification Prior to Urine Chemistry Testing

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Objectives: Stability and/or solubility of urine analytes can be affected by hydrogen ion concentration (pH). As such, urine pH adjustment post-collection may be required when clinical testing cannot be conducted immediately. This is particularly important for specimens transported to regional or national reference laboratories. While assay-specific pH requirements and instructions are available from most laboratories, there has been little published work describing how frequently these requirements are followed. The workflow impact of pH screening (usually conducted with manual pH test strips upon specimen receipt in the laboratory) has not previously been described. The objectives of this study were to: 1) evaluate what percent of specimens were received outside pH recommendations for a select set of pH-sensitive analytes, 2) conduct a performance evaluation of an automated urine pH assay, and 3) assess the cost and workflow implications of fully automating pH assessment, including the use of IT middleware rules to automatically apply disclaimers when pH is outside of recommended limits.

Methods: Using IRB-approved protocols, de-identified retrospective data from clinical urine specimens was reviewed for 6 analytes: calcium (Ca^{2+}), citric acid (CIT), magnesium (Mg^{2+}), myoglobin (MYOG), inorganic phosphate (PHOS), and uric acid (UA). Performance characterization of an automated pH assay (SVT pH; Roche Diagnostics) on a cobas c502 chemistry analyzer was conducted, assessing imprecision, linearity, accuracy, and reference interval verification. Comparison studies were completed with a small volume pH electrode (InLab Micro / SevenEasy pH meter; Mettler-Toledo) as well as manual pH test strips (pH Test 0-14, BDH).

Results: The percent of clinical urine specimens received with pH outside of laboratory recommended limits was: Ca^{2+}, 13.7%; CIT, 3.8%; Mg^{2+}, 6.5%; MYOG, 43.6%; PHOS, 7.4%; and UA, 7.4%. The automated SVT pH assay demonstrated excellent precision with %CV's of 1.1% at pH 2.8, 0.8% at pH 6.3, and 0.6% at pH 11.4. The SVT pH assay was linear between pH 3 and 11 (slope 0.977). Non-linearity was observed below pH 3. Accuracy studies showed comparability (Deming regression, slope 0.837; r=0.98; bias 0.4%) to pH electrode measurements, although overall positive bias was observed below pH 2.5. Using pH electrode results as a target, both the SVT pH assay and pH test strips produced results within acceptable total allowable error (<20%). Previously published urine pH reference intervals were verified using the SVT pH assay. Workflow and financial analyses demonstrated that a switch to automated pH testing would save 538 hrs of manual work per year for these 6 orderables at approximately equivalent cost to manual pH test strip measurements. Creation of middleware rules to automatically apply pH disclaimers, however, would enable overall cost-savings with automated testing.

Conclusion: Automated pH testing has the potential to improve workflow efficiency in a reference laboratory setting. In combination with middleware rules, it can reduce overall cost of testing. At urine pH ranges relevant to clinical processes, automated testing demonstrated results comparable to pH test strips and pH electrode measurements. Additional educational efforts are needed to improve adherence to requirements for post-collection pH adjustment, particularly for MYOG and Ca^{2+}.

A-249

Commutability - Is it important and can patient samples be noncommutable? Use of CLSI EP14-A3

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It is common practice for manufacturers to use patient samples as secondary reference standards within a calibrator traceability scheme or as trueness controls. It has been well established that the commutability of reference material received from external sources must be ensured before being used for such purposes*. However, little work has been published that shows whether internally collected/created patient samples do or do not demonstrate commutability. Often such samples need to be either diluted or spiked with analyte in order to achieve desired measurand concentrations. It is understandable that such modified samples may demonstrate non-commutability. However, interferences or other sample specific characteristics may make specimens drawn from certain subjects behave differently than specimens form other subjects.

A study was conducted over multiple immunoassays to determine if secondary reference material created from unpooled plasma and serum units were commutable. Given that most immunoassays do not have a reference measurement procedure (RMP) available, most comparisons between measurement procedures (MP) were between commercially available MPs. For those with an RMP the RMP was also used in the commutability determination. At least 20 additional neat patient specimens covering the measuring interval of each assay were included in the study to create the patient specimen distribution. The analysis techniques described in CLSI EP14-A3 were used to make the commutability determinations.

A number of different non-commutability behaviors were seen in these studies. All of these behaviors will be demonstrated vis the commutability plots recommended in EP14-A3. Some non-commutability was seen in diluted samples, but the question remains whether this was due to the difference in behavior between the two MPs used with respect to the specific diluent being used. Some relationships required a data transformations (per EP14-A3) in order to show a consistent variability across the measuring interval. Some relationships showed high variability in neat patient specimens that could be used to single out individual patient specimens that should be excluded from the secondary reference material panel. In summary, commutability analyses as recommended in EP14-A3 can be used to identify non-commutable patient specimens.

*Miller WG, Myers GL, Rej R, Why Commutability Matters, Clin Chem, 52:2006, p553-4.

A-250

Interferences on the results of the dipstick urinalysis by Vitamine C and fluorescein sodium

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Background : Dipstick urinalysis is a basic tool for testing of pH, glucose, protein, hemoglobin, leukocyte esterase and so on in urine. It is known that many materials in urine interfere the results of the dipstick urinalysis. Vitamine C is one of the important interfering materials which show false negative results and commonly used vitamine dietary supplement in Korea. Fluorescein sodium is the dye used to examine the circulation of the retina and excreted through the urine causing yellow-green appearance. This study is to analyze the interference effects on the results of the dipstick urinalysis by vitamine C and fluorescein sodium.

Methods : Study was done on the random urine specimens from 4 general hospitals in Daegu, Korea from July to November, 2015. Dipstick urinalysis was tested by Uriscan Pro III and Uriscan 11 strip(YD Diagnostics,Korea). Urine sediments were tested by the microscopic exam and Sysmex UF-1000i(Sysmex Co.,Japan).

Results : 1,110 of 5,006(18.1%) random urine specimens were positive for vitamine C. When we added vitamine C to the urine specimens for the final concentration of 12.5, 25, 50 100 and 200 mg/dl in vitro, the interferences by vitamine C were not seen on the protein and nitrite positive urine specimens but the result values of the glucose, hemoglobin and leukocyte esterase decreased as the concentration of vitamine C increased. In case of in vivo interferences by vitamine C, 74 of 175(42.3%) vitamine C positive urine specimens which have above 180 mg/dl blood glucose showed negative results on glucose of the dipstick urinalysis. 507 of 1,507(33.6%) and 503 of 1,507(33.4%) of vitamine C positive urine specimens showed more than 1 level lower results of hemoglobin and leukocyte esterase compared to results of the urine sediments. 118 of 164(72%) of vitamine positive urine specimens which have positive bacteria results of the urine sediment showed negative results on nitrite of the dipstick urinalysis. Fluorescein sodium showed false negative results on the hemoglobin and leukocyte esterase of the dipstick urinalysis.

Conclusion : Interferences by vitamine C were seen on more than 30% of the total results of the glucose, hemoglobin and leukocyte esterase. It will be helpful to measure vitamine C together on the same urine specimens for the accurate interpretation of the false negative results of the dipstick urinalysis by vitamine C. More studies are needed for a variety of the interference materials on the urinalysis results.

A-252

Carryover from HbEE Samples Increase the Percentage of HbA2 Quantified with the Bio-Rad Variant II Beta Thalassemia Short Program HPLC Method

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Background: High-performance liquid chromatography (HPLC) is frequently used for screening and diagnosis of hemoglobinopathies. HPLC can be used for provisional identification of hemoglobin variants and quantification of both hemoglobin variants and normally occurring hemoglobin fractions, i.e. HbA2. A stable assessment of HbA2 is of great importance since HbA2 is a cornerstone in the diagnosis of beta thalassemia trait. The percentage of hemoglobin variants usually range from approx 15-90% of total hemoglobin but also minor fractions might be of clinical importance, like the delta globin variant HbA2' and low expression variants like Hb Constant Spring. Since we had occasionally seen indications of carryover from HbSS and HbCC samples in subsequent HPLC chromatograms and since HbA2 and HbE coelute in the HPLC method we use, we wanted to evaluate whether carryover from HbEE samples significantly affected the quantification of HbA2.

Methods: We use one Variant™ II instrument dedicated for the Beta Thalassemia Short Program from Bio-Rad for our ordinary work flow. Our HbA2 quantification method is accredited according to ISO15189. The instrument was subjected to annual service according to the manufacturer´s maintenance program. 4 different lots of reagents (columns and buffer) were used during the 2 year study period. During the time period 2014 - 2015 we regularly reanalyzed all samples injected immediately following a HbEE sample. Reanalysis was performed on the same day or the day after initial analysis.

Results: During the study period we analyzed approx 4 800 patient samples and 37 of these samples were initially injected following a sample from a patient homozygous for HbE (HbEE). In all cases, the HbA2 result obtained at reanalysis was lower than the first result. The difference ranged from 0.1 to 0.5% HbA2 (mean ± SD being 0.3 ± 0.11%). The difference between the mean of the first and second result for the same sample was highly significant ($p<0.0001$, Student´s paired *t*-test). All reruns were performed in random order, i.e. anywhere in the injection series. Control samples were analyzed both in the beginning and end of the series and also as every 10th sample. During the study period three different lots of control material were used at each level and imprecision ranged from 2.0-2.3% (CV% coefficient of variation) at 2.8% HbA2 level and from 1.1-1.5% at 5.7% HbA2 levels. There was no consistent pattern showing differences between results of control samples analyzed in different injection positions. The same carryover effect as was seen for patient samples was found for control samples injected immediately after HbEE samples. Samples from HbE heterozygotes (HbAE) normally contain only approx 20-25% of total hemoglobin as HbE. Also when HbA2 results in samples analyzed immediately following HbAE samples were evaluated a slight carryover effect could be noted.

Conclusion: Each HPLC method used for analyzing hemoglobin fractions should be evaluated to find out whether carryover might be a problem. For users of the Bio-Rad Variant II Beta Thalassemia Short Program samples injected immediately after samples from patients likely to be homozygous for HbE (HbEE) should be routinely reanalyzed to avoid falsely increased HbA2 results.

A-253

Effects of Hemoglobin(Hb)J-Bangkok, HbE, HbG-Taipei and HbH Traits on Measurements of Glycated Hb(HbA_1c) by IE-HPLC

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Background: Glycated Hb(HbA_1c) is a marker of long-term glycemic control and diagnosis in patients with.diabetes. HbJ-Bangkok, HbE, HbG-Taipei and HbH are very common Hb variants in southern China.We investigated the accuracy of HbA_1c measurement in the presence of HbJ-Bangkok, HbE, HbG-Taipei and HbH traits.

Methods: Methodology comparison. Collected five patients whose IE-HPLC HbA1c measurement results were inconsistent with their average FBG, performed Hb electrophoresis analysis of whole blood samples using Hb capillary electrophoresis, sequenced Hb genes using dideoxy-mediated chain termination, and measured HbA1c with borate affinity HPLC and turbidimetric inhibition immunoassay (TINIA)

Results: Two patients were HbJ-Bangkok; their Hb genotypes and HbJ-Bangkok content were $\beta^{41-42}/\beta^{J\ Bangkok}$ and $\beta^{N}/\beta^{J\ Bangkok}$ and 93.9% and 52.4%, respectively. The remaining three patients were HbE (β^{N}/β^{E} Hb genotype, 23.6% HbE content), HbG -Taipei ($\beta^{N}/\beta^{G\ Taipei}$ Hb genotype, 39.4% HbG-Taipei content), and α-thalassemia HbH (6.1% HbH content, 2.8% Hb Bart's content). There was interference in both IE-HPLC and TINIA HbA1c determination in the patient with β-thalassemia with HbJ-Bangkok; in the remaining four patients, there was interference in IE-HPLC HbA1c determination but not in that of TINIA. For all five patients, there was no interference in AE-HPLC HbA1c determination.**Conclusion:** The HbJ-Bangkok, HbE, and HbG-Taipei variants and α-thalassemia HbH disease cause varying degrees of interference in IE-HPLC HbA1c detection. For such patients, we suggest using other methods free from such interference to detect HbA1c, or using other indicators to monitor blood glucose levels.

A-254

Evaluation of plasma ACTH stability using the Roche Elecsys Immunoassay

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INTRODUCTION: Adrenocorticotropic hormone (ACTH) measurement is useful in the evaluation of hypothalamic-pituitary-adrenal disorders. ACTH is labile in blood, due to proteolytic degradation, and care should be taken during the preanalytical phase to prevent in vitro degradation. Our current collection instructions indicate to collect the sample in a chilled EDTA tube, keep tube on ice after draw, and spin down immediately in a refrigerated (4°C) centrifuge. Often, the laboratory receives questions regarding sample handling requirements (need for chilled tube, refrigerated centrifuge) as well as inquiries about delayed centrifugation and separation from cells. Our laboratory does not have data to support the various scenarios encountered at the collection site in order to determine the acceptability of the specimens collected outside the current protocol.

OBJECTIVE: To investigate time and temperature effects on plasma ACTH concentration.

METHODS: ACTH was measured using the Roche Elecsys immunoassay as per manufacturer's instructions. The current collection instructions indicated as "baseline" (sample collection in a chilled tube, keep tube on ice, and spin down immediately in a 4°C centrifuge) were compared to the following conditions: condition A: immediate centrifugation versus delayed centrifugation; condition B: chilled tube and centrifuge versus ambient tube and centrifuge; and condition C: specimen stability at ambient and refrigerated temperatures after centrifugation and sample aliquot. For each condition samples from 10 healthy volunteers were drawn between 8-10 am in K_2-EDTA tubes (Becton Dickinson). All samples, including the baseline sample, were stored at -80ºC prior to testing and then thawed and tested on the same day once the study was complete. For condition A, 3 scenarios were evaluated: current protocol, collection tubes in the refrigerator for 2 or 4 hours prior to centrifugation. For condition B, 2 scenarios were evaluated: current protocol and ambient temperature sample collection and centrifugation. For condition C, ACTH stability was analyzed at ambient and refrigerated temperature after 2, 4, 8, 12, 24 and 48 hours of collection.

RESULTS: For condition A, the average % differences from baseline ACTH concentrations were 3.4% (range -1.5-14.9%) and 2.1% (range -1.4-15.6%) when samples were kept refrigerated prior to centrifugation for 2 and 4 hours, respectively. For condition B, collection and processing of the samples at ambient temperature showed an average % difference from baseline of 1.6% (range -1.4-15.3%). For condition C, the average % difference from baseline was <10% at 12 hours ambient and 24 hours refrigerated.

CONCLUSIONS: ACTH stability is not affected if the samples are collected and centrifuged at ambient temperature. A 4 hour delay in sample centrifugation does not affect ACTH concentration. Once specimens are spun and aliquoted, the acceptable ACTH stability is up to 12 hours at ambient temperature and up to 24 hours refrigerated. These less stringent collection parameters would benefit laboratories that don't have access to refrigerated centrifuges for sample processing.

A-257

Validation of reference intervals for common biochemistry analytes in a multi-ethnic population

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BACKGROUND: Reference intervals are essential for interpretation of clinical laboratory test results in patient care. It can be expensive and logistically difficult for every clinical laboratory to establish reference intervals for all tests performed within the laboratory. Reference intervals provided by manufacturers are thus usually adopted in place of laboratory-established reference intervals. However, manufacturer-

established intervals are often based on Caucasian populations. The Department of Laboratory Medicine of Khoo Teck Puat Hospital (KTPH) conducted a study to validate in-use reference intervals for common clinical biochemistry laboratory tests.

MATERIALS AND METHODS: Serum samples (n=540) were obtained from healthy volunteers (age 21 - 70 years) during the annual hospital staff health screening over three years. Samples were analysed on the Roche Cobas c501 chemistry analyser (Roche Diagnostics, Switzerland) for 24 tests: sodium, potassium, chloride, bicarbonate, urea, creatinine, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), iron, transferrin, calcium, phosphate, magnesium, C-reactive protein (CRP), amylase and uric acid. The study was approved by the Ethics Board. Results were plotted in histograms. Reference intervals were obtained using parametric analysis for analytes that follow Gaussian probability distribution and non-parametric analysis for analytes that demonstrated non-Gaussian distribution. Study-derived reference intervals were compared with existing reference intervals in the laboratory.

RESULTS: Our results showed that 18 out of the 24 analyte reference intervals in the study were generally similar to the in-use reference intervals; the latter were validated using volunteers recruited from staff screening exercises conducted in another facility between 2009 and 2011. However, there were 6 analytes whose reference intervals were different from the in-use intervals. The analytes included: (1) CRP which had a 97.5 percentile cut-off of 9.0mg/L in the study group against the recommended cut-off of 5 mg/L; (2) Amylase, whose 97.5 percentile value was 138 U/L vs the current cut-off of 100U/L; (3) CK, whose range was 24 to 200U/L vs the study-derived range of 51 to 371 U/L. Current upper limits of reference intervals for intracellular enzymes like ALT, AST and GGT were also notably lower than the study-derived intervals even after correcting for suspected transient illness.

CONCLUSION: Validation of manufacturer-provided reference intervals and previously validated-data is important to ensure reference intervals remain relevant to the population served by the laboratory, to ensure appropriate care has been prescribed based on the laboratory results. Further studies may be required to ensure data can be translated from a group of healthy, physically-active volunteers to a population with heterogeneous levels of activity as certain analytes like serum CK concentrations can be also affected by strenuous physical activity.

A-258

Method-to-Method Variability in Urine Albumin Measurements

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Background: Urine albumin (uALB) is a useful marker in screening, diagnosis, and treatment of renal microvascular disease. Beckman Coulter recently re-formulated their uALB reagent for the AU series of instruments to increase the analytical measurement range (AMR) of the assay. We aimed to determine the performance characteristics of the re-formulated AU reagent. Additionally, we evaluated the susceptibility of six uALB reagents across five commonly used analyzers to the high-dose hook effect.

Methods: Characterization of the re-formulated uALB reagent was performed on the Beckman Coulter AU680 (Brea, CA). Intra-assay precision included consecutive measurements (n=20) of two concentrations of BioRad QC material (Hercules, CA); inter-assay precision entailed analysis of each concentration (n=1/day) for 20 days. Linearity within the manufacturer's claimed AMR (0.7–45 mg/dL) was confirmed using a residual high urine sample diluted with albumin-free urine. A data-driven approach was utilized to determine the necessary reportable range. On-board and manual dilutions were validated to support this range. Inter-assay variability was assessed using 78 residual urine specimens. Specimens with sufficient volume (n=74) were also analyzed using the Siemens Vista. The hook effect was evaluated on the AU680, Roche Cobas (Indianapolis, IN), Siemens Vista (Malvern, PA), Siemens BNII (Malvern, PA), and Siemens DCA Vantage (Malvern, PA). Serum was spiked into albumin-free urine to generate a range of albumin concentrations (n=12; 0–4,500 mg/dL). Each sample was measured neat and on dilution, where applicable.

Results: Despite similar imprecision, the QC means of re-formulated reagent demonstrated a significant positive bias (~50%) compared to the production reagent. Linearity within the manufacturer's claimed AMR was confirmed (slope=1.017; y-intercept=-0.774 mg/dL), along with the accuracy of 10X and 51X dilutions to extend the technical range. Patient sample comparison demonstrated a positive bias using the re-formulated reagent (n=78; concentration range=0.7–1156 mg/dL; slope=1.110, y-intercept=0.185 mg/dL; average bias=10.6% or 13.5mg/dL), which paralleled the differences in QC material mean. When compared to the Siemens Vista, average negative biases of 10.7% and 21.2% were observed with the re-formulated and original AU reagents, respectively. Based on neat measurements of the hook effect samples, uALB exceeding 3,000 mg/dL may be erroneously depressed into the AMR of both AU reagents. The DCA Vantage assay "hooked" at even lower levels of uALB (>500 mg/dL), while the Vista, Cobas, and BNII assays were unaffected.

Conclusion: The re-formulated AU uALB reagent met the manufacturer claimed performance characteristics. A striking observation was the ~11% bias between the two AU reagents. uALB assays are clearly not standardized, yet clinical guidelines dictate result interpretation. To illustrate the potential implications, 4% of the patient samples used for method comparison would be interpreted as moderately increased albuminuria (30–300 mg/g creatinine) using the original AU reagent and severely increased albuminuria (>300 mg/g creatinine) using the re-formulated AU reagent. As these levels are typically monitored longitudinally, this could result in the appearance of disease progression and lead to potentially inappropriate changes in clinical management. While the hook effect also could obscure uALB interpretation, the DCA Vantage is the only instrument of those tested that may be affected at physiologic concentrations.

A-259

Critically Low Carbon Dioxide in Patients with Severe Hypertriglyceridemia

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Background: Hemolysis, icterus and lipemia (HIL) are common interferences in clinical laboratory testing. These interferents may absorb or scatter light in spectrophotometric methods, which can lead to erroneous results. Assay manufacturers are required to document HIL thresholds through interference studies. Unlike hemoglobin and bilirubin there is no single chemical substance available that completely mimics the heterogeneity of a lipemic sample. In an attempt to simulate lipemia, manufactures will often use Intralipid a sterile, nonpyrognic fat emulsion. The range of lipid particle sizes for Intralipid is 200-600nm and does not cover the large lipid particle sizes of chylomicrons (70-1000nm). In this study, we identified a patient with repeated critically low carbon dioxide (CO_2) concentrations on the Abbott Architect CO_2 assay with no clinical signs of hypocapnia. The patient had marked mixed hyperlipidemia (hypertriglyceridemia and hypercholesterolemia) with acidemia. Therefore, an interference was suspected with the Abbott CO_2 assay.

Objective: To investigate the performance of the Abbott CO_2 assay in the presence of increasing amounts of hyperlipidemic samples.

Methods: The effect of lipemia on the Abbott CO_2 assay was assessed by performing mixing experiments and historic data review. Residual plasma from the hyperlipidemic and normolipidemic patients' plasma specimens collected for routine patient care were utilized. Hyperlipidemic specimens from a patient with severe hypertriglyceridemia (triglycerides >3,500 mg/dL) were titrated into a normal plasma pool. Samples with increasing lipid concentrations of 0, 5, 15, 25, 35, 50, and 100% were analyzed by either the Abbott Architect or Ortho Vitros enzymatic CO_2 assays. Linear regression analysis was used to obtain expected CO_2 concentrations for each of the samples analyzed. The expected and observed CO_2 concentrations were compared and percent bias calculated. Laboratory information system (LIS) records for a 6-month time period were reviewed. Patients >18 years with simultaneous CO_2 and triglyceride measurements on the Abbott Architect were evaluated (n=22,595).

Results: The package insert for the Abbott CO_2 assay reports 0 and 2% bias with 1000 and 2000 mg/dL of Intralipid respectively. Whereas the package insert for the Ortho Vitros CO_2 assay notes no significant interference with triglyceride concentrations up to 900 mg/dL. In mixing experiments, with triglyceride concentrations of approximately 1000 mg/dL, 2000 mg/dL and 3500 mg/dL gave negative CO_2 biases of 23%, 42% and 65%, respectively on the Abbott Architect. Measurement of CO_2 on the Ortho Vitros for the same lipemic specimens demonstrated negative biases of 6%, 23% and 36% respectively. Systematic review of LIS records for the past 6-months demonstrated that all patients (n=26) with triglycerides >1500 mg/dL in this time period had low CO_2 concentrations (4-22 mmol/L).

Conclusion: The use of Intralipid does not adequately mimic all the potential interferences within a hyperlipidemic sample. Patients with severe hypertriglyceridemia will have falsely low CO_2 results when determined by the Abbott or the Ortho Vitros CO_2 assays. In patients with hypertriglyceridemia, the

use of clinical symptoms, lipemic indices and other laboratory results should be used in the context of potentially low CO_2 concentrations in order to avoid unnecessary hospital admissions.

A-260

Investigation of biotin interference in common thyroid function tests using the Roche Elecsys® immunoassay system

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Background: Many FDA-cleared automated immunoassays used in clinical laboratories allow for the rapid measurement of serum analytes through biotin-streptavidin interactions. Depending on the nature of the assay, sufficient biotin concentrations in serum can lead to falsely increased (competitive assay) or falsely decreased (sandwich assay) results. Biotin is a water-soluble B-complex vitamin found in small amounts in numerous foods, but is also commonly found in both therapeutic preparations and over-the-counter multi-vitamins and hair, skin, and nail supplements. Our core laboratory has recently identified several Mayo Clinic patients with erroneous thyroid function test results obtained on the Roche Cobas® e 601 immunoassay platform due to biotin supplement consumption. In one case, the patient was scheduled for radioiodine thyroid ablation due to erroneous thyroid function test results. However, this serious patient safety incident was averted due to additional testing performed in the laboratory and diligent communication of the interference to the clinician.

Objectives: The aims of our study were to characterize the extent of biotin-mediated interference with Roche Elecsys® thyroid assays and to develop a troubleshooting protocol for depleting endogenous biotin from serum samples using streptavidin agarose.

Methods: Biotin interference studies were performed by adding biotin (Sigma-Aldrich) in varying concentrations to a residual waste serum pool. Serum was mixed with biotin stock solution (9:1, high biotin pool) or saline (9:1, zero pool). The zero biotin and high biotin serum pools were mixed to create five samples with varying biotin concentrations (ng/mL) for each assay (free thyroxine (FT4) and thyroid stimulating hormone (TSH), 0-102 ng/mL; total triiodothyronine (T3), 0-51 ng/mL; total thyroxine (T4), 0-408 ng/mL). Biotin interference studies were performed for the following analyte concentrations: FT4 (0.8, 1.5 ng/dL), T4 (5.6, 8.4 mcg/dL), T3 (54, 129 ng/dL), and TSH (2.5, 6.5 mIU/L). Samples were measured in triplicate using a Roche Cobas® e 602. The results were analyzed using EP evaluator to determine the biotin interference threshold (+/-10% considered clinically significant bias). Biotin depletion studies were conducted by treating biotin-spiked serum (1000 ng/mL biotin) with varying volumes of Pierce™ streptavidin agarose (Thermo Fisher Scientific Inc.), incubating for 1 hour ambient, centrifuging and analyzing supernatant.

Results: The following experimentally derived biotin interference thresholds were obtained (Roche package insert cut-off): FT4 = 61 (25) ng/mL, T4 = 348 (100) ng/mL, T3 = 19 (10) ng/mL, TSH = 30 (20) ng/mL. Treatment of 0.45 mL biotin-spiked serum with 0.05 mL streptavidin agarose beads for 1 hour effectively removed biotin and eliminated interference.

Conclusions: The experimental biotin spiking studies corroborated the Roche package insert thresholds for biotin interference and showed that the FT4, T3, and TSH assays are most sensitive to biotin interference. By treating the samples with streptavidin agarose, biotin interference was eliminated. This treatment protocol may be helpful when investigating cases of potential biotin interference. However, because biotin interference can mimic results found in biochemical hyperthyroidism (elevated FT4 and decreased TSH), identifying samples with interference may be difficult. This highlights the importance of laboratory, physician, and patient awareness of potential biotin interference in immunoassays.

A-261

Factors affecting urinary Neutrophil Gelatinase Associated Lipocalin levels in stable patients with Cardiovascular Disease without Acute Kidney Injury

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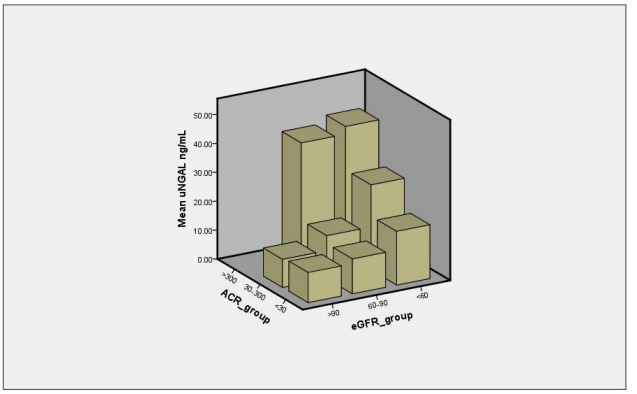
Several low-molecular weight proteins (LMWp) have been proposed as biomarkers for the early detection of acute kidney injury (AKI). Among them is neutrophil gelatinase-associated lipocalin (NGAL), produced by neutrophils and released in the circulation. Systemic-NGAL is freely filtered by the glomerulus and reabsorbed by megalin-facilitated endocytosis in proximal tubules. AKI will reduce reabsorption of systemic-NGAL, and stimulate tubular-epithelium to release NGAL increasing its urinary levels. However, filtered albumin is also reabsorbed by the same receptor. Independently of tubular-injury, competition for receptor-mediated transport between albumin and other LMWp could account for increased levels in the presence of proteinuria. Moreover chronic kidney disease (CKD) reduces filtration of NGAL. The objective of this study is to evaluate the relationship between uNGAL with urinary-albumin and GFR in patients with stable Cardiovascular Disease without AKI. We enrolled 226 patients with cardiovascular diseases. Urinary NGAL was measured with an ELISA (Bioporto, Denmark). Serum cystatine-C (sCysC) and creatinine (sCr) were measured on Architect 8200 analyzer (Abbott, USA). GFR was calculated with the CKD-EPI equation using both sCr and sCysC measurements (eGFR). Urinary-albumin and urinary-creatinine were measured on the same analyzer and the albumin-to-creatinine ratio (ACR) was calculated. We observed a negative, significant non-linear relationship between uNGAL and eGFR (r=-0.604) and a positive, significant, non-linear relationship (r=0.496) between uNGAL and ACR. Multivariate regression analysis revealed that both eGFR, and albuminuria affect baseline uNGAL levels. Our result show (figure) that levels of uNGAL increase significantly as GFR drops and albuminuria increases (MANOVA). In conclusion, albuminuria and CKD may increase the threshold for detection of AKI by increasing the excretion of LMWp. The presence of high uNGAL along with low eGFR and high ACR might also indicate progressive kidney disease. We consider that baseline measurements of uNGAL levels along with the estimation of GFR and ACR could help in risk-stratification of patients.



A-262

Decreased ascorbic acid interference by auto-oxidation of ascorbic acid in glucose, total cholesterol, and triglyceride measurement

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Background: We studied the effects of ascorbic acid interference and its auto-oxidation with the lapse of time on the measurements of glucose, total cholesterol, triglyceride and uric acid assay. **Method:** Five samples of healthy volunteers within the reference interval of glucose level (80-100 mg/dL) was used to observe for potential ascorbic acid interference. We spiked three different levels of ascorbic acid to five EDTA whole blood samples making final ascorbic acid concentration of 3, 15, and 30 mg/dL in each samples, followed by centrifugation and serial analysis on each time for each plasma sample. For control, normal saline was spiked instead of ascorbic acid solution. Each spiked volume was less than 10% of the total volume, according to

recommendations by the International Federation of Clinical Chemistry in guidelines for the evaluation of drug effects in clinical chemistry. Measurements of glucose, total cholesterol and triglyceride were perfomed both by the reagent containing ascorbate oxidase (Sekisui Medical Co., LTD., Tokyo, Japan) and the reagent not containing ascorbate oxidase (Roche Diagnostics GmbH, Mannheim, German). On the other hand, both manufacture's uric acid assays contained the ascobate oxidase. **Results:** Ascorbic acid at concentration 3 mg/dL did not interfere the measurement of cholesterol significantly (less than 5%). However, negative interferences in the measurement of cholesterol were observed in the presence of 15 mg/dL ascorbic acid when the reagent without ascorbate oxidase was used. All five cholesterol results showed significant negative biases (from -24% to -39%) when compared with controls at 1 hour after adding ascorbic acid. More negative interferences (from -45% to -75%) were shown at the level of ascorbic acid 30 mg/dL. Whereas no significant difference between the results of control and test samples was observed in measurements using the reagents containing ascorbate oxidase, which converts ascorbic acid to dehydroascorbic acid preventing ascorbic acid interference. When we repeated cholesterol assay with the same samples at 6 hours, the negative biases were less than 10% at the level of ascorbic aicd 15 mg/dL. However, persistent negative interference (around 15%) was observed up to 12 hour in the ascorbic aicd concentration 30 mg/dL. The similar pattern of interference by ascorbic acid on measurement of glucose and triglyceridewas observed. On the other hand, all the results of both uric acid assays were not showed significant difference over time. **Conclusion:** In case of using the reagents not containing ascorbate oxidase, we recommend to repeat the test after more than 12 hours when negative interference by ascorbic acid is suspected in measurements of total cholesterol, glucose, and triglyceride.

A-263

A Negative Interference Observed in an Enzymatic Creatinine Assay Due to Dopamine and Dobutamine

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Background: Measurement of serum creatinine is important for assessing renal function by estimating glomerular filtration rate (eGFR). The present study was designed after an inpatient with renal failure at our institution had a significant decrease in serum creatinine that could not be explained by medical intervention. The patient's previous creatinine was 4.25 mg/dL and the questionable result drawn 6 hours later was 2.07 mg/dL (reference interval: 0.52-1.04 mg/dL). All creatinine measurements were conducted on the Siemens Dimension Vista 1500 using an enzymatic creatininase method. After further investigation it was noted that three specimens with a questionable result were collected from a peripherally inserted central catheter (PICC) line; simultaneous arterial line samples were consistent with previous high creatinine results and the patient's clinical condition. Review of the patient's medical history revealed infusion of catecholamines (dopamine and dobutamine) during the time period the questionably low creatinine results from specimens collected from the PICC line were reported. Our objective was to investigate the extent to which catecholamines interfered with the Siemens creatinine method.

Methods: Three serum pools were made with varying concentrations of creatinine: 0.69 mg/dL (Pool 1), 3.32 mg/dL (Pool 2) and 6.89 mg/dL (Pool 3). An aliquot from each pool was spiked with dopamine (Pools 1 and 3) or dobutamine (Pool 2) to achieve a catecholamine concentration of 10 mcg/mL. Each spiked aliquot was serially diluted (x2, x4, x8, x16, x32, x64, x128) to observe the creatinine results in the presence of dopamine/dobutamine. All dilution aliquots were analyzed using the Vista enzymatic creatinine (IDMS traceable, peroxide detection) and the Abbott iSTAT enzymatic creatinine (non-IDMS traceable; sarcosine oxidase detection) methods.

Results: The patient's Vista enzymatic creatinine results demonstrate a significant decrease and deviation from previous results (difference:-2.18 mg/dL). The next draw hours later remained substantially lower (difference: -1.99 mg/dL) compared to baseline. Creatinine increased significantly to 4.95 mg/dL 4 hours later. Simultaneously paired arterial line and PICC line specimens were collected with the next draw (difference: 1.86 mg/dL). Additional specimens were collected by venipuncture and all samples were analyzed on the Vista and iSTAT. Absolute difference in creatinine across time points was minimal with the iSTAT (0.9 mg/dL) compared to the Vista (2.93 mg/dL). Serial dilutions of the 3 pools demonstrate a significant decrease in the creatinine concentrations at 10 mcg/mL of dopamine or dobutamine. Pool 1 had a decrease in creatinine of 48% and Pool 3 showed a decrease of 26% when spiked with dopamine; Pool 2 (dobutamine) also demonstrated a 22% decrease. In all cases, negative interference was minimized at higher dilutions such that the interfering substance was likely diluted out. iSTAT creatinine results were less susceptible to catecholamine interference at 10 mcg/mL or less.

Conclusions: Dopamine and dobutamine are routinely administered in intensive care settings. Significant false negative results were observed with the Siemens Vista enzymatic creatinine method (peroxidase detection mechanism) and related to PICC line draws. Enzymatic creatinine methods using sarcosine oxidase appear less susceptible to this interference.

A-264

Validation of the Calcium Correction formula using serum total calcium, albumin, corrected calcium and ionized calcium

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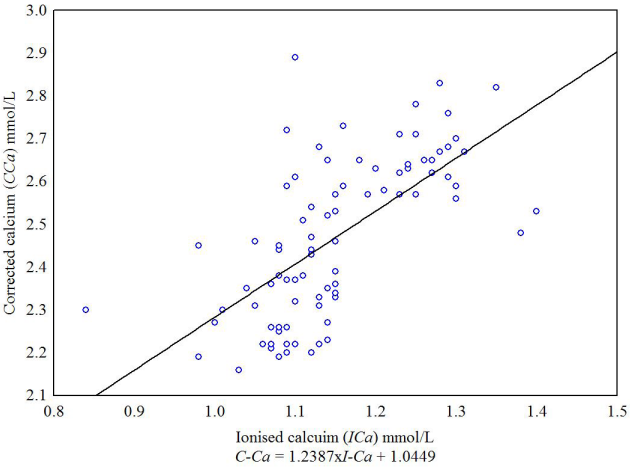
Background: The correction of serum calcium has been traditionally based on a historical equation which has not been fully validated. This study assessed the correlation between serum corrected calcium with serum ionized calcium values in an academic laboratory. Corrected calcium is derived from Payne's formula and consists of adjusting the serum total calcium levels based on the serum albumin level to reflect the "true" calcium status .The corrected calcium equation has been widely used in clinical laboratory but recent evidence indicates that some modification may be required based on the local context.

Methods: This was a prospective study using samples received at a laboratory for the measurement of serum calcium and ionized calcium. Serum albumin is measured by Bromocresol purple (BCP) in a Beckman DXC automated analyzer and the total calcium is determined by ion selective electrode method in a Beckman DXC automated analyzer. Ionized calcium is directly measured by OptiLion analyzer. The results of corrected calcium and ionized calcium will be grouped based on the different albumin cut-off levels of below 30 g/l, between 30-34 g/l, within albumin reference intervals (35-52 g/l) and greater than albumin reference intervals (> 52 g/l), respectively. Corrected calcium was then compared with ionized calcium across all levels of albumin. In total, 252 patient results were analysed.

Results: The graph illustrates the concordance between corrected calcium and ionized calcium.



For albumin levels < 30g/L; only 71% of results were in agreement; With albumin levels of 35-52 g/L only 14% of results were in agreement. Payne's formula overestimated hypercalcemia in 43% of patients and underestimated hypocalcemia in 72%.

Conclusion:

Calcium adjusted for albumin using Payne's formula fails to assess "true" calcemia. Payne's correction formulae should be abandoned in favor of uncorrected calcium in the routine laboratory. In cases of doubt , ionized calcium should be measured directly.

A-265

N-acetyl Cysteine Interferes with the Trinder Reaction Based Assays and Beyond

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Background: N-Acetyl-L-cysteine (NAC) is a sulfhydryl-containing drug that is used to treat acetaminophen poisoning and other diseases. NAC has been recognized as a

source of interference in multiple laboratory tests that are based on (triglycerides, total cholesterol, LDL, HDL, lactate, uric acid, and lipase) or not based on (creatinine, total protein, albumin, AST, ALT, ALP, total bilirubin, and glucose) the Trinder reaction, respectively. However, different assay platforms, NAC dosages, and interference criteria were employed in these studies, and therefore the effect of NAC interference on these analytes remains inconclusive. This study aims to systematically determine the clinically relevant interference of NAC in the aforementioned assays as well as insulin immunoassay performed on the Abbott Architect platforms.

Methods: Excess patient serum containing 5 different physiopathological concentrations of analytes was spiked with 5 therapeutic doses of NAC (50 - 2500 mg/L). Serum without NAC was used as a control. Analytes were measured either immediately post-NAC addition or after up to 48 hr storage at 4 °C. Samples from 6 different acetaminophen-overdosed patients collected pre- and post- NAC infusion were also assayed. The interference was defined as % difference in NAC-containing samples from the control. A bias greater than the allowable total error of each analyte according to CLSI's recommendation was considered as clinically relevant. Additionally, the inference data were subjected to multiple regression analysis to predict the true analyte value in NAC-containing samples.

Results: NAC dose-dependently reduced the analyte measurements with a maximum of 5-95% reduction among different analytes. The magnitude of interference also varied with analyte concentrations. Clinically relevant interferences were observed for triglycerides, total cholesterol, lactate, insulin, uric acid, and lipase with NAC at concentration as low as 250 - 1250 mg/L, respectively. Statistically significant but non-clinically relevant negative bias for LDL, HDL, albumin, AST, creatinine, and ALP and positive bias for total protein were also observed in the presence of NAC. In general, NAC interference was gradually attenuated by prolonged storage except that a negative bias in insulin immunoassay increased with storage time and lasted for up to 48 hrs. A significant decrease of total cholesterol and uric acid was also observed in post-NAC treatment samples from acetaminophen-overdosed patients. Additionally, the predicted analyte concentrations in NAC-containing samples using multiple variable regression model showed significant Pearson correlation with the measured values ($P<0.05$).

Conclusion: Our results indicate that therapeutic dosages of NAC can cause clinically relevant negative interference in several assays involving the Trinder reaction on the Abbott Architect platforms. The magnitude of interference can be markedly different, depending on the concentrations of NAC and analyte as well as the storage time. Additionally, this study reports for the first time the significantly negative interference of NAC in insulin immunoassay, which might be attributed to the NAC binding to insulin causing impaired antibody-insulin recognition. Therefore, particular caution should be taken when clinicians interpret these assays' results of patients receiving NAC treatment. Finally, this study demonstrates the possibility of using multiple variable regression test to predict the analytes' concentration in NAC-containing specimens.

A-266

The Effects of Hemoglobin E variant on Hemoglobin A1c measurement

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Background: Hemoglobin A1c is a marker of long-term glycemic control in diabetes patients and is linked to the risk for developing diabetic complications. Hemoglobin E is one of the most common hemoglobin variant in the world, especially in South-East Asia region. The aim of this study is to compare the HbA1c values of non-diabetic subjects with normal hemoglobin typing and heterozygous hemoglobin E variant, using two laboratory methods, which are immunoassay and high-performance liquid chromatography (HPLC)

Methods: The leftover blood samples of healthy, non-diabetic subjects, who attended the Siriraj Hospital for health screening and did not have impaired fasting glucose or diabetes (fasting plasma glucose <100 mg/dL). These subjects must not have abnormal liver or kidney function tests, as defined by having raised liver enzymes (AST/ALT) and reduced estimated glomerular filtration rate (eGFR <60 mL/min/1.73 m^2). These blood samples will be subjected to analyse using the Roche Integra 800 (immunoassay) and the Bio-rad D-10 (HPLC) analyzers.

Results: The mean±SD HbA1c values in the homozygous A (n=80) measured by the immunoassay method were 5.52±0.28 (range 4.9-6.3%), and 5.42±0.32 (range 4.7-6.4%) by the HPLC method. In hemoglobin E heterozygote, the mean±SD HbA1c values were 5.37±0.33 (range 4.4-6.6%) by immunoassay, and 5.35±0.33 (range 4.5-6.1%) by HPLC. By using unpaired t-test, HbA1c values were significantly different between homozygous A and hemoglobin E heterozygote analyzed by immunoassay method (P =.001), but not by HPLC (P=.115)

Conclusion: In normal fasting plasma glucose subjects, HbA1c values in hemoglobin E heterozygote subjects were significantly lower than homozygous A subjects by using immunoassay method. The interpretation of HbA1c values in the presence of hemoglobin E variant should be performed with caution.

A-267

Efficacy of Calcium Correction in a Cancer Population

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Background: Plasma calcium exists in three forms with approximately 15% bound to anions, 40% bound to albumin and 45% circulating as free ionized calcium. Methods that measure total calcium are affected by protein concentration fluctuations, while measurements of ionized calcium are unaffected. Studies on calcium correction for albumin levels have been extensively studied in hemodialysis patients and the critically ill, but few have investigated the utility of these equations in cancer patients. The objective of this study was to investigate the usefulness of calcium correction for patients with hypoalbunemia and the resulting potential impacts on laboratory operations.

Methods: A retrospective analysis was carried out to determine the potential value of calcium correction on cancer patients at Memorial Sloan-Kettering Cancer Center from January 2013 to December 2015. Using the hospital database information, the following data was obtained: (1) total number of critically low calcium values (<6.5 mg/dL) out of total calcium results, (2) total number of critically low calcium values with corresponding low albumin levels (<4.0 g/dL), (3) number of critical ionized calcium values (<3.3mg/dL) and (4) average time spent making critical calcium callbacks. To correct for serum albumin concentration the Payne formula was utilized:

Corrected calcium = measured Ca (mg/dL) + 0.8 * (4.0 g/dL - patient albumin (g/dL))

Results: Over a three-year period, 706,552 calcium levels were analyzed of which 1,761 (0.25%) were critically low. Of these critically low calcium results, 97.4% had corresponding low albumin levels. Using the Payne formula to correct the calcium level, the number of critically low calcium values dropped to 14% of the uncorrected critical values (248 or 0.04% of the total calcium measurements). Next, to evaluate the accuracy of calcium correction in estimating actual critical calcium events in cancer patients with hypoalbunemia, we examined those critically low calcium results that also had an ionized calcium result within an hour. Within these paired results, there were a total of 554 critically low calcium results, and of those, 99% had albumin levels below 4.0 g/dL. Importantly, within these paired results, only three had corresponding critically low ionized calcium results. To determine the potential impact that correcting calcium in patients with hypoalbunemia could have on workflow and staffing within the laboratory, the amount of time technologists spend on repeat testing of critical value specimens and critical value callbacks was estimated. On average, a total of 15 minutes was spent on each critically low calcium result, which adds up to a total of 440 hours over the three-year period. If low calcium values would have been corrected, a total of 378 hours of technologist time could have been saved over three years through the reduction of repeat critical value testing and critical callbacks.

Conclusion: In cancer patients with hypoalbunemia, correcting calcium results using the Payne formula maybe a better indicator of calcium homeostasis than measured concentrations alone. This correction has the potential to provide healthcare professionals with more accurate patient results and reduce technologist time spent responding to these clinically insignificant low calcium values.

A-268

Identification and Quality Control of FFPE and cfDNA Samples using Agena Bioscience ExomeQC Panel

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Background: Highly-annotated, well-sourced bio-specimens, are essential in elucidating the molecular mechanisms for initiation and progression of cancer along with being a highly valuable resource to validate panels of actionable mutations for clinical utilization. Formalin-Fixed Paraffin-Embedded (FFPE) tissue archives are most commonly used for oncology research, validation studies and diagnostic testing. Accurate detection of mutations is often problematic in FFPE tissues since they tend to have highly variable levels of fragmentation, which frequently limits the number of DNA template, formalin introduced sequence artifacts and the presence of PCR

inhibitors. Lack of consistency between the various protocols for sample handling and extraction for DNA is one of the major obstacles in translating biomarker analysis to clinical practice. **Methods:** Pre-analytical protocols for FFPE samples generally assess either sample identity (ID) or nucleic acid template quality (QC). Often samples are assumed to be poor quality when low call rates are observed. To address these QC and ID issues, we have designed the ExomeQC panel, a single reaction panel that will perform DNA quality assessment and template copy number enumeration across a broad dynamic range of 100-100,000 copies (0.3-300ng) as well as monitoring sample fragment size over a 100-500bp range. This is performed by competitive PCR of selected known housekeeper genes derived from cancer studies, which are largely devoid of polymorphisms and somatic mutations, and have minimal copy number variation in germline and somatic tissues. To assess the intact, amplifiable template copy number across a size range of 100-200-300-400-500 nucleotides we have 5 assays at each amplicon size, with 200, 1000, 3000, 10000 or 100,000 of input competitive template copies. Sample ID is enabled via 21 highly polymorphic exonic SNPs along with 3 XY paralogues; while localized and historical identity matching and quality assessment is enabled via a completely automated software solution. **Results:** The ExomeQC panel was applied to archived FFPE (n=48) samples to assess amplifiable DNA copy number and template fragmentation. Pre-analytical utility was determined via comparison to control samples and where available downstream analytical performance from these samples. **Conclusion:** The ExomeQC panel provides a unified resource for pre-analytical identity authentication and quality assessment of potentially degraded clinical samples.

A-269

Sample Rejection in Clinical Chemistry Assay; Causes and Trends in a Named Laboratory: A pilot Study

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Background: Laboratory operations play a crucial role in patients' management thereby improving treatment and prognosis. Delivering quality laboratory data within an acceptable turnaround time depends on the use of right sample, collected at the right time, into an appropriate sample container which is delivered to the laboratory within the acceptable time frame. Previous studies on determinates of laboratory quality have indicated that pre-analytical issues accounted for the highest number of errors; and premised on this information, proper control of these errors could be of significant importance in improving the quality of data generated from the laboratory. Data detailing major contributors to pre-analytical issues in Nigerian laboratories are limited. This pilot study was conducted to determine the causes and trends of samples rejected for analysis in a clinical chemistry laboratory.

Methods: This study was conducted in one of the laboratories within the University College Hospital, Ibadan, Nigeria. Records detailing samples meant for clinical chemistry assay, collected for the period of two years, (2012-2014) were examined retrospectively and reasons for the rejection which were documented in the sample rejection log were reviewed. The data collected was analyzed using simple descriptive statistics.

Results: For the period under review, out of the total number of 92,374 samples received in the laboratory for clinical chemistry assay, 177 samples were found to be rejected representing 0.2 % of the samples collected. Major reasons for samples rejected in the laboratory were: Insufficient sample, 58 (33%); collection into wrong samples, 39 (22%); Clotted samples, 33 (19%); fasting samples collected at the wrong time, 16 (9%); wrong labeling of sample, 13 (7%); overnight samples sent to the laboratory, 10 (6%); unlabelled samples, 4 (2%) and haemolysis, 2 (1%)

Conclusion: From this study, it was evident that among all other factors observed, collection of insufficient sample and collection into wrong sample bottles and anticoagulants were major reasons contributing to samples rejected by the laboratory. Although the percentage of samples rejected was small compared to the total samples received, yet appropriate education and development of necessary quality documents could be of high significance in reducing these trends with a view to improving quality data, turnaround time, patient management and prognosis.

A-270

A survey on Indian patients about understanding of fasting requirements before fasting glucose measurements.

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Background: Fasting is an important controllable preanalytic variable. Fasting glucose testing needs 8 to 14 hours of fasting as per WHO guidelines. Hours of fasting, water intake, sudden change in smoking or exercise, medication withholding or intake etc. affect the fasting test results. Counselling to generate patient awareness regarding above factors is important. **Methods:** In order to understand the ground reality whether patients are being explained about need for fasting, its meaning and associated requirements a survey was undertaken in the outpatient phlebotomy section of a subsidized care government hospital in Kolkata, WB, India. Exclusion criteria were health professional subjects e.g. technician, nurse etc. The questionnaire was framed in local native language (Bengali). Information collected were age, sex, perception of hours of fasting required, and perception about whether to restrict water intake, beverage, light breakfast, religious eatables (e.g. Prasad for Hindu or Sehir for Ramadan etc.), smoking, drinking, medication, exercise during the fasting and their behavior in previous three days regarding excessive exercise, change in food quantity, whether they were instructed, who instructed, was it verbal or in writing, what was the actual number of hours they fasted, whether they actually took some edibles in the morning, what food or beverage they ate last etc. **Results:** Ninety people were given questionnaire, 72 (80%) consented for the study, 42 female and 30 male. The result of the survey was very striking and only some highlights are given. Fasting duration varied from 2 hours to 16 hours. A staggering 83% of the participants perceived that nobody explained to them anything about the nature of the fasting. Of the rest 14% were explained by the doctor and 3% knew from health professional relatives. The cursory verbal instruction by doctor seemed to make either minor or no improvement or even paradoxic worsening in compliance. 69% of the uninstructed subgroup were not aware that drinking water was allowed at all, in the instructed group 57% were unaware. 13% of the uninstructed subgroup thought that drinking tea, coffee etc was allowable in the morning, a much larger proportion of 29% in the instructed subgroups also had the same concept. Even more paradoxical results were apparent in perception about snacks- the 14% of the instructed and 3% of the uninstructed thought that light morning snacks could be allowed. Only 3% of uninstructed thought religious food could be taken in morning, while a staggering 29% of the so called instructed group thought the same was allowable. **Conclusion:** Counselling for awareness about fasting and understanding of its nature need corrective interventions in the present setting. Doctors were overburdened with patients and yet the instructed minority received it from doctors. But the instructions were neither thorough nor effective, did not touch upon locally relevant religious confusion about fasting, was often misunderstood and rather gave a false confidence in wrong assumptions. It could be improved by introducing distribution of printed leaflets, posters and also training the nurses, phlebotomists and social workers who could also use audio-visual aids in addition to verbal reminders.

A-271

Evaluating microtainer neonatal whole blood minimum volume requirements

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Introduction:

While microtainers can potentially reduce the amount of blood drawn from neonates, short draws with microtainers can lead to high rates of recollects. We have re-evaluated our minimum volume requirements with a new wider bore design of BD Microtainers® (cat# BD365967). Our study asked two questions. First, when transferring separated plasma/serum using a micro-pipette, transfer pipette, or pouring, what volumes of plasma/serum can we recover from the new SST with different initial volumes of whole blood with neonatal hematocrit? Second, how many chemistry tests can be performed with different volumes of whole blood with neonatal hematocrit?

Methods:

To answer these questions, we adjusted adult whole blood to resemble that of a neonate (adjusted hematocrit: 0.61 ± 0.02 L/L) and aliquoted set volumes of the adjusted whole blood into BD Microtainer® serum separator tubes (SSTs). After centrifugation, the volume of separated plasma was measured following transfer by a micro-pipette, transfer pipette, or pouring. Using false bottom tubes (Beckman

Coulter, cat# 448774), we investigated the maximum number of tests a Roche Cobas 6000 analyzer can perform for each transfer method.

Results:

Total theoretical plasma volume in a tube was estimated as: 1-hematocrit x total blood volume. Of the different transfer methods evaluated, the micro-pipette is the most effective at transferring plasma with a mean ± avg deviation recovery of 68% ± 3% of total theoretical serum volume, but at higher volumes (300 µL, 400 µL), pouring off the separated serum is nearly as effective (67% ± 1.5%). Transferring using a plastic transfer pipette is the least effective method with recoveries of 56% ± 1.2%. Of the different whole blood/transferring methods we examined, whole blood volumes of 300 µL yielded 80 - 82 µL of plasma, which was enough for the Cobas 6000 to measure serum indices plus four tests requiring 2 - 6 µL sample/test. Lower volumes of 150 µL and 200 µL yielded 31 - 47 µL plasma. For the whole blood volumes below 300 µL, there was not enough plasma to measure serum indices (requires 6 µL) plus one chemistry test. In our study, we found that the required sample volume underestimated the total volume required for testing and that a significant volume of plasma is lost per test run (10.1 ± 0.4 µL/test) as part of the sampling process on this analyzer type and this was consistent across the run volumes.

Conclusions:

For neonates, a minimum volume of 300 µL should be drawn into the BD Microtainers®. Pouring or using a micro-pipette can transfer enough serum for 3 - 4 general chemistry tests. However, additional volume is required for each test in addition to the stated volume that is used in the reaction itself. Labs are cautioned not to set minimum volume requirements by adding up the amount of sample volume for tests as stated in the manufacturer's application on automated analyzers. Neonates require the analysis of multiple chemistry analytes, and our findings will prevent redraws in this group.

A-272

Identification of biotin interference with selected VITROS® biotin-streptavidin-based immunoassays in individuals taking supplements

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Background: Biotin is a known interfering compound in immunoassays based on biotin-streptavidin interactions. Serum biotin concentrations in individuals with normal dietary intake of this vitamin typically range between 200-500 pg/mL and are generally not found to cause interference; however, biotin supplementation is not uncommon among patients with clinical biotin deficiency and those taking over-the-counter vitamins for general health and beauty benefits. Biotin concentrations in these individuals can greatly exceed expected reference levels, presenting an opportunity for immunoassay interference.

Objective: In light of recent clinical inquiries regarding the above with assays performed in our laboratory, we sought to determine if this was an issue, and, if so, characterize the magnitude of interference at varying concentrations of biotin.

Methods: Selected immunoassays were evaluated using the VITROS 5600 (Ortho Clinical Diagnostics) chemistry system by adding biotin (Sigma-Aldrich®) to pooled plasma to span a range representative of that found in healthy individuals and those reported in individuals taking supplements. Our laboratory uses a 10% acceptability limit in interference studies.

Results: Excess biotin in pooled plasma resulted in variable but clinically significant interference with assays relying on biotin-streptavidin interactions (table).

Conclusion: Supraphysiologic serum biotin concentrations in individuals taking biotin supplements can result in clinically significant interference with common laboratory assays and must be taken in consideration when evaluating inconsistencies between clinical presentation and laboratory parameters. This is the first report of this phenomenon for the VITROS analyzer. Negative interference was observed for immunometric (sandwich) assays due to displacement of biotinylated antibodies by biotin. The competitive assays exhibited falsely positive results in the presence of excess biotin secondary to competition of free biotin for binding sites on streptavidin. Little is known about the effects of biotin metabolites on chemistry tests. Clinicians and laboratory scientists must be mindful of common potential interfering compounds with analyte quantification.

Assay	Assay type*	Units	Pooled plasma	Pooled plasma with added biotin (pg/mL)					Manufacturer published interference <10% (pg/mL)
				500	5,000	12,500	25,000	50,000	
Troponin I	IM	ng/mL	0.073	0.074	0.056	<0.034	<0.034	<0.034	2,500
TSH	IM	mIU/L	2.96	2.95	2.61	1.25	0.75	0.27	5,000
FSH	IM	mIU/mL	13.3	13.0	11.5	9.0	5.9	<0.5	10,000
LH	IM	mIU/mL	6.8	6.8	6.1	4.7	2.4	<0.5	5,000
Prolactin	IM	ng/mL	19	19	17	15	11	<1.4	10,000
bHCG	IM	mIU/mL	624	631	603	551	355	16.5	10,000
CEA	IM	ng/mL	3.7	3.8	3.7	3.7	3.4	<0.5	10,000
PSA	IM	ng/mL	1.42	1.39	1.3	1.24	1.06	0.16	10,000
Ferritin	IM	ng/mL	418	424	434	419	403	88	10,000
Progesterone	COMP	ng/mL	1.97	1.95	1.99	2.00	2.26	20.7	20,000
Estradiol	COMP	pg/mL	162	155	156	150	402	error	5,000
Testosterone	COMP	ng/dL	98	103	94	85	75	1550	10,000

*IM = immunometric, COMP = competitive

A-273

The Effect of Preservatives on Long-Term Stability of Emerging Urinary Biomarkers of Kidney Injury

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Relevance

Kidney injury and loss of function can be assessed using several emerging urine biomarkers. The urine of patients who are at risk for developing either acute or chronic loss of kidney function could be frozen for future biomarker measurement alongside newly collected urine. However, since the long-term stability (LTS) of these markers is unknown, a significant change in concentration may not necessarily indicate disease. Therefore, LTS of novel biomarkers is a critical element that needs to be verified to confidently detect risk of disease.

Objective

This study compared the LTS of five emerging urine biomarkers of kidney injury (Cystatin C (CysC), KIM-1, NGAL, osteopontin (OPN), and clusterin) using three processing methods, including a proprietary preservative.

Methods

Ten single-donor urine samples were collected and processed with three different methods: 1) neat and centrifuged (N&C), 2) pretreated with a protease inhibitor cocktail (PIC), or 3) pretreated with protease inhibitor cocktail plus carrier protein (CP+PIC). After processing, aliquots were stored at -70°C until assayed (baseline, 7 mo, 12 mo). Between-run precision (BRP) of each biomarker was determined from QCs. A sample was considered stable if the %Difference from baseline was ≤ 2*CV% of the BRP for the assay, but not to exceed >30%Difference from baseline. The biomarker was defined as stable if at least 4/6 (≥67%) samples had no individual changes exceeding the acceptance criteria, and rendered unstable if it did not meet the acceptance criteria for two consecutive time points.

Results

uKIM-1, CysC, NGAL and OPN were stable up to at least 12 months, regardless of the processing method. Although clusterin was not stable in N&C urine, as only 60% of the samples met the acceptance criteria, it was stable in PIC- and CP+PIC-preserved urine with over 70% of the samples meeting the acceptance criteria. In N&C urine, KIM-1 showed continuous degradation with a mean %Difference from baseline of -2.9% (ranging from -30.7% to 15.7%) at 7 months to -8.9% (ranging from -61.4% to 25.2%) at 12 months. In contrast, KIM-1 degradation was greatly reduced in preserved urine as evident by the condensed range. The mean %Difference from baseline in PIC-preserved uKIM-1 was 4.8% (ranging from -12.5% to 23.3%) at 7 months and 6.4% (ranging from -28.7% to 30.1%) at 12 months, while CP+PIC-preserved uKIM-1 was 4.2% (ranging from -11.8% to 24.2%) at 7 months and 9.3%

(ranging from -14.3 to 25.1%) at 12 months. A similar trend was seen with CysC and OPN in preserved urine.

Conclusion

While human KIM-1, CysC, NGAL and OPN are stable in N&C urine for up to at least 12 months, the stability of KIM-1, CysC, OPN and clusterin was dramatically improved when preserved, whereas NGAL stability was unaffected by any collection process. The addition of preservative is recommended when samples are stored at -70°C for 12 months or longer prior to analysis.

A-274

Effect of Hemolysis, Icterus and Lipemia Interference on Routine Clinical Chemistry and Therapeutic Drug Monitoring Assays

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Background: Almost 70% of all medical decisions regarding patient treatment and management are made based on clinical laboratory tests. It is, therefore, crucial that the laboratory provides accurate and reliable results. The majority (46-68%) of laboratory errors are pre-analytical, with ~13% due to specimen integrity, including the presence of endogenous compounds (hemoglobin, bilirubin, lipids) that can interfere with laboratory results. Unless the effect of these interferences is well characterized, the inaccurate result may lead to misdiagnosis or unnecessary treatment, with the potential to cause serious harm to the patient. While most manufacturers provide guidelines on interferences, the cutoffs can be inaccurate, misleading or not provided, resulting in the release of inaccurate results biased positively or negatively by the interferent. In this study, we characterized hemolysis, icterus and lipemia (HIL) interferences on 38 assays. Based on this study, our clinical laboratory practice has been adjusted to provide more appropriate guidance to the clinician.

Methods: For hemolysis interference, the hemolysate was prepared by lysing a unit of type O blood and preparing hemolysate/serum mixtures ranging from hemoglobin levels of <2 mg/dL to >2000 mg/dL. To study icterus interference, bilirubin was purchased from Sigma-Aldrich (St. Louis, MO) and used to spike pooled serum mixtures with bilirubin ranging from 0-80 mg/dL. To assess lipemia interference, specimens were prepared using Intralipid (20%) solution (Baxter, Deerfield, IL). Serum samples were spiked with varying amounts of intralipid to obtain samples with L-index ranging from 0 to 2000. All samples were tested on the Abbott Architect c16000 Clinical Chemistry Analyzer. A subset of hemolyzed specimens was also analyzed on Roche Modular systems.

Results: A total of 38 analytes were tested in our entire study. The magnitude of unacceptable interference was set using analyte specific Total Allowable Error limits as defined by CLIA. We found that hemolysis significantly interferes with the results of 10 analytes, lipemia with 8, and icterus with 5. We modified our practice based on the results of this study by including a comment alerting the clinician about potential interference if the interference results in >10% bias but still below CLIA TAE. In addition, we found that for acetaminophen, AST and ALT vendor claims regarding the magnitude of hemolysis interference may be misleading due to lack of studies covering the entire clinically relevant range.

Conclusion: In conclusion, in order to provide the highest quality patient care, it is very important for the clinical laboratory to fully characterize HIL interferences in-house and not rely completely on manufacturers' guidelines. This will ensure that the laboratory provides clear guidance to the clinician and prevent unnecessary re-draws in challenging, critically ill patients. Most importantly, it will enable the clinician to provide appropriate treatment in a timely manner. Our final analysis of impact of our practice changes indicated that by implementing precise HIL cut-off specific rejection criteria, approximately 400 re-draws per month due to hemolysis and up to 300 re-draws per month due to icterus were prevented.

A-275

Development of a Reporting Algorithm to Facilitate the Release of LDH Results from In Vivo Hemolyzed Samples

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Background: Hemolysis introduces a significantly positive bias in the LDH results and is the most frequent reason for cancellation of the LDH testing in our laboratory. Hemolysis can occur in vitro due to inappropriate blood collection, delivery, processing, and storage or in vivo because of hemolytic diseases. Rejection of in vivo hemolysis samples is considered malpractice. The subsequent follow-up calls and multiple sample recollections often interrupt clinical workflow and increases the turnaround time for patient results. In general, patients with in vivo hemolysis should have decreased haptoglobin and normal potassium concentrations in the absence of any other were comorbidities that change their concentrations. The objective of this study is to establish an algorithm for reporting reliable LDH results and to reduce the incidence of in vivo hemolysis-related cancellation.

Methods: The Abbott ARCHITECT c Systems were used in this study. Hemolysis index (HI) was assessed and expressed in ordinal values 1, 2, 3, and 4. Sixty four serum samples with elevated LDH and HI greater than or equal to 1 were collected and subjected to additional haptoglobin and potassium assays. LDH results were triaged based on HI and a cutoff value for haptoglobin (8 mg/dL) or potassium (5.1 mmol/L), respectively.

Results: The haptoglobin concentrations in 28 out of 64 specimens were less than 8 mg/dL, including 23 patients with sickle cell anemia, 2 other hemolytic anemia, and 3 patients without hemolytic anemia. The remaining 36 specimens had haptoglobin levels of greater than or equal to 8 mg/dL. Among them, 33 samples were from patients with non-hemolytic diseases and only 3 samples were from sickle cell disease patients. The sensitivity, specificity, positive and negative predictive values of haptoglobin in detection of in vivo hemolysis were 89.3%, 91.7%, 89.3%, and 91.7%, respectively. In contrast, only 2 out of 28 patients with hemolytic diseases and 4 out of 36 patients without hemolytic diseases had potassium concentrations greater than 5.1 mmol/L. The sensitivity, specificity, positive and negative predictive values of potassium in detection of in vivo hemolysis were 92.9%, 11.1%, 44.8%, and 66.7%, respectively.

Conclusion: We have developed an algorithm using serum hepatoglobin as a reflexive test for patients with HI greater than or equal to 1 and elevated LDH to identify in vivo hemolysis. Based on the preliminary data, approximately 39.1% unnecessary cancellations of LDH testing can be avoided using this algorithm. However, serum potassium is not a specific marker for identifying in vivo hemolysis.

A-276

Evaluation of the Short and Long Term Stability of Common Clinical Chemistry Analysis for Human Biobanking Specimen

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Background: The necessity of biobanking is growing more and more nowadays for clinical research. Since most of the researches with biobanking specimen are for later use, the stability of specimen is the most important factor for maintaining quality of research and biobanking. However, there are few researches on the stability of biobanking specimens, particularly long term stability. This study performs the evaluation of the short term and long term stability of common clinical chemistry tests for biobanking specimen.

Methods: Blood specimens are drawn from healthy volunteers. For short term evaluations, specimens are stored in four different temperatures (20, 4, -20, -70 degree of Celcius), and analytes are measured at basal, 2, 6, 24, 48, 72 hours and 1 week for 28 kinds of analytes including calcium, phosphorus, glucose, urea nitrogen, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, gamma GT, creatinine, sodium, potassium, chloride, total carbonates, iron, total iron binding capacity, triglycerides, HDL, LDL, Immunoglobulin G, A, M, C3 and C4. For long term evaluations, specimens are stored in 2 different temperatures (-20 and -70 degree of Celcius), and analytes are measured at 1, 3, 6 months, 1 and 2 years intervals for same analytes as short term evaluations. Percent differences from basal level for each analyte are evaluated.

Results: For short term evaluation, percent changes on all analytes for all temperatures shows less than 10 % from basal levels which are within the range of total error of laboratory tests. However, for long term evaluations, alkaline phosphatase, AST, and ALT shows percent changes greater than 10 % from 6 months storage. Particularly, result changes on -20 degree of Celcius are much greater.

Conclusion: From this study, we can predict how biobanking specimens can be used in clinical researches. Furthermore, alkaline phosphatase, AST, and ALT can be suggested as easily available biomarkers for evaluation of the stability of biobanking specimen.

A-278

Icterus (Total Bilirubin) Measurement Without Reagent and *In-situ* Bias Correction To Chemistry Assays

B. Lee, S. Park. *Samsung Electronics, Suwon, Korea, Republic of*

Background: One of the pre-analytical factors related to sample integrity, "Icterus (Total Bilirubin)", is both a clinical parameter and a major interference to chemical assays. The spectrophotometric interference is due to the fact that the absorption of Total Bilirubin and chromophore typically used in reaction with hydrogen peroxide overlap. Many commercial chemistry analyzers measure Total Bilirubin with reagent assay and add typical additives, such as Bilirubin oxidase, to remove the icteric interference. Our research provides the direct measurement of Total Bilirubin without reagents and its application to remove the icteric interference in the other chemistry assays by *in-situ* correction in a thin film-based clinical chemistry analyzer.

Methods: The thin film-based clinical chemistry analyzer is composed of a spectrophotometer-installed reader and a thin film-based cartridge with one sample injection port and 16 micro-wells connected by microfluidic channels. The dimension of single well is 1.2 x 2.0 x 0.15 mm (length/width/ height) and the volume is 0.32 uL. First, Total Bilirubin value without reagent (TBIL_B) for plasma samples was measured in the blank well by a dose-response curve between the blank-well absorbance for plasma sample and reference TBIL concentration. The blank-well absorbance was calculated by the combination of multiple wavelengths (450, 535, 630, 810 nm). Second, we verified *in-situ* bias correction algorithm of icteric interference in the other chemistry assay by using blood urea nitrogen (BUN) assay and plasma samples (4.9 ~ 88.2 mg/dL). The absorbance of BUN assay was measured in one reagent well and the absorbance of Total Bilirubin was measured in one blank well in the same cartridge simultaneously. The *in-situ* bias correction was done by using the explicit removal of the icteric interference from BUN absorbance, such as OD(BUN assay) - alpha x OD(TBIL_R) and the BUN values were evaluated based on the reference values before and after correction.

Results: For 42 plasma samples (0.17 ~ 10.6 mg/dL), TBIL_B shows the Passing-Boblok regression of Y=1.03X-0.06 (X; reference, Y; our system), and the correlation coefficient of R=0.9981 (P<0.0001). For the case of the narrow TBIL concentration range (0.17 ~ 4.16 mg/dL), the results are Y=1.04X-0.07 and R=0.9947 (P<0.0001). By using BioRad MultiQual controls, the precisions were evaluated as 5.4 %CV for 0.61 mg/dL of TBIL and 2.5 %CV for 7.00 mg/dL. The in-situ bias correction algorithm was verified by 49 plasma samples (4.9 ~ 88.2 mg/dL of BUN measured by Cobas P-modular). The correlation coefficient for non-corrected BUN is R=0.9792 (95%CI : 0.9632 to 0.9883) and, for corrected BUN, R=0.9938 (0.9889 to 0.9965). The ranges of absolute bias based on the reference values are 0.01 to 18.52 for non-corrected BUN, and 0.06 ~ 5.97 for corrected BUN.

Conclusion: This TBIL measurement without reagent can be one of alternative methods in real clinical usage because it is simple and cost-effective. The *in-situ* bias correction algorithm was verified for BUN measurement and it shows significant reduction of absolute bias compared to non-corrected BUN value. This algorithm can be extended to the other chemistry assays, which have highly icteric interference.

Tuesday, August 2, 2016

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

Hematology/Coagulation

A-280

Performance Evaluation of the Beckman Coulter DxH 500 vs. the COULTER HmX Five-part Differential

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Introduction:

The DxH 500 analyzer from Beckman Coulter is a quantitative, multi-parameter, automated hematology analyzer for in-vitro diagnostic use in clinical laboratories. These clinical laboratories include low-volume hospitals, small reference laboratories, and physician's office laboratories. The DxH 500 is used to identify the normal patient with normal system-generated parameters from patients with abnormal parameters and/or flags that require additional studies.

The DxH 500 analyzers identify and enumerate the complete blood count (CBC) parameters and five-part differential in only 12 microliters (µL) of whole blood (venous or capillary) sample types making it ideal for pediatric and geriatric populations.

Methods:

The purpose of the study is to evaluate the DxH 500 performance in a clinical lab setting and to assess acceptance of the instrument. We utilized our established method, the COULTER HmX, as the comparator system. Simple Deming regression were applied for method-comparison data analysis.

Results:

Using Deming approach to estimate regression parameters on 436 specimens, the correlation coefficient for the five-part differential percentage were as follows: Neutrophils = 0.990; Lymphocytes = 0.993; Monocytes = 0.939; Eosinophils = 0.882; and Basophils could not be estimates since most of the results were near zero. The WBC Differential parameter regression scatterplots are included in Figure 1.

Figure 1. WBC Differential Parameter Regression Scatterplots

DxH 500 vs. COULTER HmX

Conclusion: The DxH 500 is a small footprint five-part differential hematology analyzer designed for low-volume laboratories that provides differential results comparable to larger hematology systems.

Footnotes

[1] K. Linnet. Stat Med. 9,1463-1473.1990

A-281

Reliability Proof of the Beckman Coulter DxH 500 System[1]

E. Lin, G. Scott. *Beckman Coulter, Miami, FL*

Background: Reliability is a key differentiator for Beckman Coulter products and is a vitally important factor for customers when selecting diagnostic instrumentation. To address the needs of the low-volume user for hematology testing, Beckman Coulter developed the DxH 500, a highly reliable automated hematology analyzer and WBC differential counter for use in the clinical laboratory. Reliability represents the probability of components, subsystems and system to perform intended functions for a desired period of time without failure in specified environments with a confidence level. To ensure that the critical components and the system meet or exceed the reliability requirements, the reliability testing of the DxH 500 was comprised of multiple test stages with rigorous test plans and methods.

Methods: To efficiently identify and correct all potential malfunctions and design weakness, reliability testing focused on hardware issues, confirmed software, system, and user interface performance. A reliability growth model was developed using Army Material Systems Analysis Activity (AMSAA) for reliability improvements and predictions after design changes and corrective actions. Prior to the DxH 500 commercial release, external reliability studies were conducted at 21 clinical sites across five continents and multiple countries to ensure the reliability requirement was met or exceeded. **Results:**

To demonstrate an ESC (Emergency Service Call) rate of < 2 per instrument per year with 80% confidence level, up to 1 ESC was allowed, the total accumulative test time of 294 days and 12,558 cycles are required.

The actual accumulative test time from the 21 instruments were 1,342 days and 36,932 cycles with just 1 ESC incident reported. Therefore, the results had surpassed the test criteria. In addition, there were no hardware parts replaced on any of the instruments tested.

The system interruption rate due to some error events was predicted to be < 1 per 1,000 operational cycles after the corrective actions have been implemented after the external reliability testing, with the majority of the errors recoverable via the user interface diagnostic tool.

Conclusion:

The reliability of the DxH 500 was validated using in-house metrics that ensured critical components and the system met or exceeded key reliability requirements. External site testing further verified the high level of confidence in the reliability performance of the DxH 500 expected in the clinical laboratory.

[1] Not available for sale in the US

A-282

Effect of −158 Gγ (C→T) Xmn1 Polymorphism on HbF level in a goup of sickle cell disease patients from Siwa oasis Egypt

P. Moez, R. F. Moftah, H. Hamouda. *Faculty of Medicine - Alexandria University, Alexandria, Egypt*

Background: Sickle cell disease (SCD),an autosomal recessive disorder is caused by a single point mutation in position 6 of the β globin gene. The World Health Organization (WHO) estimated that each year about 300,000 infants are born with major haemoglobin disorders - including more than 200,000 cases of sickle-cell anaemia .It is the most common genetic disease in Africa, the Caribbean, the Americas, the Middle East, and India.In Oasis Siwa Egypt ,the prevalence rate of SCD (trait and anemia) is approaching 20%.Clinical severity of sickle cell disease is extremely variable. higher expression of fetal haemoglobin (HbF) in adulthood ameliorates morbidity and mortality in sickle cell disease. The −158 Gγ (C→T) Xmn1 Polymorphism -a common sequence variant in all population groups is known to influence the γG gene expression. It predisposes carriers to increased Hb F concentrations in particular when they are under conditions of erythropoietic stress ameliorating the clinical phenotype. The present study aims to investigate the frequency of −158 Gγ (C→T) Xmn1 Polymorphism and its association with high HbF level in sickle cell disease patients from Siwa oasis in Egypt .

Methods: This study was performed on 65 SCD cases and 65 age and sex matched healthy controls. Both cases and controls were selected out of a screening program conducted on primary school children in Siwa oasis by Alexandria Faculty of Medicine during years 2011- 2012. All patients and controls were subjected to complete blood count,capillary electrophoresis for the detection of HbS and HbF levels and PCR-RFLP for detection of −158 Gγ (C→T) Xmn1 Polymorphism using the XmnI restriction enzyme. The mean age of SCD patients was11.3 ± 2.4 years and controls was10.1 ± 2.5 years. Because of the influence of age on the HbF level, patients younger than five years were excluded from the study. Genomic DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). A 650-bp fragment 5' to the γG gene was amplified using the primer 5'- AAC TGT TGC TTT ATA GGA TTT T-3' and 5'- AGG AGC TTA TTG ATA ACC TCA GAC-3'.

Results: Genotype frequencies of the −158 Gγ (C→T) Xmn1 Polymorphism among SCD cases revealed that 84.6% of cases were homozygous for the wild-type allele (CC) and 15.4% were heterozygous (CT), The genotype frequencies among controls were 83.1% homozygous for the wild-type allele (CC) and 16.9% heterozygous (CT). In cases and controls there was no significant difference between the wild-type and heterozygous genotypes as regard HbF level.

Conclusion: From the current study we conclude that the −158 Gγ (C→T) Xmn1 Polymorphism has no effect on HbF level in sickle cell disease patients from Oasis Siwa Egypt. A wide range of HbF was obtained both in the presence and absence of this site. Further studies with a larger sample size are needed for a better understanding of the association between the −158 Gγ (C→T) Xmn1 Polymorphism and Hbf level in sickle cell disease patients from Siwa Oasis .

A-284

IKZF-1 and BCR-ABL gene expression signatures in acute lymphoblastic leukemia

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BCR-ABL1 gene fusion [t(9;22)] is rare in B-cell-progenitor acute lymphoblastic leukemia (pB-ALL) children but frequent in adults and it is associated with poor outcome. Microarray expression data showed that a group of high-risk patients, negative for BCR-ABL1, presents similar expression profiles to the BCR-ABL1 carriers, therefore becoming known as BCR-ABL1-like. In the past decade, studies have tried to define genetic hallmarks of BCR-ABL1-like, and one of them is the presence of IKZF1 deletions. The IKZF1 gene is an essential transcription factor in hematopoiesis involved primarily in lymphoid differentiation. IKZF1 deletions are highly incident among BCR-ABL1-positives and BCR-ABL1-like cases. Some studies have shown that IKZF1 deletions are significantly associated with increased relapse rate and adverse events in both pediatric and adult patients. Aims: To gain further comprehension of the biology of these genetic aberrations, we compared the gene expression signatures of BCR-ABL gene fusion and IKZF1 deletions inpB-ALL. Patients and Methods: Thirty-four Brazilian pB-ALL patients (25 adults and 9 children) were retrospectively and non-consecutively included in this analysis. Ten of them carried a BCR-ABL gene fusion, six of whom also carried an IKZF1 deletion, and four carried IKZF1 deletions only. RNA was extracted from bone marrow samples obtained at diagnosis using PAX gene Bone Marrow RNA (QIAGEN/BD). RNA integrity was determined with Bioanalyzer2100 (Agilent Technologies). Gene expression was carried out using the Low Input Quick Amp Labeling kit One Color and the Sure Print G3 Human GE 8 x 60K array (Agilent Technologies). Data were extracted with the Feature Extraction Software v7.5 and normalized using the Gene Spring software v12.5 (Agilent Technologies). Machine-learning supervised analysis was performed in order to build a classifier based on the differential gene expression signatures from carriers and non-carriers of each alteration. The K-nearest neighbors prediction algorithm was selected to identify the 40 informative probes to build the classifiers. Performance of each prediction model was assessed by leave-one-out cross-validation assay through the Gene Pattern platform (Broad Institute).DAVID v6.7 software (NIH) was used for functional annotation analysis. Results: The prediction model based on the gene signature established for IKZF1 deletions had accuracy and precision rates of 93% and 89%, respectively. For BCR-ABL presence, accuracy and precision were 93% and 100%. Hierarchical clustering analysis using probes selected for IKZF1 grouped patients carrying deletions in two neighbor clusters, one of them also included in a sub-cluster patients who were positive for BCR-ABL but negative for IKZF1 deletions. When using probes selected for BCR-ABL, all fusion carriers were grouped in a separate single cluster. Five genes (12.5%) were common to both IKZF1 and BCR-ABL gene signatures: SNORA26, C8orf38, AGFG2, FAM78A and SLC9A9. Both IKZF1 and BCR-ABL signatures contained genes involved in GTPase activity regulation, the function annotation of these clusters, though, were not statistically significant when p-values were corrected. Conclusion: Although IKZF1 deletions are a common finding in BCR-ABL-like patients, we were able to identify distinguished gene signatures for IKZF1 deletion carriers and BCR-ABL carriers. The role of these genes in pB-ALL need to be further investigated.

A-285

Impact of serum free light chains in the screening of acute kidney injury of unknown origin

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Background: The "International Kidney and Monoclonal Gammopathy Research Group (IKMG)" have defined an algorithm that included the quantification of serum free light chains (FLC) for the screening of monoclonal gammopathies in the study of acute kidney injury (AKI) of unknown origin. This algorithm allows us a rapid identification of a monoclonal FLC as the possible cause of a tubular interstitial process:

1.Clonal FLC>500 mg/L: probable FLC tubular interstitial pathology. Requires hematology work-up and initiation of disease-specific treatment to reduce serum FLC levels.

2.Clonal FLC<500 mg/L: alternative monoclonal FLC pathology (amyloidosis, light chains deposition disease, cryoglobulinemia) or incidental MGUS. Requires renal biopsy.

3.Absence of clonal FLC: AKI of another cause.

Our aim is to show the utility of this algorithm in the study of AKI of unknown origin.

Methods: Descriptive study of eight patients with AKI of unknown origin where this algorithm was applied. Serum FLC were quantified by the assay Freelite (The Binding Site).

Results: The results are shown in the table.

Case	Patient findings	Serum FLC	IKMG Algorithm	Diagnosis
Female, 74 years	AKI, proteinuria, edema	kappa=17.7 mg/L, lambda=180 mg/L, ratio=0.09	Positive Clonal FLC<500 mg/L	Primary Amyloidosis
Female, 75 years	AKI, anemia, back pain	kappa=22.4 mg/L, lambda=3510 mg/L, ratio=0.01	Positive Clonal FLC>500 mg/L	Lambda Light Chain Multiple Myeloma
Male, 58 years	AKI, proteinuria, dyspnoea	kappa=81.5 mg/L, lambda=82.3 mg/L, ratio=0.99	Negative Absence of clonal FLC	IgA Nephropathy
Female, 82 years	AKI, proteinuria, edema	kappa=477 mg/L, lambda=23 mg/L, ratio=20.73	Positive Clonal FLC<500 mg/L	Light Chain Deposition Disease
Male, 51 years	AKI, proteinuria, bone pain	kappa=3600 mg/L, lambda=6.74 mg/L, ratio=534.12	Positive Clonal FLC>500 mg/L	IgA Kappa Multiple Myeloma
Male, 53 years	AKI, proteinuria, hematuria	kappa=12.1 mg/L, lambda=13.9 mg/L, ratio=0.87	Negative Absence of Clonal FLC	IgA Nephropathy
Male, 81 years	AKI, back pain	kappa=332.5 mg/L, lambda=29.4 mg/L, ratio=11.31	Positive Clonal FLC<500 mg/L	Monoclonal Gammopathy of Renal Significance
Female, 67 years	AKI, proteinuria	kappa=19810 mg/L, lambda=5.9 mg/L, ratio=3357.67	Positive Clonal FLC>500 mg/L	Kappa Light Chain Multiple Myeloma

Conclusions: Nephrotoxic serum FLC can cause a progressive and irreversible kidney damage in patients with AKI of unknown origin. The IKMG algorithm is easy and quick that can help us to guide the study of a patient with AKI.

A-286

Comparative Study of the Sysmex CS-2100*i* and CS-2500 Coagulation Analyzers

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Background: The objective of this study was to compare the performance of the Sysmex® CS-2100*i* System and the new Sysmex CS-2500 System, automated coagulation analyzers from Siemens Healthcare, using different Siemens reagents and applications. Performance characteristics of the two systems for prothrombin time (PT sec and INR), activated partial thromboplastin time (APTT), fibrinogen, antithrombin (AT), D-dimer, and coagulation factor VIII (FVIII) were evaluated.

Methods: Result comparability of the two devices was investigated using previously frozen clinical samples purchased from commercial vendors. Method comparison studies were carried out at Sysmex Corporation, Kobe, Japan, according to CLSI guideline EP09-A3. The method comparison was based on a total of 2184 results (PT_{sec}: n = 302, PT_{INR}: n = 300, APTT: n = 304, fibrinogen: n = 300, AT: n = 301, D-dimer: n = 302, and FVIII: n = 375) distributed over the clinical reportable range (CRR).

Precision studies were performed according to CLSI guideline EP05-A2 and followed the scheme of 20 x 2 x 2 testing. 55 samples (PT_{sec}: n = 10, PT_{INR}: n = 9, APTT: n = 7, fibrinogen: n = 9, AT: n = 7, D-dimer: n = 6, and FVIII: n = 7) covering important medical decision points and the total CRR were used. The complete data set contained 13.200 results (4.400 for each instrument).

In addition, performance data for the Sysmex CS-2500 System regarding linearity and limit of quantitation (LOQ) for fibrinogen, AT, D-dimer, and FVIII were determined according to CLSI guidelines EP06-A and EP17-A2 respectively.

Results: Analysis of method comparison data by Passing-Bablok regression and difference plot revealed very good agreement of the Sysmex CS-2500 System to the Sysmex CS-2100*i* System, showing slopes between 0.990 and 1.026 and correlation coefficients ≥0.998. CVs for between device precision varied from 0.00 to 6.46%, with a median CV of 1.07% (depending on application, sample, and instrument).

Conclusion: Method comparison results for the two systems were in very good agreement. Precision for the new devices/reagents combination showed low CV values and linearity, and LOQ proved to be adequate for use of the Sysmex CS-2500 System in the clinical routine (in combination with the reagents/applications tested). Combined with enhanced functionality and ease of use compared to the Sysmex CS-2100*i* System, the Sysmex CS-2500 System provides improved performance, quality, and efficiency to clinical laboratories.

The products/features mentioned here are not commercially available in all countries. Due to regulatory reasons, their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.

Sysmex is a trademark of Sysmex Corporation.

A-287

Genome-wide analysis of molecular characterization and classification in Myelodysplastic syndromes (MDS)

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Background: Myelodysplastic syndromes (MDS) contain a group of bone marrow disorders with massive variability of cytogenetic abnormalities of significant prognostic and therapeutic development importance. The molecular pathogenesis of the syndromes is poorly understood. Conventional metaphase cytogenetics (MC) reaches 10% sensitivity combination with Fluorescence In Situ Hybridization (FISH), can detect only around 50% of primary MDS, and are the focus of attention at clinical research society.

The International Prognostic Scoring System (IPSS) is the most widely used prognostic scoring system, which incorporates karyotype (MC & FISH). IPSS has been the most critical in prognostication system with clinical needs to develop innovative scoring systems, as well as continuing efforts to improve itself.

The availability of advanced molecular techniques, such as SNP-array, has allowed the discovery of additional genetic mutations for improving better diagnosis of MDS, as well as developing an enhanced prognostic system to guide therapy selection.

Objective: To discover novel MDS specific genomic mutations and develop a classification model for a better prediction of the individual prognosis for MDS.

Methods: Bone marrow aspirates (total N=208) were collected from MDS patients with chromosomal abnormalities detected on standard metaphase karyotyping. Sample preparation and microarray analysis were followed by manufacture's manual. Segmented copy number variations were calculated from SNP-array data using manufacture's power tool suite. They were further processed to illustrate the large scale genomic alterations (>4 Mbp) and clusters. Novel alterations were identified using statistical algorithms developed in house.

Results: Total 7269 chromosomal deviations were observed with 3510 segment gain abnormalities and 3759 loss abnormalities from the dataset. The most of the gain abnormalities were detected at chromosome 8 and chromosome 1, meanwhile chromosome 5 and chromosome 7 were harbored the most of the losses.

Through detailed analysis of the deviations, thousands genes were affected including 225 proto-oncogenes or tumor-suppressor genes. The well-studied MDS associated genes, such as ASXL1, CBL, DNMT3A, EZH2, KRAS/NRAS, RUNX1, SETBP1, SRSF2, TET2, TP53 and U2AF1 were in the distressed gene list. Aberrant methylation of tumor-suppressor genes are drivers of MDS pathogenesis. Many epigenetic regulation associated genes were discovered in this study also.

Subsequently, eight sub-groups were proposed via an in-depth analysis by clustering of genome-wide alterations. The sub-groups are clustered and characterized by genetic segment variations. Such classification system demonstrates significant insights into underlying molecular mechanisms in disease development, and provide a promising dynamic tool during the disease course to monitor disease development in real time.

Conclusion: MDS is a biologically heterogeneous clonal disease. Molecular mutations in many pathways have been identified. It can be proposed that potential every MDS patients carries at least one pathogenic mutation in one gene.

Recurrent genetic mutations have been used for risk stratification, treatment selection and therapy response monitoring. The proposed classification model could be one of the highly relevant independent prognostic parameters in individual risk assessment. This study demonstrates that SNP-array based genetic mutation analysis provides a better understanding of the molecular pathogenesis of MDS. However, more clinical studies are needed to support the proposed classification model for clinical practice.

A-288

Reporting Of Critical Test Values for Hematological Parameters. A Large-Scale Laboratory Results

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Background: Critical test values can be defined as a life threatening state that requires an urgent reply, which is an important post analytical process for a clinical laboratory. The aim of this study is to evaluate reporting of the critical test values for hematological parameters in a large-scale university hospital. **Methods:** One-year data (2015) of hematology tests from Central and Emercency Laboratories of Hacettepe University Medical School was evaluated. Data comprised all reported, non-reported and dropped calls for critical test values. Among hematology parameters hemoglobin (Hb) (<7 g/dL and >20 g/dL), hematocrit (Hct) (<20% and >60%), platelet (Plt) (<20000/uL, and >100000/uL,) were taken references. Depending on the decision made by clinicians in our hospital, the critical test reporting for white blood cell (WBC) count has not been performed. Only verbal reports were taken into account. **Results:** During 2015, total 12.483 critical test reporting were performed in total 1.369.283 hematology test results (0.92%) in Hacettepe University Laboratories. Critical value reporting percentage was 94.8% for total tests whereas dropped call was only 4.2%.. Based on analytic test, reporting percentage was 92.87% for hemoglobin, 94.6% for hematocrit and 91.5% for platelets. The largest part of the reporting was to Adult and Pediatric Hematology Departments (80%) followed by Nephrology, Cardiology, General Surgery and Gynecology Departments, and Intensive Care Units (ICUs). Reporting percentage was found >98% for Hematology, Pediatric ICU, Adult Emergency Department (ED) and Neurosurgery, and >95% for Pediatric ED, Pediatric ICU, Newborn ICU, General Surgery, and Pediatric Surgery. The lowest reporting percentage was found for Pediatric Hematology and Pediatric Bone Marrow Transplant Unit as 41.8% and 40.3%, respectively. For inpatient clinics the mean percentage was 95.3% whereas for outpatient clinics it was 89.7%. The mean and median reporting times were 26.38 min. and 15 min. for hemoglobin; 25.62 min and 13.5 min for hematocrit, and 25.18 min and 14 min for platelets, respectively. For all, delay was mostly due to lack of the name of the doctor who ordered the test, the changes in secretariat of that clinic or coincidence with the lunchtime. **Conclusion:** Based on these results, the reporting percentage of critical test values for hematological parameters in our hospital was adequate, but it needs to be improved for pediatric hematology and transplant units. It would be better to shorten the median reporting time, in particular for ED and ICUs. Furthermore, clinical laboratories may determine their own recording policy by collaboration with clinics.

A-289

Use of Sigma-Based QC to Monitor Hematology and Coagulation Testing in an Expanding Multi-Instrument, Multi-Site, Integrated Healthcare System

H. H. Harrison, M. Adler, D. Kimmich, M. Knowles-Tuchman, D. Costello, R. Nowak, A. Mika, S. Rockwell, L. Sheaffer, P. Dorion, T. Grifone, J. Olson, P. Tsang, M. Wilkerson, E. Yu, &. Analytical QC Team. *Geisinger Medical Laboratories, Danville, PA*

Background: The Geisinger Medical Laboratories support an integrated healthcare system in Central PA which, over the past three years, has expanded from 11 to 17 testing sites, including 6 hospitals, 3 specialty clinics, 8 regional clinic laboratories and a core reference laboratory. The objective of this project was extension of our enterprise-wide Westgard sigma statistics and OPSpecs chemistry QC program to routine hematology and coagulation assays, including hemoglobin, hematocrit, RBC count, WBC count, platelet count, and prothrombin time. We performed monthly monitoring of sigma statistics for 1) ongoing evaluation of hematology and coagulation instruments at all integrated partner sites, and 2) monitorIng and verification of performance after conversion to system standard instruments (Sysmex, Inc. and Diagnostica Stago). **Methods**: Prior to performing sigma calculations and standardized QC evaluation, we validated systemwide reference ranges and standardized control materials and lots. The Sysmex sites used tri-level Sysmex QC materials with peer data derived from company provided Insight reports. Instrumentation included XN-9000 (3), XN-3000 (4), XN-2000 (2), XE-5000 (3), XT-4000 (1), and XS-1000 (10). The coagulation sites used the same lot of Neoplastine activation reagent and same lots of bi-level Stago QC materials for prothrombin

time assays. The Stago instrumentation included the Compact (11), Evolution (6), Satellite (3), and Start4 (3). Peer data were autogenerated for the system. **Results**: Sigma values were calculated, tabulated, and graphed monthly for the instruments and control materials listed above. Across platform average sigmas and ranges for 2015 were: Hgb 13.5 (8.6 to 15), Hct 6.0 (5.2 to 8.1), RBC 8.9 (6.5 to 13.5), platelets 10.9 (4.8 to 15.1), WBC 12.3 (8.4 to 15.7), and prothrombin 8.0 (6.4 to 9.6). To better illustrate the time and specific instrument type-related data we developed a clustered scatterplot graph that allows visualization of sigma performance over time by site and by instrument. The data show steady performance with minimal variation among instrument types which, despite minor variations in averages and ranges, was consistently above the threshold of acceptablility. As expected, the lowest sigmas were observed for the hematocrit determination. **Conclusions:** All instruments yielded consistent world class performance with average Sigmas in excess of 6, and QC precision well within CLIA error limits. Overall this level of performance allows us to use the 1-3s rule throughout, with concomitant reduction of approximately two-thirds of false QC flags and associated workflow stoppage. Monitoring sigmas over time yields an additional dimension of assessment of integrated process stability in comparison to the single "slice in time" view afforded by one-time measurements. In addition, the improved graphical representation (dashboard) allows a cleaner look at the data across the system to better manage multiple instruments and identify nonrandom excursions.

A-290

GloCyte: A New Automated Technology for Cerebrospinal Fluid (CSF) Cell Counts

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Background: GloCyte is a new semi-automated analyzer that uses fluorescent microscopy and digital image analysis to enumerate total nucleated cells (TNC) and erythrocytes (RBC) in CSF. This study aimed to (1) compare the GloCyte with manual and Sysmex XN methods and (2) determine the clinical impact of replacing the manual method by either automated method.

Methods: 57 samples from 39 patients with a variety of benign and malignant conditions were sequentially analyzed by hemocytometer, GloCyte, and Sysmex XN body fluid mode (Sysmex) within 4 hours of receipt in the laboratory. The average of duplicate manual cell counts using all 9 squares of Levy-Neubauer hemocytometers was used as the "gold standard", and compared to the Sysmex and GloCyte counts. Cytospin smears were reviewed by a pathologist and correlated with cell counts and clinical history. To assess precision, 4 samples were analyzed by all methods by 5 different technologists.

Results: Pearson correlation and Passing-Bablok regression estimates (confidence intervals) for TNC are shown in the table. In comparison to the manual method, Sysmex had a positive proportional bias, whereas GloCyte did not. When 0-5 TNC/µL was used as the reference range, sensitivity was 97% for GloCyte and 100% for Sysmex. Specificity was 91% for GloCyte and 70% for Sysmex. Eight samples had RBC/TNC ratios that suggested traumatic tap or intra-cranial hemorrhage by all methods. 19 samples were from patients with meningitis or encephalitis, all of which would have been similarly classified by all 3 methods. The precision for GloCyte and Sysmex were consistently better than manual counts, with Sysmex slightly better than or equal to GloCyte.

Conclusion: GloCyte TNC counts have less variability than manual counts and appear to be more accurate than Sysmex. Replacing manual TNC and RBC counts with either automated method would improve consistency of results without compromising diagnostic accuracy.

Regression Estimates for GloCyte and Sysmex TNC Compared to Manual Method

	R	Intercept	Slope
GloCyte	0.988	0.00 (-0.22 - 0.95)	1.049 (1.000 - 1.238)
Sysmex	0.980	0.356 (-0.462 - 0.959)	1.205 (1.091 - 1.667)

A-292

Performance Evaluation of a New INNOVANCE Heparin Assay* for the Quantitative Determination of Both Unfractionated Heparin and Low-molecular-weight Heparin Using a Hybrid Calibration Curve

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Background: Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) are frequently used as prophylactic and therapeutic anticoagulants. They considerably accelerate the inactivation of thrombin and coagulation factor Xa by antithrombin. Therefore, they are used in prevention and treatment of venous thromboembolism, in certain types of coronary artery syndrome, and in thrombotic stroke. **Objective:** The objective of this study was the performance evaluation of the INNOVANCE Heparin assay against a similar assay and on different analyzers. The INNOVANCE® Heparin assay* from Siemens Healthcare uses a hybrid curve that enhances patient safety by eliminating errors due to sample mix-up in treatment with either UFH or LMWH. **Methods:** For performance testing of the assay, we conducted multicenter studies with 313 UFH and LMWH samples on the BCS® XP System (Siemens Healthcare) and compared the results with those of the HemosIL Liquid Anti-Xa assay on the ACL TOP system (Instrumentation Laboratories, USA). We measured both UFH and LMWH samples. The precision of the new assay was determined by testing both plasma pools and controls covering the entire measuring range over 20 days in two runs with two single determinations (20 x 2 x 2 scheme). Additionally, we tested the assay with 171 samples on the Sysmex® CS-2100*i* System* in comparison to the BCS XP System.

Results: The overall correlation between the INNOVANCE Heparin assay on the BCS XP System and the HemosIL Liquid Anti-Xa assay was high, with a correlation coefficient of 0.981, slope of 1.10, and intercept of 0.01. Both repeatability and total precision were below 7% for investigated controls and pools on the BCS XP System. Comparability of results obtained on the BCS XP System versus the Sysmex CS-2100*i* System was excellent (correlation coefficient: ≥0.998; slope: 1.00; intercept: 0.05).

Conclusion: The INNOVANCE Heparin assay is well suited for the measurement of both UFH and LMWH. It demonstrated excellent precision, correlated well with other commercially available assays, and showed excellent comparability among different analyzers.*Not available for sale in the U.S. The products/features mentioned here are not commercially available in all countries. Due to regulatory reasons, their future availability cannot be guaranteed. Please contact your local Siemens organization for further details.

Sysmex is a trademark of Sysmex Corporation.

A-293

Assessment of trough rivaroxaban concentrations on coagulability in a nonvalvular atrial fibrillation population

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Background: Whether trough-phase rivaroxaban concentrations provide sufficient anti-coagulation needs more study. We evaluated coagulability marker levels in the trough concentration phase in nonvalvular atrial fibrillation (NVAF) patients, and the correlation between these markers and rivaroxaban concentration.

Methods: Fifty-five Japanese NVAF patients (mean age, 70 years; CHADS2 score, 2.5) received 24-week rivaroxaban treatment of either 15 or 10 mg once daily. Of these, 26 patients had no history of anticoagulant therapy (naive group) and 29 had switched from warfarin (warfarin group). D-dimer levels, prothrombin fragment 1+2 (F1+2) levels, protein C activity, and antithrombin activity were measured at 0 (baseline), 12 and 24 weeks of rivaroxaban treatment just before the patient's regular dosing time (trough phase). For 49 patients, D-dimer, F1+2, and rivaroxaban concentrations were also measured twice between 28 and 32 weeks of rivaroxaban treatment at non-trough times to achieve a range of drug concentrations for correlation anaylsis.

Results: For the naive group, D-dimer and F1+2 levels were significantly reduced ($p < 0.01$) from baseline at 12 and 24 weeks (Table). For the warfarin group, these values were unchanged for D-dimer and significantly increased ($p < 0.01$) for F1+2. Protein C activity was unchanged in the naive group and was increased ($p < 0.01$) in the warfarin group. Antithrombin activity was unaffected by rivaroxaban in either group. Prothrombin time ($r = 0.92$, $p < 0.0001$) and activated partial thromboplastin

time ($r = 0.54$, $p < 0.0001$) correlated with rivaroxaban concentration, but not D-dimer and F1+2 levels.

Conclusion: Rivaroxaban in the trough phase is comparable to warfarin in reducing D-dimer levels, but suppresses F1+2 levels less. Lack of correlation between rivaroxaban concentration and D-dimer and F1+2 levels suggests that the mere presence of rivaroxaban reduces their concentrations to therapeutic levels.

	Baseline	12 weeks	24 weeks
Naive group			
D-dimer (μg/mL)	0.45	0.31*	0.24*
F1+2 (pmlo/L)	235.0	164.0*	183.5*
Protein C activity (%)	104	100	103
Warfarin group			
D-dimer (μg/mL)	0.20	0.23	0.22
F1+2 (pmlo/L)	76.0	162.0*	163.0*
Protein C activity (%)	34	94*	95*

Data were presented as median value. *$P < 0.001$ compared with baseline.

A-294

Assessing Falsely Elevated Mean Cell Volume (MCV) Due to Sample Transport Delay

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Background: The MCV is the least stable CBC parameter and is known to be temperature and time sensitive. CBC samples are commonly transported to centralized clinical laboratories, during which time samples might not be consistently maintained under appropriate conditions to preserve sample integrity. We sought to determine whether transport-related variables might account for an apparently high rate of unexpected macrocytosis from a physician practice location.

Methods: The University Hospitals Health System is an integrated health network with a courier system to transport laboratory samples from many practice locations to the main hospital laboratory for analysis. We queried the Laboratory Information System for all CBC results and turnaround times (collect - result) (TAT) from a single physician practice for 6 months. Pearson correlation of MCV with TAT was done and a correction formula for MCV was developed based on linear regression. A sample stability study was performed to confirm the interference of time and temperature on MCV on the Sysmex XN system.

Results: The sample stability study confirmed that MCV of samples stored at room temperature (RT) significantly increased as early as 4hs post collection. 697 sample from one physician office had a mean MCV = 95.2 fl and mean TAT = 8.57 hr. By regression analysis, a strong linear relationship of TAT and MCV ($r^2 = 0.6$) was demonstrated when TAT was expressed as discrete time intervals of one hour and MCV was represented by the mean MCV for samples collected over each successive hour. Based on this analysis, a correction of MCV over TAT can be calculated as: $MCV_{corrected} = MCV_{tested}$ - 7.4X TAT. Using this correction formula, 67/103 (65%) of samples with elevated MCV corrected to normal. Investigation of transport practices revealed that samples were transported in cool-bags, but typically sat at RT for variable periods of time before pick-up and after delivery to central hubs. As a result of this investigation, CBC samples are now kept in the refrigerator at all physician practice locations until pick-up and cool-packs are monitored for temperature. A follow up study 3 months after this intervention showed the average MCV dropped from 95.2 fl to 93.9 fl while mean TAT remained the same . By regression analysis, no significant correlation was observed between MCV and TAT (r^2=0.068).

Conclusions: We determined that inappropriate sample handling during transport caused false elevation of MCV in some samples and developed a mathematical model to estimate the incidence and magnitude of the errors. This method could be applied to other healthcare systems and reference labs that rely on sample transport as a quality assurance tool to monitor sample integrity.

A-295

Sensitivity of Screening Panels For Monoclonal Gammopathies

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Background: Monoclonal gammopathies are caused by the production of a homogeneous monoclonal immunoglobulin or immunoglobulin part by a single clone of plasma cells. We evaluated the diagnostic sensitivity of serum protein electrophoresis (SPE), selective use of immunofixation electrophoresis (IFE) and immunoglobulin free light chain (FLC) assays that are recommended for identifying monoclonal gammopathies.

Methods: We investigated the clinical diagnosis and results of the 520 samples retrospectively sent to our laboratory on the same day for SPE, serum and urine IFE, serum FLC, over a 1-year period. These samples translated into 250 unique patients: Of the 250, 179 were excluded because their monoclonal protein had been previously recognized, leaving 71 newly diagnosed patients: 27 who had a newly identified monoclonal band on SPE and 44 who had no monoclonal band detected. 36 patients of these 71 patients were clinically diagnosed as monoclonal gammopathy. The sensitivity of each test alone or in a combination was calculated according to the diagnosis confirmed with biopsy. The detection of positive bands was confirmed by two experienced investigators, a positive FLC result was defined as an abnormal FLC κ/λ ratio (normal = 0.26-1.65).

Results: SPE, serum IFE, urine IFE and FLC assays did not perform well as single tests (72%, 86%, 44% and 75%, respectively). SPE, serum IFE, and serum FLC testing in combination is slightly more sensitive than SPE, serum IFE, and urine IFE in combination (90.6% vs. 89.6%, respectivey). Despite this observation, the difference in sensitivity for the detection of a monoclonal gammopathy comparing SPE + serum IFE + serum FLC testing versus SPE + serum IFE + urine IFE is not statistically significant (P>0.5). It can be argued that ordering only a serum sample (without a 24 h urine sample) is easier for the patient. To detect intact M-proteins, many researches use SPE, followed by serum IFE if an abnormality is detected. Although this approach is widely used, serum and urine IFE in addition to FLC provide the highest detection sensitivity (93.1%) and might be adequate without SPE, since the addition of SPE does not increase sensitivity (93.1 %). In our cohort of 520 patients, the ratio of elevated serum κ-FLC (high κ/λ ratio and high free kappa chains) in κ positive IFE samples was 67%, while the ratio of elevated serum λ -FLC (low κ/λ ratio and high free lambda chains) in L positive IFE samples was 73%.

Conclusion: In conclusion, depending on the availability of tests, combining serum IFE, urine IFE, and serum free light chain testing provides the highest sensitivity for the detection of a monoclonal gammopathy (93.1%), and addition of SPE to this panel does not increase the sensivity in detecting monoclonal gammopathies. Therefore, it is reasonable to consider IFE and serum free light chain testing as complementary tests and that each test provides important information.

A-296

Evaluation of D-Dimer calibration verification sets to validate the linearity of the assay's analytical measurement range

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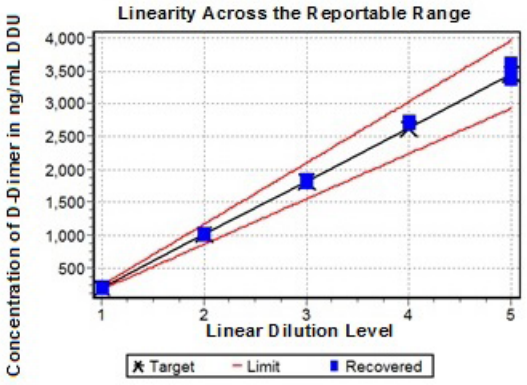
Background: Select coagulation/fibrinolysis assays were added to the College of American Pathologists (CAP) Checklist for calibration verification and Analytical Measurement Range (AMR) validation. Our objective was to address the CAP requirement for D-Dimer calibration verification and AMR validation.

Methods: D-Dimer was derived from human fibrin clots and five levels of equal delta concentrations were made in a human plasma matrix for the AMR from 150 to 3680 ng/mL, DDU, for the Instrumentation Laboratory (IL) systems and from 0.27 to 4.00 μg/mL, FEU, for the Diagnostica Stago systems. Each system used a single lot of D-Dimer reagents, calibrators and controls for these studies. We used the CLSI, EP05-A3 guidelines for Reproducibility, using the 3x5x5 (3 Lots of VALIDATE® D-Dimer x 5 days x 5 replicates/run on 1 instrument) and Precision using the 20x2x2x3 (1 Lot of VALIDATE® D-Dimer x 20 days x 2 replicates/run x 2 runs/day on 3 instruments) formats, respectively. Analyze-it, v4.60, was used for data analysis.

Linearity of sets was evaluated using MSDRx software. Three different IL systems were used at two laboratory sites.

Results: Reproducibility results for Levels 1 through 5, n = 75/Level, for the IL systems were 7.2, 4.1, 5.6, 3.7, and 4.2 total %CV. Precision results for Levels 1 through 5, n = 240 per Level, were between 2.9 and 10.0 total %CV for all Levels on the three lots of D-Dimer tested. Example of D-Dimer linearity on an IL instrument is shown below. Three different Stago systems were used at two separate laboratory sites and ran the identical study protocols. Reproducibility results were 15.5, 5.5, 4.7, 3.1, and 8.6 total %CV. Precision results were between 2.8% and 18.6 total %CV for all Levels.

Conclusion: The five level D-Dimer sets are acceptable for calibration verification for these manufacturer's claimed AMR.



A-297

Effects of Centrifugation, Freezing, Thawing, and Re-centrifugation As Confirmed By Pro-coagulant Phospholipids on Specific Coagulation Parameters

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Background: Specific coagulation markers are generally aliquoted, stored and batch analyzed for economic efficiency or because they are sent to central laboratories. Pre-analytical factors should be carefully considered for these frozen samples. The presence of pro-coagulant phospholipids derived from circulating (platelets, white blood cells, red blood cells) and non-circulating (endothelial) cells within the vasculature may affect the results. In our study, we aimed to look for the effect of centrifugation, freezing, thawing conditions and double centrifugation or re-centrifugation -as confirmed by pro-coagulant phospholipid measurements- on specific coagulation markers.

Methods:

Blood samples were drawn from 10 non-smoking healthy donors after informed consent into citrated vacutainer tubes (Becton Dickinson, NJ, USA). None of the donors had a known coagulation defect or were treated with drugs that might affect coagulation or platelet function. Fresh samples were centrifuged at 2000xg for 15 min at room temperature once or twice and analyzed within two hours for FV, FVIII, FIX, FX, lupus anticoagulant, activated protein C-resistance (APC-R), and pro-coagulant phospholipids (Diagnostica Stago, France). At the same time, plasma samples aliquoted in polypropylene tubes (Eppendorf, Germany) were frozen at −20°C for 24 h. For observing the effects of thawing and re-centrifugation, frozen samples were thawed 24 h later in a 37°C thermostat controlled water bath in a duration of 5, 15 and 30 minutes, then either mixed by gentle inverting or re-centrifuged before analysis. Relative bias percentages from the baseline (as measured by immediate analysis after centrifuging either once or twice, according to manufacturer's suggestions) was calculated for each condition and compared with the current analytical quality specifications for desirable bias from the Westgard QC.

Results:

Phospholipid particles measured after double centrifugation as recommended by the manufacturer and the results were consistent and not affected from freezing and different thawing durations (all<±4%). Single centrifuged samples should either be analyzed immediately or centrifuged again after thawing for 5 min (-3.1% and -4.2% with respect to the baseline, respectively). Freezing significantly affected the results for FV activity for each tested condition and should be analyzed fresh. For FVIII measurements, although single centrifugation is recommended before freezing, we observed that only double centrifugation before freezing (3.8%), an additional centrifugation after thawing for 5 min (-4%) or double centrifugation without extending thawing duration more than 5 min (-7.5%) had acceptable biases (<8.9%). For FIX; analysis after double centrifugation without freezing was identical to baseline. Also, re-centrifuging after single centrifugation and thawing for 5 min (-2.3%), double centrifugation with a 5 min thawing (3.5%), and re-centrifugation after a 5 min thawing of frozen sample with double centrifugation (0.5%) gave acceptable results. FX seemed to be stable under every condition. Although double centrifugation is recommended for lupus anticoagulant assay, it was stable in each condition including single centrifugation (biases range between 0.003-0.78%). On the other hand APC-R should be analyzed fresh.

Conclusion: Increasing storage time will facilitate sample processing from off-site laboratories. In our study we demonstrated that the different storage and thawing conditions might affect coagulation testing. Laboratories should consider the pre-analytical variables accordingly during analysis and interpretation.

A-299

Standardization and implementation of an eight color panel for flow cytometric immunophenotyping of bone marrow samples

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Background: Flow cytometry is a method that allows the multiparametric analysis of suspended cells. Recent advances in the use of flow cytometry made it possible to diagnose some hematopoietic pathologies by combining several antibodies in a single tube to evaluate all markers (antigens) of interest in the same sample fraction. Based on the Euroflow consortium, we have implemented an 8-color panel and new instrument standardization procedures for the immunophenotyping of bone marrow samples. **Objective:** To improve immunophenotyping processes and reduce overall time spent from instrument setup to sample acquisition and analysis by implementing new standardized procedures for instrument setup and an 8-color panel to evaluate the maturation of cell lineages in bone marrow samples. **Methods:** 20 bone marrow samples from different donors and with normal cell distribution were evaluated under two different protocols: **1)** 3- or 4-color panels manually compensated and multiple tubes per sample; **2)** 8-color panel automatically compensated and a single tube per sample. For the first protocol, three tubes were used to evaluate each sample: Tube 1, for granulocyte maturation (CD11b-FITC/CD13-PE/CD45-PC5); Tube 2, for erythrocyte maturation (CD71-FITC/CD36-PE/CD45-PC5); and Tube 3, for monocyte maturation and detection of CD34+ immature cells (CD64-FITC/CD34-PE/CD14-PC5/CD45-APC). The second protocol used a single tube to evaluate maturation of granulocytes, monocytes and erythrocytes as well as to detect CD34+ immature cells (CD36-FITC/CD13-PE/CD34-PerCP-Cy5.5/CD14-PE-Cy7/CD11b-APC/CD71-APC-H7/CD64-V450/CD45-V500). All samples were acquired on a three-laser BD FACSCanto II™ instrument with FACSDiva™ v6.1.3 software (BD Biosciences, San Jose-CA, USA). Instrument setup was performed with BD™ Cytometer Setup & Tracking (CS&T) beads and compensation was defined with BD™ CompBeads. Data files were analysed using Infinicyt v1.8 software (Cytognos S.L., Salamanca, Spain). **Results:** Following standardized procedures, automatic compensation of the 56 possible overlays from the 8-color panel (8 x 7 compensation matrix) was easily defined and in reduced time. It also hapened to be more reproducible than procedures used in the 3- or 4-color panels, therefore reducing the frequency in which a new compensation matrix needed to be defined (daily for 3- or 4-colors vs. monthly for 8-colors). Average time spent with data acquisition was measured in seconds (s) and results are expressed as mean ± SD. Protocol 1: 108s ± 148,7 ; Protocol 2: 35s ± 49,6. The expression pattern of the main cell populations was also compared by evaluating their Mean Fluorescence Intensity (MFI) and are reported as mean ± SD in Protocol 1; mean ± SD in Protocol 2. CD14+CD64+ (5.09 ±3,62 ; 4.99 ±3,60); CD71+CD36 (10.88 ±7,66; 10.14 ±7,15); CD11b+CD13 (64.49 ± 13,53; 65.12 ± 13,24); pan-leukocyte gating CD45+ (13.92 ± 10,48; 14.36 ± 10,45) and CD34+ immature cells (0.55 ± 0,45; 0.66 ± 0,45). Analysis time per sample was also improved since the number of data files was reduced from 3 tubes to 1 between protocols. **Conclusion:**These results demonstrate an improved overall productivity with the 8-color panel compared with the 3- and 4-color panel. Data quality was comparable between the protocols but the 8-color panel and its standardized procedures improved the efficiency by reducing time from instrument setup to data acquisition and analysis.

A-300

Hope Hemoglobin interfering with the measurement of Hba1c by HPLC ion exchange

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Background: Hemoglobinopathies involve genetic changes that determine hemoglobin variants which could produced clinical variations in their carriers. The main form of hemoglobin variant detection is made from the change in electric charge due to the exchange of amino acids. The Hope Hemoglobin is a variant hemoglobin. The high performance liquid chromatography (HPLC) - ion exchange, hemoglobin electrophoresis gel in alkaline pH and acid pH are used in the quantification of the anomalous fractions. Glicated Hemoglobin (HbA1c) is a molecule comprised of a glucose irreversibly bound at the terminal NH 2 (valine residue) of the beta globin chain of hemoglobin A. HbA1c is used to monitor long-term glycemic control and for diagnosing diabetes mellitus. Hemoglobin gene variants/modifications can affect the accuracy of some methods. The aim this study were demonstrated the interference and identify the variant hemoglobin in the measurement HbA1c.

Methods: Samples of 2 patients (a man, 40 years old and a japanese woman,69 years old) were sent to the laboratory for screening tests. Peripheral blood samples were collected in tubes containing EDTA at 5%. For the CBC test were used the XE-5000 hematology analyzer interconnect to the and SP.1000i analyzer (Sysmex Corporation, Japan). Serum levels of iron (Ferrozine) were performed on the Cobas 8000 P702 module and ferritin (electrochemiluminescence) in the Cobas e411 analyzer (both manufactured by Roche's Diagnostics Division, Basel, Switzerland). The Hemoglobin electrophoresis (agarose gel) in alkaline pH and acid pH using in SPIFE / REP equipment (Helena Laboratories Beaumont, Texas U.S.A.). To test the molecular DNA was isolated by QIAamp DNA Blood kit was amplified exons 1, 2 and 3, and introns 1 and 2, the beta-globin gene. The amplicons generated were subjected to DNA sequencing by the Sanger method (ABI-DNA sequencer, Model 3130 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) .The data were analyzed using the program Sequencer. The A1C on ion exchange (HPLC) Variant II and D-10 (Bio-Rad Laboratories, Inc., CA). **Results: :** The chromatograms from patient 1 and 2, it was observed A1c values of and 49.1% and 54.2% in HPLC Variant II and were confirmed in HPLC D-10. This findings showing inconsistent results with the biochemical parameters and clinical conditions. In agarose gel electrophoresis the variant fraction is faster than hemoglobin A at alkaline pH and it has a position similar to fetal hemoglobin in acid pH. In the exon 3 beta chain found a mutation where there is an exchange of G to A, position 136 with exchange of the amino acid glycine by Aspartic Acid.This features Hope hemoglobin. It has similar electrical charge to the glycated hemoglobin (A1C), performing the same retention time. Being hemoglobin with physiological changes has decreased affinity for oxygen. This mutation produce falsely elevated results because it has a similar retention time to Hba1c**.** Hope hemoglobin has increased affinity for oxygen and to be clinically silent heterozygous. **Conclusion:** Spurious HbA1c results can occur in the presence of hemoglobin variants as described in these two patients, bringing analytical challenges for the lab staff.

A-301

Redraw in The Hematology laboratory: Accuracy, Cost, and Necessity.

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Background: It is estimated that more than 70% of the clinical decisions for patient management are based on laboratory results; and ensuring that the laboratory delivers accurate results is very crucial to the patient and the doctor. However, producing accurate results require sometime a redraw of the sample either to confirm a result or because it is compromised. Beside the delay in reporting results which could cause delay in treatment for the patient, the redraw cost the laboratories an enormous amount of money, in addition to the frustration to the patients.

Method: more than 33,208 samples were collected for CBC from resident in Long-Term Care facilities, all tests were done using Beckman Coulter, DxH. Redraws were separated into preanalytic and analytic; they were separated further by the reason for the redraw. Statistical analysis was done using Analyse-it.

Results: 475 redraws were generated, 396 redraw were generated before analysis and 79 during analysis. More than 60% of the redraw before analysis were due to either missing lavender tube or drawing the wrong tube; 51.9% of the redraw upon analysis was due to platelets clumping, 46.8% due to failed delta and 1.3% was due to clotted specimen.

Conclusions: Preanalytical causes were responsible for the majority of the redraws. Laboratories should increase awareness of the effect of preanalytical factors on hematology results. Redraws can be reduced by developing clear procedure, enhancing employees training and periodic retraining, improve communication between laboratory sections, between laboratories and health care providers, and increase error detection by implementing and monitoring quality indicators. By lowering the preanalytical errors, patients will receive fast and accurate care, laboratory will save on the cost, and the relation between the laboratory and the requesting doctor/facilities will be enhanced.

A-302

Evaluation of the Sebia® CAPILLARYS™ 2 Flex Piercing analyzer for measurement of Hemoglobin A1c

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Background: Hemoglobin A1c (HbA1c) is integral for monitoring long-term glycemic control in diabetic patients and has also been recommended for use as a diagnostic test for diabetes. Methods for measuring HbA1c include immunoassays, ion exchange high performance liquid chromatography (HPLC), boronate affinity HPLC and more recently, capillary electrophoresis. It is imperative that methods used to measure HbA1c meet established performance goals and readily differentiate hemoglobin variants that may affect HbA1c quantitation. Objective: To evaluate the analytical performance of the Sebia® CAPILLARYS™ 2 Flex Piercing instrument for HbA1c measurement and compare HbA1c results obtained to ion exchange HPLC and boronate affinity HPLC methods. Methods: Precision studies were conducted using commercial quality control material (Sebia and Bio-Rad Laboratories) (intra-assay n=20, inter-assay n=40, 10 days). Limit of quantitation (n=32, 4 days), analytical measurement range (determined by mixing residual EDTA whole blood samples with high and low HbA1c concentrations) and analyte stability (n=10) were evaluated. Residual EDTA whole blood samples with the following characteristics were obtained: (i) samples without hemoglobin variants (non-variant, n=100); (ii) variants including HbC, HbD, HbE, HbS, HbAD Punjab samples; beta-thalassemia and high HbF samples; rare hemoglobin variant samples including Hb Wayne, Hb Athens Georgia, Hb Constant Springs, Hb Hope, Hb G Philadelphia, and Hb K Woolwhich (variant, n=95); (iii) samples provided and analyzed by the National Glycohemoglobin Standardization Program (NGSP, n=45); (iv) samples containing carbamylated, labile, and hemoglobin F peaks (interference, n=44). HbA1c was measured using Bio-Rad Variant™ II Turbo ion-exchange HPLC (VariantII), Trinity Biotech™ Ultra^2 boronate affinity HPLC (Ultra2), and Sebia CAPILLARYS 2 Flex Piercing capillary electrophoresis (Capillarys2) instruments. The NGSP sample group was also analyzed by the NGSP Tosoh G8 (Tosoh Bioscience) ion-exchange HPLC assay. Results: Imprecision studies demonstrated intra-assay and inter-assay coefficients of variation (CVs) of <2% across the measurement range using the Capillarys2. The CV at the limit of quantitation (3.8% HbA1c) was <2%. The verified analytical measurement range was 4.3-13.0% (slope=0.98, y-intercept =0.11, r^2=0.999). HbA1c was stable in whole blood stored ambient (20-25ºC), refrigerated (2-8ºC), and frozen (-80ºC) for 3, 7, and 60 days, respectively. Comparisons to the ion-exchange and boronate affinity HPLC methods using the non-variant and NGSP sample groups demonstrated excellent agreement (non-variant: Capillarys2=1.03(VariantII) - 0.18, r^2=0.998, range=3.1-16.5% and Capillarys2=0.99(Ultra2)-0.25, r^2=0.997, range=3.7-16.8%; NGSP: Capillarys2=1.02(VariantII)-0.12, r^2=0.998, range=4.6-10.4% and Capillarys2=1.08(Ultra2)-0.64, r^2=0.996, range=4.6-10.4%) with bias ≤0.3 units at 4.0, 5.6, and 6.5% HbA1c concentrations. Overall, samples with common hemoglobin variants including HbAS, HbAC, HbAD, HbAE, HbAD Punjab, beta-thalassemia, and HbF <15% demonstrated satisfactory agreement (bias ≤0.3 units) between the Capillarys2, VariantII and Ultra2 with the exception of HbAE and HbAD Punjab which were elevated by 0.7-1.4% HbA1c using the VariantII method. HbA1c measurements on the Capillarys2 were not affected by the presence of carbamylated, labile, or HbF fractions up to the manufacturer-established thresholds. Conclusion: The Sebia® CAPILLARYS™ 2 Flex Piercing instrument met established performance goals for HbA1c measurement with acceptable imprecision and bias compared to ion-exchange HPLC and boronate affinity HPLC methods. The Capillarys2 was also able to readily identify common hemoglobin variants and interferences.

Tuesday, August 2, 2016

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

Immunology

A-303

Molecular Allergology - A 'MUST' Clinical Laboratory Service for Correct Allergy Diagnosis and Targeted Patient Management

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Background: While measuring allergen specific immunoglobulin E (IgE) can help allergy diagnosis, it does not differentiate genuine allergens from cross-reacting species and cannot distinguish heat stable from labile proteins. Thus an incomplete assessment of an allergic patient makes clinical management suboptimal.

Method: In October 2012 our laboratory was the first in a Hong Kong private hospital to provide a molecular allergology service using Immunocap 100, Phadia (Pharmacia Diagnostics), to measure IgE to five peanut components, namely rArah1, rArah2, rArah3, rArah8, and rArah9. rArah1, rArah2, rArah3 and rArah9 are heat stable proteins, while rArah8 is heat labile and cross reacting component. Cases were referred after screening positive for allergen specific IgE by blood and/or skin prick test, or if there was a strong history of symptoms elicited by peanut.

Results: Fifty-eight out of 795 peanut allergic cases, 7.3%, were received, 38 were boys and 20 were girls, aged 1-10 years. Patients were categorized into three groups according to their responses to each of the allergen component. Group 1 was composed of fifty-two children, they were genuine peanut allergic with IgE level at RAST score 3 - 6 to either one or all of rArah1, rArah2, rArah3 and rArah9. Group 2 consisted of four children, they showed response to heat-labile cross-reacting component, rArah8, only with IgE level at RAST score 2 - 3. Group 3 had two children demonstrated no response to peanut specific markers or cross-reacting components available for testing. Different group associated with different targeted treatment regime. Group 1 children with moderate to high levels of IgE to the respective heat stable storage proteins (rArah1-3 and 9) were advised to avoid peanut entirely. As the risk of anaphylaxis is small in group 2 children with only IgE to the heat labile PR-10 protein rArah8, eating peanuts is allowed but only when they are well cooked and after a supervised oral challenge in the Allergy Centre has shown that this is safe. Since peanut allergic is not ruled out, group 3 children were advised to avoid peanut entirely.

Conclusions: Precise identification of sensitization to specific allergen components by molecular allergology can facilitate targeted patient management.

A-304

Urinary interleukin(IL)-18 as an early predictive biomarker of subclinical proximal tubular dysfunction in HIV-infected patients exposed to Tenofovir.

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BACKGROUND

Proximal tubular dysfunction (PTD) is a frequent complication of HIV-infected patients and lack of early biomarkers for PTD has impaired our ability to intervene in a timely manner considering the increasing number of HIV-infected patients in Nigeria.

AIM

In this present study, we tested if interleukin- 18 (lL-18) is a predictive biomarker for PTD in HIV-infected patients on tenofovir (TDF).

SUBJECTS AND METHOD

HIV-infected patients on tenofovir (TDF) and Non-tenofovir (N-TDF) antiretroviral therapy were recruited for this study, taking as control HIV treatment-naive patients. Exclusion criteria included pre-existing renal insufficiency and nephrotoxin use. Serial urine samples were analyzed by enzyme-linked immunosorbent assay for lL-18 in 254 HIV- infected patients at three different points (at baseline, at 4 weeks and at 12 weeks of follow-up).

RESULT

Using eGFR values, marked decrease in kidney function was detected only at 12 weeks in the TDF regime group (p=0.003) as compared to other study groups. In contrast, urine lL-18 increased at a much early time (at 4 weeks) particularly in the TDF regime group (p=0.000) followed by the naïve group (p=0.02) and continued to increase up to 12 weeks of follow up. This marked elevation is progressive.

CONCLUSION

Our results indicate that lL-18 is an early, predictive biomarker of PTD and that this biomarker may allow for the reliable early diagnosis of PTD at all times in HIV-infected patients on TDF at risk of proximal tubular dysfunction, much before the rise in serum creatinine.

Keywords: Urinary-interleukin-18; Biomarker; HIV-infected-patients; Proximal-tubular-dysfunction.

A-305

Evaluation of the AdvanSure Alloscreen max panel for detecting multiple allergen-specific IgEs in Korean allergic patients: Comparison of AdvanSure Alloscreen max panel, Polycheck Allergy, and ImmunoCAP

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Background: Allergen-specific IgE blood tests for diagnosing allergy are less affected by administration of antihistamines than skin prick and display numerical results that may be important for consecutive monitoring. The ImmunoCAP (Phadia, Uppsala, Sweden) is accepted as a standard allergen-specific IgE measurement, but it is expensive and detects only one allergen-specific IgE. In contrast, multiallergen IgE screening assays are more cost-effective because they detect IgE antibodies to common allergens in a single test. A new generation AdvanSure Alloscreen max panel (LG Life Science, Seoul, Korea) has been introduced as a multiallergen IgE screening assay. This study evaluated performance of the AdvanSure Alloscreen max panel compared with that of Polycheck Allergy (Biocheck Co, Munster, Germany) and ImmunoCAP in Korean patients with allergy.

Methods: A total of 185 patients who showed class 1 or higher results to one or more allergens on the Polycheck Allergy assay and who were diagnosed with an allergic disease were included. The AdvanSure Alloscreen max panel was performed on 185 serum samples. A total of 58 allergens with similar reagent codes in the AdvanSure Alloscreen max panel and Polycheck Allergy were selected to compare matched results, and 7,151 matched result sets were obtained. Among the 588 matched result sets with discrepancies (class 0 or 1 in one assay and class ≥ 2 in another assay), 566 available cases were tested with ImmunoCAP.

Results: Positive rates to allergens varied between the AdvanSure Alloscreen max panel and Polycheck Allergy assays. The 0-3-year age group showed high positive rates for milk, egg white, peanut, cheddar cheese, and house dust on both assays. The ≥ 3 year age group showed the highest positive rates for Dermatophagoides farina, D. pteronyssinus, and house dust. The AdvanSure Alloscreen max panel showed higher positive rates than those of Polycheck Allergy for those three allergens. Agreement rates (kappa values) between AdvanSure Alloscreen max panel and Polycheck Allergy for each of the 58 allergens were 53.5-98.4% (−0.12-0.85). The overall agreement rate (kappa value) between the two assays was 91.8% (0.53). Fifteen allergens had Kappa values > 0.6. The overall agreement rate (kappa value) between the AdvanSure Alloscreen, and Polycheck Allergy assays compared with ImmunoCAP were 64.5% (0.29), and 35.5% (−0.3), respectively, when the 566 matched result sets with discrepant results were analyzed by ImmunoCAP. Agreement between the AdvanSure Alloscreen max panel and ImmunoCAP was better than that between Polycheck Allergy and ImmunoCAP for 31 of 51 allergens.

Conclusion: AdvanSure Alloscreen max panel is a very convenient and fully automated test requiring only a 100 µl blood sample to simultaneously measure up to 92 allergens. The AdvanSure Alloscreen max panel showed better agreement with ImmunoCAP than Polycheck Allergy. Therefore, the AdvanSure Alloscreen max panel would be a useful screening tool for detecting allergen-specific IgE on multiple allergens.

A-306

The associations of *TLR7* polymorphisms in autoimmune thyroid diseases

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Background:

Graves' disease (GD) and Hashimoto's disease (HD) are autoimmune thyroid diseases (AITDs), and the prognosis of AITD is different from each patient. Toll-like receptors (TLRs), which play a key role in innate responses, have been identified as pattern-recognition receptors and trigger activation of signaling pathways to produce proinflammatory cytokines. It is possible that a substance coming from self becomes the endogenous ligand and causes chronic inflammation such as the autoimmune disease by TLR. Therefore, TLR7 which recognize single-stranded RNA (ssRNA) are possibly associated with the pathogenesis of autoimmune diseases. To clarify the association between TLR7 and AITD, we genotyped *TLR7* rs179009 C/T, rs179010 C/T, rs179019 A/C and rs3853839 G/C polymorphisms.

Methods:

Among GD patients who had a clinical history of thyrotoxicosis with positive for anti-thyrotrophin receptor antibody (TRAb), we screened patients who had been treated methimazole for at least 5 years and were still positive for TRAb (intractable GD) and patients with GD who had maintained a euthyroid state and were negative for TRAb for more than 2 years without medication (GD in remission) and patients who could not be categorized to intractable GD or GD in remission groups at the time of analysis. Among HD patients, who were positive for anti-thyroid microsomal antibody (TgAb) and/or anti-microsomal antibody (McAb), we genotyped patients who developed moderate to severe hypothyroidism before 50 years of age and were treated daily with thyroxine (severe HD), and untreated euthyroid patients who were over 50 years of age (mild HD) and patients who could not be categorized to severe HD or mild HD groups at the time of analysis. Healthy volunteers were euthyroid and negative for thyroid autoantibodies. We genotyped *TLR7* rs179009 C/T, rs179010 C/T and rs3853839 G/C polymorphisms used by PCR-RFLP method and genotyped rs179019A/C polymorphism used by Taqman PCR mathod in AITD and healthy control subjects.

Results:

Because *TLR7* gene is located in X chromosome, we analyzed the genotype and alleles of each polymorphism by sex.

1. In male, rs3853839 G allele was significantly more frequent in GD, AITD and intractable GD than that in control subjects (P=0.0062, 0.0232, and 0.0173 respectively).

2. In female, the rs179009 TT genotype was significantly less frequent in HD patients than that in control subjects (P=0.0494). In male, we could not find any differences among each group.

3. In female, rs179010 CT genotype was significantly less frequent in severe HD than that in control subjects (P=0.0479).

4. Both in male and female, we found no associations between rs179019 polymorphism and the pathogenesis of AITD.

Conclusion:

TLR7 rs3853839 polymorphism was associated with the pathogenesis of GD, and *TLR7* rs179009 and rs179010 polymorphisms were associated with that of HD.

A-308

Natural antibody assays and Immune Status Measurement

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Background: Naturally occurring common-antigen antibodies (CaAb) are raised in response to infection or environmental exposure. Antibody deficiency disorders represent the most common type of immunodeficiency disease. Measurement of antibody binding to common exposure antigens could be used as surrogate markers of immune status. Aim: Determine whether CaAb activities could be used as markers of antibody deficiency.

Methods: An enzyme immunoassay (EIA) to detect anti-IgG and anti-IgM CaAb against bacterial (Pneumococcal C-polysaccharide and Tetanus toxoid) fungal (yeast β1-3 glucan) and viral (Cytomagalovirus, CMV and Epstein-Barr virus) antigens were developed and optimised. Serum samples from primary (primary antibody deficiency (PAD; n=20) and common variable immunodeficiency (CVID; n=20) and patients with hypogammaglobinaemia (multiple myeloma; MM, n=20) patients were analysed and compared to healthy controls (n=53). CaAb IgG was also monitored in healthy controls (n=7) for 0, 7, 14, 21, 28, 90, 180, and 360 days. Total serum IgG and IgM were measured by turbidimetric immunoassay. IgG CaAb subclass compositions were determined using subclass-specific titre matched antisera.

Results: IgG and IgM CaAb levels were detected in healthy adult controls for all 5 specificities using a single sample dilution (1/100). The intra-assay precision ranged from 1.8 to 4.5 %CV, and 0.73 to 4.9 % for inter-assay. There was a variable and weak correlation between the different IgG specificities (Spearman's Rho -0.302, p=0.0278 to Rho 0.525, p<0.001). Whereas, the IgM values were more closely correlated (Rho 0.66 to 0.84, p<0.001). IgG subclass analysis showed an IgG2 bias (>76% of total IgG) towards polysaccharide antigens. Correlation between total serum IgG and IgG CaAbs were weak (Rho -0.075 to 0.457), but somewhat higher (0.602 to 0.775) for total IgM. CaAb IgG levels were remarkably stable in healthy controls for up to one year. Although the titre of each CaAb was specific to that individual the comparable order of the 5 different antigens was maintained throughout the year. 9/10 CaAb IgG and IgM titres were significantly reduced in the PAD and CVID populations compared to controls (Mann-Whitney U, all p<0.02). Within the MM population IgG and IgM titres against the bacterial and fungal antigens was reduced compared to the control (P<0.003). However, there was no reduction for IgG and IgM CaAbs against viral antigens. Interestingly CMV IgG levels were not suppressed in any of the immunodeficiency populations. Correlations between CaAbs and total serum immunoglobulins in immunodeficient patients were moderate for IgM (Rho 0.174 to 0.627) but weak for IgG (-0.61 to 0.03).

Conclusion: We have developed a multiple EIA to simultaneously measure naturally occurring antibody IgG and IgM levels to some common microbial antigens. Our results indicate that measurement of these natural antibodies can differentiate between a normal and suppressed humoral immune system and are relatively independent of total immunoglobulin concentration.

A-309

A new improved method to Human leukocyte antigen (HLA-B27) genotyping

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Human leukocyte antigens (HLA) are the products of genes of the major histocompatibility complex. Some of these antigens are related to the presence of certain diseases. HLA-B27 allele is associated with ankylosing spondylitis (relative risk of 95%). The HLA-B27 is present in over 90% of caucasian individuals affected by this disease and can predict risk of transmission to their offspring. Increased incidence of HLA-B27 has been reported in Reiter's syndrome, uveitis, psoriatic arthritis and reactive arthritis. This antigen is not a disease marker, since it is present in approximately 10% of normal individuals. The result should be associated with the clinical and radiological findings suggestive of these diseases. Several methods have been developed for identification of HLA-B27. Initially, flow cytometry was used, but nowadays, due to their specificity and speed, molecular methods are used preferably. Among the main molecular methods disclosed for HLA-B27 genotyping are SSP-PCR, RFLP and PCR in real time. Several advantages are presented in these methods, as reduction the execution time of 5 hours in the PCR-SSP to 2 hours only in the real time, specificity of PCR primers and low cost.This work present a method developed in-house for the detection of HLA-B27 allele which combines PCR facilities in real time, as speed and sensitivity, with improvements over last published methods. The study has approved by ethics committee and the informed consent has dispensed because the type of sampling. A total of 186 samples that were requested for HLA-B27 typing were included with 139 negatives and 47 positives. The reaction was performed in multiplex containing the primers for detection of HLA-B27 and STR marker TPOX (AATG) as the internal control. The use of this marker as an internal control (IC) proved to be valid because its thermodynamic properties that increase the distance between the target and IC melting curves, making the test more reliable. HLA-B27 alleles and IC were identified with peaks at 87.32 ± 0,28°C and 76.34 ± 0,31°C, respectively. Furthermore, the modifications on the extraction method using FTA Card® provided improvements, making the process faster.One hundred and eighty six samples genotyped previously by PCR allele-specific standard method (Steffens-Nakken, 1995) were assessed by the new method, and the concordance between the two methods was 100%. We conclude that the method developed was more efficient for genotyping HLA-B27.

A-310

Evaluation of the ARCHITECT B•R•A•H•M•S PCT (Procalcitonin) Assay

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Background: Our objective is to develop and evaluate a procalcitonin (PCT) method for the ARCHITECT *i* platform family of instruments.

Procalcitonin (PCT) is a 116 amino acid protein prohormone of calcitonin. Under normal conditions intact PCT is not secreted from the thyroid and levels in circulation are very low (< 0.5 ng/mL). Circulating PCT concentrations may increase up to several hundred ng/mL in severe sepsis and septic shock. After successful treatment intervention, PCT values decrease, indicating a positive prognosis. Persistent high or further increasing levels of PCT are indicators for poor prognosis. PCT can be a useful test for diagnosis and prognosis of bacterial infection and is usually ordered along with other tests to help detect or rule out sepsis, bacterial meningitis, or bacterial pneumonia in those that are seriously ill.

Methods: The ARCHITECT B•R•A•H•M•S PCT assay is a fully automated two-step chemiluminescent microparticle immunoassay (CMIA) to determine the presence of procalcitonin in human plasma and serum. The method features a highly specific anti-h-Calcitonin rat MAB coupled to paramagnetic particles and serves to capture the PCT from the specimen. After incubation and wash, the 'sandwich' completes with anti-h-Katacalcin mouse MAB conjugated with an acridinium-derivative. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of PCT in the sample and the RLUs detected by the ARCHITECT *i* System optics.

Results: The sensitivity, imprecision, and linearity estimates utilized procedural guidance from the Clinical and Laboratory Standards Institute (CLSI). The measuring range extends from 0.02 to 100.0 ng/mL. The limit of blank (LoB) and limit of detection (LoD) are estimated at 0.001 and 0.005 ng/mL, respectively. The limit of quantitation (LoQ) defined by the lowest concentration where imprecision is less than or equal to 20% is <0.02ng/mL. Dilutions of disease state plasma demonstrated linearity from 0.02 to 115 ng/mL. The total within-lab 20-day imprecision is <5% for patient panels from 0.04 to 90 ng/mL. The auto-dilution protocol extends the upper measuring range from 100 to 1000 ng/mL

In a reference range study (n=300) with half males and half females, the 97.5th percentile was 0.07 ng/mL. The 90%CI range of the 97.5th percentile was from 0.05 to 0.06 for females and from 0.08 to 0.09 ng/mL for males. The method was compared to B•R•A•H•M•S PCT sensitive Kryptor with n=107 plasma specimens both fresh and frozen with concentrations from 0.01 to 59.13 ng/mL. The Deming slope to B•R•A•H•M•S PCT sensitive Kryptor was 1.03 with a correlation coefficient of 0.99.

Conclusion: The ARCHITECT B•R•A•H•M•S PCT assay has low imprecision, a limit of quantitation below the typical normal range of PCT, good linearity, and compares well with the B•R•A•H•M•S PCT sensitive Kryptor assay.

A-311

Capillary Electrophoresis Quantitation of Clonal Protein in the Gamma Zone

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Clonal immunoglobulins (Ig) are produced by benign and malignant disorders, mostly in the electrophoresis (ELP) gamma zone as monoclonal peaks. Accurate quantitation of clonal protein is needed for diagnosis, for gauging progression of clonal disorders, and for measuring the effectiveness of malignant disease treatment. If clonal protein is a large fraction of gamma zone protein, an accurate measure is needed to calculate the non-clonal, healthy Ig by subtraction, and to warn of a high risk of bacterial infection if the healthy Ig drops below 500 mg/dL. The most common methods of estimating clonal protein are manual, graphic methods that show significant variation among users. OBJECTIVE: To evaluate two automated procedures developed for estimating gamma zone monoclonal protein. METHOD: A common manual method for peak sizing involves "skimming" - creation of a straight baseline connecting the edges of the peak, and integration of the area between the baseline and the peak. In the automated skimming method, the peak edges are found by a curve analysis algorithm, and a third degree polynomial creates the connecting baseline. The second automated method involves fitting a clonal peak to a simulated peak and calculation of the area of the simulated peak. Most clonal peaks are similar to Gauss error probability curves. A Gaussian curve fit is used by the method reported here. The analysis uses second derivatives of points at the top of a clonal peak for fitting to a Gauss error curve, and an integral of the fitted curve. To compare the methods, ELP data from three clinical specimens with low, normal and high levels of gamma zone polyclonal Ig were downloaded from a CapillaryS(tm) analyzer. Monoclonal similar Gaussian peaks of 62, 125, 250, 500 and 1000 mg were added to the sides, shoulders or top of the gamma zones, and recovery of the added peaks was measured. RESULTS: The peak finding algorithm found 65 of the 75 peaks. It found all of the peaks on the low Ig curves, but did not find some of the smaller peaks on the steeper sides of the normal and high Ig curves. Recovery of the added peaks by the two methods was similar. Both methods handled peak distortion well. The Gaussian fit method's average recovery was +28 mg, and the skim method's average recovery was -18 mg. The Gaussian fit method performed better near the beta-gamma boundary if the beta-2 peak overlapped the synthetic peak, but worse at the top of the gamma curve due to positive covariance of the gamma curve and the Gaussian peak. CONCLUSION: In the absence of a gold standard for clonal peaks, simulated peaks of known size are excellent alternatives for method validation. The skimming method is more robust - it adjusts better to asymmetric peaks - but the Gaussian fit method is simple and is a good estimator of symmetrical peak size. Automated peak skimming is now used routinely for our ELP interpretations. Graphic illustrations are part of this study.

A-312

Diagnostic Performances of Cell Bound Complement Activation Products Stratified by ANA Titers in Systemic Lupus Erythematosus

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Background: Abnormal complement activation and elevated anti-nuclear antibody (ANA) titers are well-documented features of Systemic Lupus Erythematosus (SLE). Clinical studies have demonstrated that complement C4d deposited on erythrocytes (EC4d) and B-cells (BC4d) are sensitive and specific biomarkers for SLE. We sought to establish the performance characteristics of these biomarkers in the context of stratified ANA titers.

Methods: Anticoagulated blood was collected from 462 subjects with SLE (91% females, mean age 40 years) and 436 subjects with other rheumatic diseases (non SLE: 86% females, mean age 54 years). All subjects provided informed consent. EC4d and BC4d expression was determined by fluorescent activated cell sorting (FACS) analysis and reported as net mean fluorescent intensity (MFI). ANA was measured by ELISA (Quanta Lite®, INOVA Diagnostics, San Diego, CA) and titers were categorized as negative (<20 U), positive (20-59 U), or strongly positive (≥60 U). Positive CB-CAPs consisted of either positive EC4d (>14 Net MFI) or positive BC4d (>60 net MFI). Sensitivity, specificity, likelihood ratios (LR), and Diagnostic Odds Ratio (DOR) were calculated.

Results: Positive ANA (≥20 U) was 88% sensitive and 55% specific in distinguishing SLE from the non-SLE group. Strong positive ANA (≥60 U) was observed in 69% SLE and 22% subjects with diseases other than SLE. Positive CB-CAPs was 62% sensitive and 88% specific in distinguishing SLE from the non-SLE group (positive LR=5.1; negative LR=0.43). As presented in the Table, the performance characteristics of CB-CAPs were higher among subjects with elevated ANA, with DOR increasing from 2.6 (ANA negative) to 12.6 (strong ANA).

Conclusion: CB-CAPs levels have value in diagnosing SLE and can be particularly helpful in differentiating SLE from non-SLE in the context of high ANA titers.

Performance Characteristics of CB-CAPs Stratified by ANA Titers

ANA Status	Sensitivity	Specificity	LR + CI 95%	LR - CI 95%	DOR
Negative (<20 U)	20% (11/55)	91% (220/241)	2.3 (1.2-4.5)	0.88 (0.76-1.0)	2.6
Positive (20-59 U)	47% (42/90)	86% (84/98)	3.3 (1.9-5.6)	0.62 (0.50-0.77)	5.2
Strong Positive (≥60 U)	74% (235/317)	81% (79/97)	4.0 (2.6-6.1)	0.32 (0.26-0.39)	12.6

A-314

Screening Pharmacologically Active Compounds Against TSG-6, A Therapeutic Target for The Treatment of Asthma

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Background: Asthma is a disorder of the respiratory system that inflames and narrows the airways. One of the key factors driving inflammation in asthma is

the increased leukocyte infiltration into the airway wall. These cells are trapped in a hyaluronan (HA)-rich extracellular matrix produced by smooth muscle cells surrounding airway vasculature and bronchi. This 'sticky' form of HA is the result of a unique protein modification. The enzyme "tumor-necrosis-factor-stimulated-gene-6" (TSG-6) transfers the heavy chains (HCs) from inter-α-inhibitor (IαI) to form the pathologic variant of HA known as the HC-HA matrix. Previously our group showed that TSG-6 not only possesses the enzymatic ability to covalently bind HC to HA, it also significantly stimulates HA synthesis by airway smooth muscle cells (ASMCs) when they are challenged by a viral mimetic. Moreover, we found that TSG-6 knockout mice develop a markedly milder form of asthma, including less HA, reduced leukocyte infiltration, and lower airway hyper-responsiveness. These findings indicate that TSG-6 has a vital role in asthma pathobiology. Therefore, we proposed to lower the levels of HC-HA crosslinking by inhibiting the action of TSG-6. **Methods:** Accordingly, we have screened a total of 7927 compounds from the Molecular Screening Core at the Cleveland Clinic using a TSG-6 activity assay. Briefly, the cross-linking chemistry of carbodiimide is used to covalently attach HA to amine groups on NH-Covalink plates. 40 µL of recombinant TSG-6 solution (12.5 µL; 0.016 µg/µL in 5 mL PBS with Mg^{+2}, pH 7.4) is incubated with each compound (10 µL; 0.04 mM) for 30 min at 25° C. Then the mixture (50 µL) is transferred into the wells that already contain 50 µl of PBS having 4% human serum, a source of the HC donor IαI, followed by incubation at 37°C for 2 hours. The relative amounts of HCs transferred to HA on the plates were detected using a polyclonal antibody that binds to the HCs. An infrared secondary antibody is then used as a reporter of HC transfer. The 96-well plates were scanned on a LI-COR CLx Odyssey infrared scanner, and the relative amount of HCs per well were determined by densitometry. **Results:** Our initial results show 43 small molecule inhibitors (antagonists) against TSG-6. The percent decrease of TSG-6 activity ranged between 67% and 408%. Currently we are in the process of *in vitro* validation of 3 of the highest hit compounds. **Conclusion:** We identified 43 small molecules that inhibit HC transfer to HA via TSG-6 by screening chemical libraries in a TSG-6 activity assay.

A-315

Diagnostic challenges in the study of patients with monoclonal proteins

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Background: The detection of a monoclonal protein is fundamental in the diagnosis of patients with monoclonal gammopathies such as Multiple Myeloma, Primary Amyloidosis and light chain deposition disease. When the monoclonal protein is presented in low concentrations, it may be difficult to detect using conventional methods based on electrophoretic techniques like serum protein electrophoresis (SPE) and serum immunofixation (IFE). However, the quantification of serum free light chains (sFLC) is more sensitive than conventional methods. A quick and easy algorithm based in the combination of sFLC and SPE presents a high sensitivity in the diagnostic study of monoclonal gammopathies. We report our experience with six patients where the monoclonal protein was either undetectable or barely detectable by conventional methods.

Methods: study of six patients with suspect of monoclonal gammopathies where the algorithm (sFLC+SPE) was applied. Serum FLC were quantified by the assay Freelite (The Binding Site) and SPE were performed on Capillarys 2 (Sebia).

Results: The results are shown in the table.

Case	Clinical findings	Algorithm (sFLC+SPE)	Diagnosis
Man, 68 years	Macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia.	Small peak in SPE (0.10 g/dL), sFLC ratio very abnormal (kappa=14450 mg/L, lambda=4.9 mg/L, ratio=2949) and immunoparesis	Light Chain Kappa Multiple Myeloma Stage 3 ISS
Female, 75 years	Acute kidney injury, edema and proteinuria	SPE negative, sFLC ratio very abnormal (kappa=17.7 mg/L, lambda=1800 mg/L, ratio=0.009) and immunoparesis	Primary Amyloidosis
Male, 57 years	Intense back pain	SPE negative, altered sFLC ratio (kappa=31.6 mg/L, lambda=15.4 mg/L, ratio=2.05)	Non Secretory Multiple Myeloma Stage 1 ISS
Male, 47 years	Pathological fracture	SPE negative, altered sFLC ratio (kappa=148 mg/L, lambda=5.6 mg/L, ratio=26.3)	Light Chain Kappa Multiple Myeloma Stage 1 ISS
Female, 82 years	Acute kidney injury, dyspnoea, edema and proteinuria	SPE negative, altered sFLC ratio (kappa=477.5 mg/L, lambda=23.3 mg/L, ratio=20.49)	Light Chain Deposition Disease
Male, 54 years	Severe bone pain in the chest, anemia and thrombocytopenia	Small peak in SPE negative (1.17 g/dL), altered sFLC ratio (kappa=3.22 mg/L, lambda 4025 mg/L, ratio=0.0008)	IgD Lambda Multiple Myeloma Stage 3 ISS

Conclusions: Freelite allows us an accurate quantification of serum FLCs in the diagnostic study of patients with suspect of monoclonal gammopathies. Due to the high specificity and sensitivity of this assay, it allows us to detect the presence of small amounts of monoclonal proteins that couldn´t have been detected by conventional methods.

A-316

Performance Evaluation of Siemens ADVIA Centaur PIGF and sFLT-1 Assays

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Background: Preeclampsia (PE) is a disease found in pregnant women that is characterized by hypertension and proteinuria. PE is caused by an imbalance of the pro- and anti-angiogenic factors PlGF and sFlt-1, respectively, which can adversely impact the health of both mother and fetus. The rapid and accurate diagnosis of PE is essential for best clinical practice and maternal/fetal care. PlGF and sFlt-1, both produced by the placenta, regulate angiogenesis in the developing fetus. The sFlt-1/PlGF ratio becomes elevated when PE is present; therefore, the sFlt-1/PlGF ratio is a valuable tool to aid in diagnosing PE in pregnant women. Siemens Healthcare Diagnostics (Tarrytown, NY, U.S.) has developed ADVIA Centaur® PE assays* for detection of PlGF and sFlt-1 in serum and plasma to be used in tandem for a clinically relevant ratio of diagnostic value. The objective of this study was to evaluate the performance and demonstrate the clinical utility of the Siemens ADVIA Centaur PlGF and sFlt-1 assays.

Methods: The assays were evaluated on the Siemens ADVIA Centaur Immunoassay System for repeatability and within-laboratory precision, method comparison, linearity, limit of detection (LoD), limit of quantitation (LoQ), calibration interval, onboard stability (OBS), hook effect, and endogenous interferences. The clinical agreement with Roche ELECSYS PE assays with >150 clinically relevant samples was also evaluated. In addition, the clinical sensitivity and specificity of the assays using already-established cutoff ratios were evaluated in early gestational (≤34 weeks) and late gestational periods (>34 weeks) from normal and physician-diagnosed preeclampsia patients.

Results: The overall analytical performance of the ADVIA Centaur PE assays was excellent. The PlGF and sFlt-1assays have ranges of 10-10,000 pg/mL and 30-85,000 pg/mL, respectively. From a clinical accuracy perspective, the ADVIA Centaur PE assays demonstrated acceptable clinical sensitivity and specificity within early and late gestational windows. The repeatability was <7% CV, within-laboratory precision was <8% CV and both were linear within each assay's range with no observed hook effect. The LoD and LoQ were <10 pg/mL (PlGF) and <30 pg/mL (sFlt-1). The calibration interval and OBS for both ADVIA Centaur PlGF and sFlt-1 assays were >14 and >28 days, respectively. Less than10% endogenous interference was observed for all tested interferents for both assays. In addition, the ADVIA Centaur and ELECSYS PE assays showed strong positive (98.2%) and negative (98.8%) agreement by clinical outcome.

Conclusion: Overall, the ADVIA Centaur PE assays are a rapid and accurate tool to aid the physician in diagnosing PE. They show very good clinical agreement with

the Roche ELECSYS PE assays. These studies indicate that the ADVIA Centaur PE assays are precise and sensitive for measuring PlGF and sFlt-1 across a wide range of concentrations. *ADVIA Centaur and all associated marks are trademarks of Siemens Healthcare Diagnostics or its affiliates *, Under development. Not available for sale. Product availability will vary by country*

A-317

Comparison between two fully automated immunoassay systems for the determinations of IgA anti-tissue transglutaminase antibody in celiac disease

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Background: An endoscopy with small intestinal biopsy has generally been considered the gold-standard for celiac disease (CD) diagnosis. With increasing prevalence and awareness of CD, less invasive serologic testing would be of significant value in clinical practice to minimize biopsies especially in pediatric patients. IgA anti-tissue transglutaminase antibody (anti-tTG) is recommended as the initial test for CD. Our laboratory compared and validated the performance between two fully automated immunoassay systems on anti-tTG determinations- the INOVA Quanta Lite h-tTG IgA (INOVA) assay (INOVA Diagnostics, San Diego, CA) and the EliA Celikey IgA (EliA) assay (Thermo-Fischer Scientific, Waltham, MA).

Methods: We ran 138 serum samples on both assays and compared the results qualitatively using concordance analysis. The INOVA assays are ran on the ESP600 QuantaLyser automated platform (INOVA Diagnostics, San Diego, CA) and the EliA assays are run on the Phadia 250 analyser (Thermo-Fischer Scientific, Waltham, MA).

Results: 19 anti-tTG determinations were negative by both assays and 52 were positive by both for overall agreement of 51.4% when INOVA reference range is set as <20 U/mL. 63 anti-tTG determinations were negative by both assays and 52 were positive by both for overall agreement of 83.3% when INOVA reference range is set as <40 U/mL. 63 anti-tTG determinations were negative by both assays and 69 were positive by both for overall agreement of 95.6% when INOVA reference range is set as <40 U/mL and equivocal range in EliA is interpreted as positive.

Conclusion: Both assays demonstrate a better correlation when increasing the cut-off on INOVA. These results imply that performance difference mainly originate from the different cutoffs and implicate for test interpretation and utilization to reduce the number of unnecessary false-positive biopsies.

A-320

Profile analysis of the urea breath test and antibody tests for Helicobacter pylori in a large-scale Korean population

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Background: Helicobacter pylori (H. pylori) infection is associated with a wide range of upper gastrointestinal tract disorders including gastric cancer. South Korea has been previously known to have high rates of H. pylori infection with decreasing seroprevalence in the past decade due to improvement of general hygiene and socioeconomic status. The stool antigen test, serological markers, urea breath tests (UBT) are commonly used noninvasive tests whereas gastroendoscopy, rapid urease test and culture are invasive methods for diagnosing H. pylori infection. Among these various testing methods, we analyzed the results of the urea breath test and H. pylori antibodies in relation to the patient population characteristics.

Methods: Samples referred to a commercial laboratory for H. pylori testing between January and December 2015 were reviewed. A total of 5,004 samples were tested for ^{13}C-UBT (HeliFinder$_{cap}$), which utilizes a low-dose ^{13}C-urea capsule and breath samples collected in test tubes through a straw. Helicobacter antibody testing for IgM was done by ELISA (n=2,882) and IgG was done with a chemiluminescent immunometric assay (n=8,792). The distribution of positive results were compared among age groups and to data previously reported in the literature.

Results: The overall positive rate for the UBT method was 21.2% with a slightly lower prevalence in children and teenagers (17.9%, 18 years old or younger) but was not statistically significant (P>0.05). The seropositive rate for all tested samples was higher for IgG (50.9%) than IgM (5.46%), as expected. The seropositivity for IgG increased with age, with the highest positive rate in the 50 to 59 year-old group (61.2%). In contrast, the IgM positive rate was the highest in children and teenagers (11.1%, 18 years old or younger) and showed a steady decrease with increasing age.

Conclusion: Due to the relative feasibility of obtaining serum samples in infants and toddlers, testing for H. pylori antibodies was done in all ages (ages 0 to 93) whereas UBT testing was done in only a handful of children under 10 (n=4). The presence of the H. pylori IgM antibody has been linked to current infection and symptomatic patients and our results demonstrated the presence of an IgM antibody in children as early as 4 years of age. In addition, the overall seropositive rate of IgG in the Korean population was similar to those described in previous studies. The high level of seropositive IgG results may indicate the use of UBT as the noninvasive testing method of choice in areas or countries with a widespread H. pylori infection for discrimination between current and previous infection. The current analysis on a large-scale Korean population demonstrated interesting findings for the H. pylori IgM antibody among age groups, and may suggest the need for follow-up or monitoring in children with positive results.

A-321

Direct measurement of polyclonal IgG4 fab-arm exchange in serum

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Background: Human IgG4 molecules are dynamic and have been shown to exchange half molecules to become bi-specific IgG4 hybrid antibodies in a process termed Fab-arm exchange. This process is independent of immunoglobulin light-chain type and can result in the formation of IgG4κ\λ molecules. Bi-specific molecules cannot cross-link antigen nor elicit lymphoid cell activation. It has been proposed that this mechanism may dampen-down unnecessary inflammatory responses. Measurement of serum IgG4 hybrids has previously been limited to either monoclonal antibodies binding to known antigens or, required the purification of total IgG4 as a first step. Aim: The development of an immunoassay that can directly measure polyclonal IgG4κ\λ hybrids in serum.

Methods: We have developed an enzyme immunoassay (EIA) to detect IgG4κ\λ hybrids in serum. A specific sheep polyclonal antisera recognizing IgG4λ was raised and processed to specificity. This was used to capture IgG4λ molecules directly from serum. Bound IgG4κ\λ hybrids were then detected using a total light chain kappa conjugate. Total IgG4 was quantified with a separate EIA. This allowed the calculation of an IgG4κ\λ hybrid/total IgG4 serum ratio. Purified polyclonal IgG4κ\λ hybrids were used as a calibrator which was quantified using the Binding Site IgG4 turbidimetric immunoassay. Serum samples from patients with 4 different IgG4-related diseases; Pemphigus/Pemphigoid (n=5), Autoimmune Pancreatitis (n=9) and Primary Sclerosing Cholangitis (n=30, PSC) were analysed and compared to healthy controls (n=95).

Results: The concentrations of IgG4κ\λ hybrids were measured in 95 healthy controls. Using a 1/5,000 sample dilution the assay had a range of 18.5 – 1500 mg/L. The intra assay precision was 8.7, 5.6 and 6.9 %CV using samples that had a high (580 mg/L), medium (85 mg/L) and low (25 mg/L) IgG4 hybrid concentrations, respectively (n=16). A normal range of between 15.2 and 538.8 mg/L of IgG4κ\λ hybrids was observed in 95 healthy controls. The corresponding total IgG4 concentrations were 17.25 to 1684 mg/L. A first order polynomial fit of total IgG4 against IgG4κ\λ gave an R^2 of 0.9466 and an intercept of 0.28. The median IgG4κ\λ hybrid/total IgG4 ratio was 0.33 (range 0.16-0.94). This suggests that the amounts of IgG4κ\λ hybrid relative to total IgG4 in normal healthy controls appears to be conserved at approximately 30%. This agrees with previous work using serum fractionated IgG4. IgG4κ\λ hybrid/total IgG4 ratios were lower in IgG4 related disease samples from Pemphigus/Pemphigoid (Mann-Whitney U, p=0.023), and Autoimmune Pancreatitis (p=0.042) compared with healthy controls. However, IgG4κ\λ hybrid/total IgG4 ratios in PSC samples with either raised or normal IgG4 concentrations were similar to heathy controls.

Conclusions: We have created an EIA to directly measure IgG4κ\λ hybrids and total IgG4 in serum. The ratio of IgG4κ\λ hybrid/total IgG4 is reasonably conserved in healthy controls but may vary in particular IgG4 related-disease state populations.

A-322

Distribution of antinuclear autoantibody patterns between the year of 2014 and 2015, according to IV brazilian guidelines for autoantibodies on HEp-2 cells

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Background: Antinuclear autoantibody (ANA) is performed by indirect immunofluorescence (IIF), and a standard screening method for the presence of ANA in patient serum. With large variety of consensus included the pattern cytoplasmic Rods and Rings, nuclear quasi-homogeneous (QH) and the mixed pattern observed with the presence of anti-CENP-F antibodies. Given this general consensus, we

conducted a survey about the prevalence of all patterns found in a large support laboratory of Brazil.

Methods: Using the database we assessed the patients results for ANA screening between the year of 2014 and 2015. All tests were performed by IIF microscopy method existing patterns, it was necessary to create a Brazilian consensus in order to standardize reading, interpretation, dilution and titration for ANA. Since the first consensus, five main groups of patterns were established - nuclear, nucleolar, cytoplasmic, mitotic apparatus and mixed. In the year 2013, the IV based on the use of HEp-2 cells (Inova NOVA LITE™ IFA Substrate Slide - ANA HeP-2), as substrate and using a screening dilution of 1/160.

Results: We had a total of 956.996 tests, from all regions of Brazil, where 238.433 results were ANA positive and 718.563 negative. The six most frequent ANA pattern obtained were Nuclear fine speckled, Nuclear dense fine speckled, Nuclear coarse speckled, Mixed Pattern, Cytoplasmic reticulated speckled and Nucleolar. All other positive ANA results were distributed in 19 different patterns that are described in table 1.

Conclusion:The most frequent ANA pattern present in 10,022% patients was the Nuclear fine speckled, that can be seen in healthy individuals, but either is associated to patients with immune rheumatic disease. Due to the high sensibility of IIF assay and the great variability of patterns described, a correct clinical interpretation of ANA screening and complementary confirmatory tests are essential for physicians assertivity in diagnostic and treatment of immune rheumatic disease.

Distribution ANA patients accordingly to the pattern on the ANA-HEP2 x Brazilian 4th consensus.

Pattern	Total	%	Pattern	Total	%
Non Reagent	718563	75,085	Cytoplasmic dense fine speckled	1583	0,165
Nuclear Fine Speckled	95910	10,022	Cytoplasmic Fibrillar	1475	0,154
Nuclear dense fine Speckled	61771	6,455	Cytoplasmic few dots speckled	1372	0,143
Nuclear coarse speckled	15891	1,661	Cytoplasmic fine speckled	1248	0,130
Mixed Pattern (Pattern data not avaliable)	15677	1,638	Nuclear Mitotic apparatus (NuMA 1)	1099	0,1115
Cytoplasmic reticulated Speckled	11929	1,247	Cytoplasmic Linear Fibrillar	894	0,093
Nucleolar	10099	1,055	Polar staining speckled	761	0,08
Nuclear centromeric	6756	0,706	Type 2 nuclear mitotic apparatus (NuMA-2)	626	0,065
Nuclear homogeneous	2212	0,231	Mitotic apparatus (Intercellular Bridge)	601	0,063
Nuclear Coarse speckled reticulated	2184	0,228	Pleomorphic speckled nuclear	582	0,061
Multipe Nuclear dots speckled	1817	0,190	Cytoplasmic Rods and Rings	378	0,039
Nuclear quase-homogeneous speckled	1656	0,173	Mitotic spindle apparatus (centriole)	194	0,02
Nuclear membrane	1609	0,168	Anti-vinculin antibody	109	0,011

A-323

Performance of BhCG, Free T4 and Cortisol assays on Abbott's next-generation immunochemistry analyzer

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Objective/Background: To evaluate the analytical performance of representative immunoassays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for detection of analytes in human plasma/serum on the next-generation immunochemistry analyzer. The sample and microparticle are combined in a reaction vessel. Analyte present in the sample binds to the microparticles. In the second step, conjugate is added to the reaction vessel; the contents are mixed and incubated. After washing, pre-trigger and trigger solutions are added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A relationship exists between the amount of analyte in the sample and the RLUs detected by the optical system.

Methods: Key performance testing including precision, linearity, limit of quantitation (LoQ), and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range across which acceptable performance for bias, imprecision and linearity was met.

Results: Total imprecision, LoQ, and linearity results along with the defined measuring interval are shown for representative assays in the table below. Results versus the on-market comparator assay demonstrated a slope 0.99 - 1.01 and r = 1.00.

Assay	Total %CV	LoQ	Linearity	Measuring Interval
Cortisol Serum	≤ 3.7	1.0 ug/dL	0.4 -67.5 ug/dL	1.0 – 59.8 ug/dL
Cortisol Urine	≤ 7.9	1.0 ug/dL	0.0 -74.3 ug/dL	1.0 – 59.8 ug/dL
Stat Total BhCG	≤ 5.5	2.30 mIU/mL	1.50 to 16,424 mIU/mL	2.30 to 15,000 mIU/mL
Free T4	≤3.1	0.4 ng/dL	0.4 to > 7.85 ng/dL	0.4 to 6.0 ng/dL

Conclusion: Representative immunoassays utilizing Chemiluminescent Microparticle Immunoassay (CMIA) technology tested on Abbott's next-generation immunochemistry analyzer demonstrated acceptable precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market comparator assay.

A-324

Study of the clinical sensitivity and specificity of autoantibodies in patients with suspect of Rheumatoid Arthritis in Primary Care

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Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis. In 2009, the new RA criteria released by the American College of Rheumatology (ACR) and the European League Against Rheumatism were revised to include the measurement of anti-CCP antibodies to aid in the classification of RA. The aim of our study is to evaluate the diagnostic value of antibodies in primary care in patients with suspected RA to be remit to a specialist in Rheumatology.

Material and methods: Anti-CCP antibodies and rheumatoid factor (RF) were measured in 211 patients with suspected RA. The ACR criteria for RA were fulfilled for 106 patients. We study the diagnostic value (sensibility, specificity, positive predictive value and negative predictive value) for anti-CCP antibodies, RF and their combinations "anti-CCP and RF" and "anti-CCP or RF".

Results: Results are shown in the table.

Antibodies	Sensitivity	Specificity	PPV	NPV
Anti-CCP	66	98	95	82
Anti-CCP and RF	60	98	94	80
Anti-CCF or RF	86	81	74	90
RF	81	81	73	87

Conclusion: In primary care, the combination "anti-CCP or RF" exhibits the better diagnostic value with the higher sensitivity than the others combinations.

A-325

Prognostic value of anti-CCP antibodies in patients with Rheumatoid Arthritis under treatment with disease modifying antirheumatic drugs

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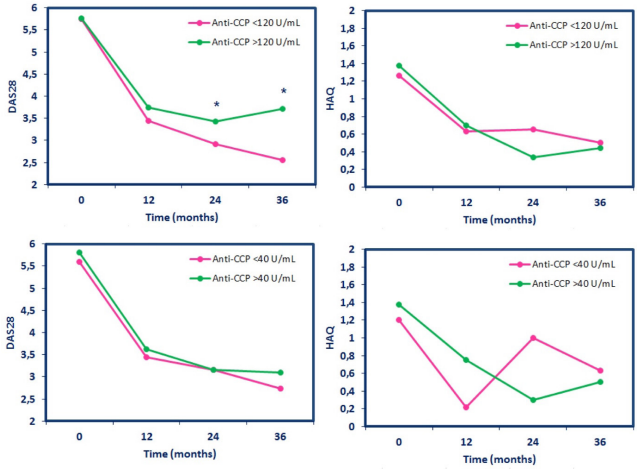
Background: Prognostic value of autoantibodies in patients with Rheumatoid Arthritis (RA) is unclear. Studies shows conflicting results regarding the prognostic value of anti-CCP antibodies. The new classification criteria for RA (EULAR/ACR 2010) introduced a weighting for anti-CCP as they are positive at low titers (<3xReference value or VR) and high titers (>3xVR). The aim of our study is to study the prognostic value of anti-CCP antibodies in patients with RA.

Methods: 59 patients with recent diagnosed RA and median age of 53 (42-59) years were followed up during 3 years after diagnosis. The patients were treated with disease modifying antirheumatic drugs (DMARDS). Disease activity was assessed by the disease activity score (DAS28) and the functional ability was evaluated by the Health Assessment Questionnaire (HAQ). Patients were stratified in two groups according the cut-offs of 40 U/mL and 120 U/mL (3xreference value). The Wilcoxon test was used to assess changes in parameters (DAS28 and HAQ) during follow up

while to study differences between groups over time we used the Mann-Whitney test was used. A value of p <0.05 was considered statistically significant.

Results: The results for the monitoring of DAS28 and HAQ for the cut-offs of 40 U/mL and 120 U/mL are show in the figures.



Conclusion: A cut-off of 40 U/mL has not prognostic value in the patients with RA according to the evolution of DAS28 and HAQ during the three years of study. However, a cut-off of 120 U/mL allows us to stratify patients in two groups with different response to the treatment according to the DAS28 value.

A-326

Correlation of Beckman Coulter DxI's Total Serum IgE measurement with Siemens Centaur and Thermofisher Phadia platforms

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Background: The measurement of serum IgE aids in the diagnosis and management of atopic allergic disease and hyper-IgE immunodeficiency syndromes. Total IgE is measured on several FDA approved platforms by immunoassay technique. Beckman Coulter DxI is one of the widely used platforms for Total IgE measurement. Recently Beckman Coulter has announced their decision to discontinue the Total IgE assay kit effective 4th Quarter, 2016. The objective of this study is to determine whether either Siemens Centaur and/or Thermofisher Phadia instrument platforms are equivalent to Beckman Coulter DxI for measurement of Total serum IgE concentration.

Method: Measurement of Total IgE on all three instruments is based on sandwich immunoassay using direct chemiluminescence technology (DxI and Centaur) or fluorescence technology (Phadia). Assay times, Analytical Measuring Ranges (AMR) and Reference Ranges (RR) are 75 min, 0.25 - 3000 IU/mL and 1.3 - 165.3 IU/mL with DxI; 18 min, 1.5 - 3000 IU/mL and <158 IU/mL with Centaur; and 105 min, 2 - 5000 kU/L and <114 kU/L on Phadia, respectively.

Serum samples were measured to determine Total IgE on DxI versus Centaur (N=122) and DxI versus Phadia (N=120). Samples that exceeded AMR on the respective instruments were excluded from the statistical analysis. Results were entered into EP evaluator (version 9.4.0.457; Alternate Method Comparison module) to determine the slope, intercept and correlation coefficient between DxI vs. Centaur and DxI vs. Phadia.

Results:

Method Comparison	N	Range (IU/mL)	Slope	Intercept	Corr Coef., R
DxI vs. Centaur	107	1.21 - 2517.34 (AMR)	0.974	1.932	0.9862
	82	1.21 - 188.10 (RR)	1.015	-1.402	0.9950
DxI vs. Phadia	112	2.38 - 3641.28 (AMR)	1.034	-2.553	0.9973
	86	2.38 - 180.83 (RR)	1.055	0.105	0.9866

Conclusion: Overall both Centaur and Phadia platforms presented an equivalent performance to Beckman DxI platform for measurement of Total serum IgE.

A-327

Recognition of the dense fine speckled (DFS) pattern remains challenging: Results from an international internet based survey

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Background: The dense fine speckled (DFS) pattern as detected by indirect immunofluorescence (IIF) on HEp-2 cells has been associated with several inflammatory diseases but is most commonly observed in individuals that do not have a systemic autoimmune rheumatic disease and even in apparently healthy individuals. Consequently, the accurate identification and correct reporting of this IIF pattern is of outmost importance which has been recognized by several international study groups for the detection of antinuclear antibodies. The DFS pattern is included in the International Consensus on Antinuclear Antibody (ANA) Pattern (ICAP, http://www.anapatterns.org/) referred to pattern AC-02. The objective of this study was to use an internet-based survey to assess how accurately the DFS IIF pattern was recognized by experienced technologists.

Methods: High resolution digital IIF images were captured using the automated IIF NOVA View instrument (Inova Diagnostics, San Diego, CA). Ten images were posted in an anonymous, international, internet-based interpretive survey as completed by IIF technologists. Four of the images in the survey were from previously characterized serum samples with classic anti-nuclear antibody (ANA) IIF patterns (nucleolar, centromere, homogeneous, and speckled) and two of the images were from samples with a DFS IIF ANA pattern and monospecific anti-DFS70 antibodies as determined by a chemiluminescence immunoassay. The remaining four images were from sera with the classic IIF ANA patterns referred to above and mixed with the anti-DFS70 positive sample. The survey included multiple choice selections: homogeneous, DFS, centromere, nucleolar, speckled, other, or unrecognizable.

Results: 125 of the 230 participants who completed the survey had diverse levels of experience in IIF pattern recognition on HEp-2 cells ranging from <1 year to >10 years experience (average >10 years). Participants had a high concordance in correctly classifying the classical ANA IIF patterns: ranging from 95.2% for centromere to 74.4% for nucleolar patterns. The unmixed DFS pattern was recognized with significantly lower accuracy (~50%; $p<0.05$). However, less than 10% correctly identified mixed patterns derived from the sera containing clinically relevant and anti-DFS70 antibodies.

Conclusions: Recognizing the DFS ANA IIF pattern and mixed IIF patterns composed of DFS + clinically relevant ANA poses a significant challenge. Consequently, it seems imperative that specific immunoassays are needed to confirm the presence of anti-DFS70 antibodies before definitive results are reported to clinicians.

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A-329

A Novel Method in Developing Allergen-Specific IgE Artificial Positive Control Serum

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Background: Serum allergen-specific IgE testing is the most frequently used method in in vitro diagnosis of allergy. Positive control sera, important in developing and manufacturing such tests, are very limited availability from natural sources, e.g., patient serum samples. Here we report an innovative method to prepare allergen-specific IgE artificial positive control sera that can be used to prepare calibrators, controls and serum panel for assay development and manufacture to ensure the lot-to-lot consistency.

Methods: Specific allergen extracts including E1 (Cat dander), F2 (Milk), W1 (Ragweed), I6 (Cockroach, German), and M6 (Alternaria alternata) purchased from various vendors were used to immunize healthy animals and obtain anti-sera. The allergen-specific IgG antibody from animals, purified through the protein A affinity column, was coupled to the human IgE Fc fragment and form the IgG-IgE conjugate. Titers of the IgG-IgE conjugate were evaluated by HOB CLIA-4G Allergy and the results were compared with ImmunoCAP.

Results: Five positive artificial control sera have been successfully prepared. The sIgE values of E1, F2, W1, I6 and M6 measured by HOB CLIA-4G Allergy were 914 kU/L, 520 kU/L, 81.4 kU/L, 549 kU/L and 94.9 kU/L, respectively, while the values by ImmunoCAP were 865 kU/L, 565 kU/L, 92.8 kU/L, 470 kU/L and 84.5 kU/L,

respectively. The results showed that these allergen-specific IgE positive control sera have been successfully prepared with high sIgE titers. These artificial IgE sera perform as good as human specific IgE sera in our study.

Conclusion: We reported a novel and high-quality method to prepare allergen-specific IgE positive control sera. This method can be easily applied to prepare high-titer and stable positive control sera for various allergens, especially for the alternative replacement of rare allergen-specific IgE control serum. It can be used to manufacture calibrators, controls, and serum panels to keep lot-to-lot consistency well controlled and is easy to scale up. This standardized and reproducible method will greatly contribute to the quality of allergen-specific IgE in vitro diagnostic.

A-330

A Sensitive and Quantitative Method for the Determination of anti-dsDNA IgG antibody on the HOB BioCLIA-1200 Automated Immunoassay Analyzer

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Background: Anti-dsDNA is one of the primary auto-antibodies present in patients with systemic lupus erythematosus (SLE). At present, the main methods of detecting anti-dsDNA antibodies are ELISA, indirect immunofluorescence (IIF), radioactive immunoassay (Farr), and dot immuno-gold filtration assay (DIGFA). Farr is considered to be the golden standard for detection of anti-dsDNA antibodies, while it has isotope radioactive. Recently, the innovative HOB anti-dsDNA testing, coupling with the fully automated, random-access BioCLIA 1200 system has been launched.

Methods: In this study, the analytical performances of HOB anti-dsDNA Kits including LOD, intra-assay, inter-assay, linearity, accuracy were evaluated. 300 clinical samples from disease group with SLE (n=100) and healthy individuals (n=200) were collected and measured by Farr, HOB anti-dsDNA Kits and ELISA (an internationally renowned manufacturer).

Results: 300 clinical samples were analyzed to determine the concordance among Farr, ELISA and HOB anti-dsDNA kits. Compared with the result of Farr, the clinical samples analyzed by ELISA, the data showed positive agreement = 58.0% (58 / 100), negative agreement = 98.5% (197 / 200), while the clinical samples analyzed by HOB anti-dsDNA kits, the data showed positive agreement = 86.0% (86 / 100), negative agreement = 98.0% (196 / 200). The performance results of HOB anti-dsDNA kit were shown in Table 1.

Conclusions: The results showed HOB anti-dsDNA kits has good precision, wider dynamic range compared with ELISA. There was poor correlation between Farr and ELISA in detecting anti-dsDNA antibodies in patients with SLE. However, HOB anti-dsDNA kits offered a better clinical relevance and technical performance for SLE diagnosis compared with ELISA.

The analytical performance of HOB anti-dsDNA kits

Company	LOD (IU/mL)	Intra-Assay (%)	Inter-Assay (%)	Linearity (IU/mL)	Accuracy (%)
HOB	0.03	5.30	6.57	1-800	101.5-103.8
ELISA	1.00	4.40	7.20	10-800	/

A-332

Performance Evaluation of a New, Stable, Ultrasensitive Chemiluminescent Substrate-APSH™

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Background: Chemiluminescence immunoassay (CLIA) is a highly sensitive technology compared to Enzyme-linked immunoassay (ELISA), Radioimmunoassay (RIA) and Immunofluorescence Assay (IFA). Substrate plays an important role in the sensitivity of CLIA. There are several commonly used luminescent substances including luminol, isoluminol, acridinium ester, and 1, 2-dioxetane compound. The 1, 2-dioxetane compound can be triggered by alkaline phosphatase (AP) and emit sensitive chemiluminescence. The 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane (AMPPD) has been widely used in different automatic chemiluminescence systems such as those from Siemens, Beckman Coulter, BioMerieux and Olympus. Recently, an innovative & Ultrasensitive HOB AP substrate-APSHTM was developed, and its performance was evaluated by the fully automated, random-access BioCLIA 1200 chemiluminescence immunoassay system. **Methods:** The substrate has been reformulated with: 1) AMP, a buffer to hold pH basic at 9.8±0.02; 2) AMPPD, a substrate, when subjected to AP cleavage, emits photos as light; 3) sodium fatty alcohol polyoxyethylene ether carboxylate, an anionic surfactant, was coupled with a series of fluorescent compounds (denoted as HOBEP series compounds, HOBEP-1 to HOBEP-5). 4) chemiluminescence enhancer. With the newly formulated substrates, critical assay performance parameters such as LOD, stability and reaction kinetics were assessed by BioCLIA 1200, and compared with an international renowned manufacturer (Company B) substrate. **Results:** The new chemiluminescent substrate-APSH™ has a background signal lower than 200 relative light units (RLU), and its detection sensitivity reaches 10^{-19} mol AP. The LOD of HOB TSH kit with APSH™ substrate can lower down to 0.005 ~ 0.012 IU/mL, while that of Company B's is 0.026 IU/mL. APSH™ showed very stable for 18 months at room temperature as its signal retention was above 95% in real-time stability study. The longer term study is still in progress. In addition, APSH™ has a similar reaction kinetics curve to Company B that signal strength reached a plateau at 15 min and duration up to 120 min, while APSH™ has higher signal strength than that of Company B.

Conclusion: The new chemiluminescent substrate-APSH™, with high strength and sensitivity, long duration time and long-term stability, satisfies the need of clinical testing on fully automated instruments. It showed an outstanding performance and as good as or better than Company B. The in-house manufacture capability also greatly reduces the cost. In summary, APSH™ is considered as a highly stable and ultrasensitive AP substrate for chemiluminescence immunoassay systems such as HOB BioCLIA-1200.

A-334

Anti-nuclear antibody testing by indirect immunofluorescence and a multiplexed immunoassay: Clinical performance of discordant results against chart review by a Rheumatologist.

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Background: Testing for anti-nuclear antibodies (ANA) by indirect immunofluorescence (IFA) using HEp2 cells is labor-intensive and expensive and suffers from poor specificity and high inter- and intra-laboratory variability. As a result, many laboratories have moved away from IFA testing and are relying on automated solid-phase assays for the detection of ANA. However, IFA is touted as the gold standard and many clinicians and laboratorians are wary of ANA results from non-IFA assays. In this study, we assess the agreement between ANA IFA and a multiplex solid-phase immunoassay (Bio-Rad BioPlex® 2200) results over a one year period and compare all discordant results from patients seen at a rheumatology clinic to the presence or absence of connective tissue disease (CTD) as determined by a Rheumatologist. **Methods:** Results from patients tested for ANA simultaneously with IFA and BioPlex between May 1, 2011 and April 30, 2012 were analyzed for concordance. Patients with discordant results ($ANA^+/BioPlex^-$ or $ANA^-/BioPlex^+$) had their electronic medical records reviewed by a Rheumatologist to identify the presence of CTD (subdivided into those meeting classification criteria and those with incomplete, but clinically suggestive, presentations) at the time of discordant results until their most recent visit up to mid 2015. **Results:** A total of 1,206 patients had ANA assessed simultaneously by both methodologies. The overall agreement between the two assays was 76.3% (920/1,206). Of the 286 discordant results, 230 were rheumatology patients whose charts were reviewed in detail. 160 were $IFA^+/BioPlex^-$ and 70 were $IFA^-/BioPlex^+$. The negative BioPlex result was clinically concordant in 39/160 cases (24.4%), reflecting an absence of autoimmune disease despite IFA positivity (with titres up to >1:640). The positive BioPlex result was clinically concordant in 61/70 cases (87.1%); 38 (62.3%) of which had established CTD meeting accepted classification criteria. In total, the BioPlex result reflected the patient's clinical presentation in 100 of the 230 charts reviewed (43.5%). **Conclusions:** The IFA and BioPlex assays showed good agreement (76.3%). Among the patients with simultaneously discordant ANA results, 80.4% (230/289) were rheumatology patients subjected to chart review. Of these, the BioPlex agreed with clinician impression 43.5% of the time (100/230). These findings suggest equivalency in the clinical performance of the BioPlex and IFA methods, although neither can be considered a true gold standard.

A-335

Cryoglobulinemia typing using Matrix Assisted Laser Desorption/ Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS).

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Background: Cryoglobulins are immunoglobulins that precipitate in a reversible- and temperature-dependent process at temperatures below 37°C. There are three main types of cryoglobulinemias. Type I consists of a monoclonal immunoglobulin, typically IgG or IgM and rarely IgA. Type II and III cryoglobulinemias are also known as mixed-type containing more than one immunoglobulin type in the cryoprecipitate. Type II is characterized by a monoclonal and a polyclonal fraction while type III is associated with polyclonal immunoglobulins. In some instances the presence of cryoglobulins does not have clinical consequences. However, in cryoglobulinemias, the insoluble globulins can be associated with weakness, purpura, arthralgias, peripheral gangrene, vasculitis, nephropathy and neuropathy, with symptoms depending on the type of cryoglobulinemia. Consequently, typing is integral to the diagnosis and management of cryoglobulinemias. Cryoglobulinemias are diagnosed by cooling serum to 4°C for up to 7 days in order to observe a protein precipitate which re-dissolves upon warming to 37°C. Cryo-precipitates are then typed using IFE. We recently demonstrated that serum monoclonal and polyclonal immunoglobulin species can be identified from immunoglobulin-enriched samples using MS detection with higher sensitivity. Here, we show that this protocol can be optimized in order to type cryoglobulinemias using MALDI-TOF MS.

Methods: 82 residual waste cryoglobulin samples were analyzed using IFE and MALDI-TOF MS, including 35 type I, 30 Type II, and 17 Type III. Serum cryo-precipitates were washed in saline and immunoglobulins from each sample were enriched in separate purifications using nanobody-coupled sepharose beads against IgA, IgG, IgM, κ and λ. Immunoglobulins were eluted with 5% acetic acid and reduced with Tris (2-Carboxyethyl) Phosphine (TCEP) to separate heavy and light chains before MALDI analysis. Saturated α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile/5water +0.1% trifluoroacetic acid was used as the matrix. MS data were collected using a Microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics) in positive ion mode.

Results: 80% of the type I cryoglobulinemia samples analyzed agreed between IFE and MALDI-TOF MS. Similarly, 81% of the type II cryoglobulinemia samples analyzed agreed between IFE and MALDI-TOF MS. Discordant results included; two type III samples observed to have a monoclonal protein by MALDI-TOF MS (IgG-λ and IgM-λ), but not by IFE, six samples where IFE and MALDI-TOF MS identified discrepant monoclonal proteins in Type I or Type II cryoglobulinemias, and multiple samples where MS could not assign a immunoglobulin type based on either low signal intensity or non-specific carryover at the purification step.

Conclusion: Cryoglobulinemias can be typed using MALDI-TOF MS with up to 80% agreement with IFE. Our initial study suggests feasibility of using the described MS method for cryoglobulineamia typing will require further optimization in order to reduce non-specific carryover at the nanobody purification step. MS detection is attractive based on the potential for automation and reagent cost savings.

A-337

Evaluation of autoantibody screening and disease-specific methods for autoimmune disease diagnosis

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Objective: To characterize differences from three facilities utilizing different methods for measuring autoantibodies.

Relevance: Investigations of autoimmune disease include measurement of autoantibodies with immunofluorescence microscopy and specific solid phase immunoassays. A better understanding of the performance of different autoantibody methods is needed to employ an effective autoimmune disease diagnostic strategy tailored to the local patient population and scope of service.

Methodology: 100 patient serum samples were analyzed for autoantibodies by computer-aided indirect immunofluorescence (IFA) by two methods (Euroimmun and Inova) at different sites and by multiplex bead-based assays (BioPlex and TheraDiag/FIDIS) at three different sites. Agreement between methods were assessed qualitatively (positive/negative) and by computer-aided interpretation of patterns as shown by immunofluorescence microscopy. Specific autoantibody results were interpreted both quantitatively (between BioPlex instruments) and qualitatively (between BioPlex and TheraDiag/FIDIS).

Results: Comparison of the BioPlex instruments at two different locations employing 44 of the 100 patient samples demonstrated good concordance (95.5%) and quantitative correlation (slopes ranging 0.82 - 0.98 with R^2 values between 0.840 - 0.998 for specific autoantigens). Conversely, BioPlex and IFA (Euroimmun) methods showed relatively poor concordance at 61.0 %; however IFA methods showed good agreement for staining pattern (80.8 %). Common autoantigens between BioPlex and TheraDiag/FIDIS demonstrated good, although varied, concordance for the various analytes (81 - 97 %). The differences between BioPlex and Theradiag/FIDIS were attributed to differences in autoantigen preparations.

Conclusions: This study demonstrated some differences between platforms for autoantibody methods. BioPlex instruments were consistent between two sites; however comparison of IFA and specific autoantibody methods from two different manufacturers demonstrated differences. This is attributed to the general lack of standardization of autoantibody analysis diagnostic kits as it pertains to autoantigen preparations. Given these differences, it is important that laboratory results should be interpreted in the context of the clinical picture and that specific diagnostic performance of autoantibody methods should be taken into account for effective delivery of laboratory services.

A-338

Islet Autoantibody Selection for Population Screening

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Background: When type 1 diabetes (T1DM) becomes preventable, screening for islet autoantibodies will be used to detect individuals at risk for T1DM. Such individuals would then undergo further testing prior to receiving preventative therapy. Presently there are 5 major autoantibodies that are used to define risk for T1DM: ICA, IAA, GADA, IA-2A and ZnT8A. The goal of this study was to determine if any one autoantibody or set of autoantibodies are more common in sera positive for at least one autoantibody that might allow more focused testing (e.g., single or dual screening versus screening for all 5 autoantibodies). Methods/Results: The initial analysis involved 71 sera tested for all 5 autoantibodies. These samples were submitted for the presumed evaluation of islet autoimmunity. Of these 71 sera, 33.8% sera were positive for at least one autoantibody. Positivity rates were: ICA 22%; IAA 8.4%; GADA 23%; IA-2A 22%; and ZnT8A 29%. IAA positivity was not significantly less common than that of the other autoantibodies (P = 0.1). Only 16.7% of sera were positive for a single islet autoantibody and 83.3% of sera were positive for =>2 autoantibodies. For samples with at least one positive autoantibody, the most common pattern was positivity for ICA, GADA, IA-2A and ZnT8A in 33% of samples. Because of this limited sample size, 854 sera were analyzed that were all tested for ICA, IAA, GADA and IA-2A. 72.8% of sera were positive for at least one autoantibody. Positivity rates were: ICA 37%; IAA 45%; GADA 52%; and IA-2A 50%. Compared to the other 3 autoantibodies, ICA was less common (P <0.0001). GADA, compared to the other 3 autoantibodies, was more common (P <0.0001). GADA and IA-2A were equally common (P = 0.38). If the goal of testing were to identify 2 islet autoantibodies in a subject's serum, by screening for GADA and IA-2A and not further testing people with both GADA and IA-2A or neither GADA or IA-2A, only 1.1% of double autoantibody positive subjects could be missed with a 36% reduction in test volume versus testing for all 4 islet autoantibodies in all subjects. Conclusion: We conclude that while GADA is positive more often than other autoantibodies, the differences in positivity rates are minimal and it is not possible with the available data to choose a single "best" panel of tests to screen for islet autoimmunity. Future analysis of cascade testing may allow more prudent use of testing to recognize islet autoimmunity.

A-339

Using anti-GD1a, GD1b, GB1b antibodies to diagnose Guillain-Barre syndrome, a case report.

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Guillain-Barre syndrome (GBS) is a disease presenting as rapid-onset muscle weakness. Although the exact etiology remains unknown, it is believed that GBS is an autoimmune disease caused by antibodies against the peripheral nerves. Diagnosis of GBS is dependent on clinical evaluation and electrophysiological studies. It is debated if, for challenging cases, ancillary laboratory tests on serum anti-ganglioside antibodies

may help establishing the diagnosis. The patient is a 78-year-old man with past medical history of hypertension and prostate carcinoma status post brachytherapy. He presented to the Emergency Department with progressive difficulty with ambulation for one week. Physical examination showed weakness in all four extremities. Guillain-Barre syndrome was suspected. Head CT-scan and MRI scan were negative for acute pathology. Electromyography showed no evidence of myopathy, but scattered evidence of neurogenic pathology. Tests on serum anti-Jo, anti-acetylcholine receptor, anti-MUSK and anti-campylobacter antibodies were negative. Patient was given intravenous immunoglobulin treatment for five days and improvement in muscle strength was observed. In order to support the clinical diagnosis of Guillain-Barre syndrome, serum tests on anti-GD1a, GD1b, and GB1b was performed by ELISA and the results turned out to be negative. Gangliosides are components of cell membranes found mostly in nervous system where their function may include regulation of neuronal growth and development. Anti-ganglioside complexes (GSCs) antibodies, including anti-GD1a/GD1b, GD1a/GM1, GD1b/GT1b, GQ1b/GM1, and GQ1b/GD1a antibodies are associated with several syndromes of peripheral neuropathy including but not limited to GBS. The sensitivity of this antibody test for GBS reported in the literature is low. One study showed that only about 38% of GBS patients have detectable titer of serum anti-ganglioside antibodies, while anti-GM1 antibody is seen in about 20-30% of the GBS patients. Another study showed that only 17% of 234 GBS patients had serum anti-GSCs antibodies detected. High titer of anti-GM1 antibody (> 1:2000) may be seen in GBS, as well as multifocal motor neuropathy, motor neuron disease, and non-specific idiopathic neuropathy, therefore, the antibody test may not be specific for GBS too. Our current patient did not show the presence of anti-GD1a, GD1b, and GB1b antibodies in the serum, even though he had clinical and electrophysiological suspicion for GBS, which is in accordance with the low sensitivity of the test reported in the literature. Therefore, the increased cost of this serological test may not justify its use due limited sensitivity and specificity.

A-340

Pleiotropic effects of 4-Hydroxynonenal on Oxidative Burst and Phagocytosis in Neutrophils

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Metabolic control of cellular function is significant in the context of inflammation-induced metabolic dysregulation in immune cells. Generation of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide are one of the critical events that modulate the immune response in neutrophils. When activated, neutrophil NADPH oxidases consume large quantities of oxygen to rapidly generate superoxide and hydrogen peroxide, a process that is referred to as the oxidative burst. These ROS are required for the execution of efficient processing and removal of phagocytized cellular debris and pathogens. In chronic inflammatory diseases, neutrophils are exposed to increased levels of oxidants and pro-inflammatory cytokines that can further prime respiratory burst responses. However, the effects of metabolism on the oxidative burst in response to ROS inducing conditions are not well understood. The purpose of this study was to investigate the effects of the nonenzymatic lipid peroxidation product 4-hydroxynonenal (HNE), a diffusible reactive aldehyde generated endogenously under conditions of increased oxidative stress, on neutrophil oxidative burst and cellular proteins. The oxidative burst was determined in freshly isolated healthy donor neutrophils using 13-phorbol myristate acetate (PMA) and the extracellular flux analyzer. Neutrophils pretreated with HNE caused a significant decrease in oxidative burst response in a dose dependent manner. Mass spectrometric analysis of alkyne-HNE treated neutrophils followed by click chemistry detected modification of a number of cytoskeletal, metabolic, redox and signaling proteins that are critical for NADPH oxidase mediated oxidative burst. These modifications were confirmed using a candidate immunoblot approach for critical proteins of the active NADPH oxidase enzyme complex (p47phox subunit, Rac1 of the NADPH oxidase etc.) and glyceraldehyde phosphate dehydrogenase, a critical enzyme in the metabolic regulation of oxidative burst. Taken together, these data suggest that lipid peroxidation-induces damage to NADPH oxidase and other critical cellular proteins and enzymes. These mechanisms may contribute to the immune dysregulation associated with chronic pathological conditions.

Tuesday, August 2, 2016

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

Mass Spectrometry Applications

A-341

A Novel LC-MS/MS Method for the Quantitation of Abiraterone Metabolites in Patients with Castration-Resistant Prostate Cancer: Innovation of Separation of Diastereoisomers Without Using a Chiral Column

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Background: Prostate cancer tumor progression depends on androgen receptor (AR) presence and function. Testosterone (T) and 5α-dihydrotestosterone (DHT) are the endogenous ligands for fueling the growth of malignant cells. In most advanced cases, the cancer initially responds to treatment with medical or surgical castration, but after time, castration-resistant prostate cancer (CRPC) develops, and patients eventually die from their disease. In 2011, the US FDA approved abiraterone acetate (AA) in combination with prednisone for the treatment of CRPC. Abiraterone (Abi) is a steroidal compound; it is administered as the prodrug AA. Abi inhibits CYP17A1, an enzyme required for androgen biosynthesis. We have recently reported that a novel Abi metabolite (3-keto-Δ^4-Abi (D4A)) is more potent than the parent drug (Abi) in blocking enzymes essential for DHT synthesis and also more potent in inhibiting tumor growth in xenograft mice (Li et al, Nature. 2015). The D4A structure is similar to that of testosterone and androstenedione, which may enable further metabolism of D4A. **Methods**: A Liquid chromatography mass spectrometry method was developed and validated to detect Abi and its metabolites in human serum using an AB Sciex Qtrap 5500 mass analyzer coupled with a Shimadzu Nexera UPLC station. The mass spectrometer was operated in positive ion mode using electrospray ionization (ESI) source. Tuning parameters were optimized for the analytes by infusing a solution containing 200 ng/mL of each. The separation of the analytes was achieved using a Zorbax Eclipse Plus C18 150*2.1mm, 3.5µm column at 40°C, and isocratic mobile phase 35% A (H_2O), 65% B (methanol:acetonitrile; 60:40) with 0.1% (formic acid) in both and flow rate 0.2 ml/min. Analytes and the IS (Abiraterone-d4) were extracted from 100µl human serum (collected from CRPC patients treated with AA) with 2 ml methyl tert-butyl ether. After evaporation, the residue was reconstituted using 300 µl 50% methanol and 10µl was injected. A 7-point calibration curve for all analytes was constructed, and six quality control samples; 2 (low, mid, and high) were injected with the samples. The analytes were quantified using multiple reaction monitoring (MRM). **Results**: Data were processed using Analyst 1.6.2 software (AB Sciex). All the analytes were separated with the developed method despite their closely related structures. Analysis in human serum showed that Abi was linear in the range of 2.0-400 ng/ml, and all the metabolites were linear in the range of 0.1-20 ng/ml the analysis also show that the samples were free from interference. Intra and inter day precision and accuracy results were within the 15% CV for the three QC levels, the results also show that the analytes were stable in serum at different conditions and also in solution; no matrix effect was found in all analytes. In all samples obtained from CRPC patients, Abi and its metabolites were all detected. **Conclusion**: The validated LC-MS/MS method resolved and quantitated all the metabolites despite the similarity in their structures, including resolving diastereoisomers, which precludes analysis of co-eluting isomers based solely on their MRM transitions. Reversed-phase chromatographic conditions were identified to accomplish the separation of all metabolites and their subsequent accurate quantification.

A-342

Simple and Sensitive Method for Quantitative Measurement of Methylmalonic Acid by Turbulent Flow Chromatography and Tandem Mass Spectrometry

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Background: Methylmalonic acid (MMA) is elevated in patients with inherited defects in methylmalonyl CoA mutase or due to inherited or acquired deficiencies of vitamin B12. MMA levels are measured in serum to evaluate individuals with deficiencies of vitamin B12 or to assess children with symptoms of methylmalonic acidemia. Although methods to measure MMA by mass spectrometery have been previously developed, we utilized turbulent flow liquid chromatography (TFLC) to simplify the sample preparation procedure. The sample was prepared by precipitating serum proteins using methanol and directly injecting the supernatant into the turbulent flow column after centrifugation. The objective was to develop an MMA assay performed by TFLC-MS/MS with the following characteristics: analytically sensitive with a clinically useful dynamic range; good specificity with no cross-reactivity with succinic acid (SA); and suitable analytical transferability for a high volume clinical laboratory.

Methods: Serum samples were prepared by protein precipitation using methanol containing deuterated MMA as an internal standard. TFLC-MS/MS analyses were performed on a Thermo Scientific TLX-2 HPLC system (TurboFlow® technology) interfaced to a TSQ Quantum Ultra mass spectrometer operated in the negative ion ESI mode. Chromatographic separation was achieved using a Cyclone MAX TurboFlow®column (50 X 0.5 mm) and an Allure Organic Acids analytical column (150 X 3 mm). The HPLC elution occurred with a mobile phase composition of 50% methanol that was held for 1.3 minutes. Calibrators (6) were prepared in blank human serum.

Results: The analytical measurement range (AMR) for MMA was 30-1000 nmol/L with a CV less than 20% at the lower limit of quantitation (LLOQ); the calibration curves were linear over the AMR with correlation coefficients $R^2 \geq 0.995$. Dilutions of 1:2, 1:10 and 1:20 were validated giving a clinically reportable range of 30-10^5 nmol/L. The accuracy of the MMA assay was evaluated by comparing results of 63 residual patient specimens to the results obtained from a national reference laboratory utilizing LC-MS/MS methodology. The accuracy was further evaluated through recovery experiments. The slopes of the linear regression curves comparing the assays was +/- 1% with excellent correlation coefficients. MMA recoveries at concentrations spanning the AMR were between 96 and 111%. Within-day and between-day (N=20) CVs at concentrations spanning the AMR were less than 12%.

Conclusion: We have developed an accurate and sensitive assay to measure MMA levels in serum by TFLC-MS/MS. The method showed excellent correlation with previously established mass spectrometry-based methods and has been fully validated for imprecision, accuracy, linearity, recovery, carryover, specificity and matrix effects. The assay is more simple to perform than previously published methods and has proven very accurate and robust and cost effective.

A-343

Validation of a rapid liquid chromatography tandem mass spectrometry method for serum 25OHD &evaluation of the necessary to separate 3-epi $25OHD_3$

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Background: Increasing clinical implementation of liquid chromatography tandem mass spectrometry (LC-MS/MS) for measuring serum 25-hydroxyvitamin D (25OHD) has revived interest in separating and measuring 3-epi $25OHD_3$, which was neglected because routinely used LC-MS/MS could not separate it (called NEPI-LC-MS/MS) from $25OHD_3$. However, the necessary to separate 3-epi $25OHD_3$ in clinical practice is controversial. **Methods:** We developed and validated a rapid LC-MS/MS method to separate 3-epi $25OHD_3$ (called EPI-LC-MS/MS) and compared the results with those from routine NEPI-LC-MS/MS. And 982 clinical samples were analyzed by both the methods. Results: Both methods showed a linearity coefficient correlation exceeding 0.999 in the 2.5-200 ng/mL concentration range for $25OHD_2$ and $25OHD_3$. Moreover, they showed between run coefficient variation (CV) and total CV of <5% for $25OHD_2$ and $25OHD_3$. Accuracy test results showed that the accuracy bias was below 3.5% in the absence of 3-epi $25OHD_3$. Comparing the25OHD results obtained by the two methods for 982 patients (age 1 -100 years) showed excellent clinical agreement (Cohen's kappa = 0.875) and correlation (R2 = 0.973). Our data showed that among the 982 patients, only 73 patients had 3-epi $25OHD_3$ (>2.5 ng/mL); out of these 73, the 3-epi 25OHD3 level in 58 patients was between 2.5 and 5 ng/mL. In patients with less than 150 ng/mL 25OHD (25OHD2+25OHD3), only 8 had 3-epi $25OHD_3$ exceeding 5 ng/mL (ranging from 5.3 to 11.0 ng/mL). Among samples containing 3-epi $25OHD_3$, only three were separated into different 25OHD-deficiency groups using the above methods. **Conclusion:** A rapid and precise EPI-LC-MS/MS method with efficient separation of 3-epi $25OHD_3$ for measuring 25OHD was developed. Our results showed that 3-epi $25OHD_3$ had little effect on routinely used NEPI-LC-MS/MS.

A-344

Validation of Posaconazole Quantification Using LC-MS/MS

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Background: Posaconazole is a triazole antifungal agent used for prophylaxis & treatment of invasive fungal infections in severely immunocompromised patients. Large variations in inter-individual posaconazole pharmacokinetics may be associated with decreased efficacy leading to breakthrough infections and, therefore, monitoring of posaconazole levels is highly recommended. The objective of this study was to validate the performance of a newly developed method for measuring posaconazole concentrations using LC-MS/MS according to CLSI EP5 A standards.

Material and Methods: Serum samples from patients receiving posaconazole therapy were collected according to our institution standards protocols. A 100 µL aliquots of patient sera, calibrators (0, 0.190, 2.85, and 5.54 µg/mL posaconazole), and controls (0.465, 1.84, and 4.61 µg/mL posaconazole) were each deproteinized with 300 µL of methanol containing d3-voriconazole (50 ng/mL) as an internal standard, vortexed for 1 minute and centrifuged at 4,000 rcf for 10 minutes. Following centrifugation, 10 µL of the supernatant was transferred to an autosampler tube and diluted with 990 µL of water. The deproteinized samples (10 µL injection) were analyzed by a Shimadzu Nexera LC with a ThermoFisher "Cyclone" (50 x 0.5mm) guard column and Thermo "Accucore" C18 (3x50mm) separation column heated to 30°C. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Total elution time was 4 minutes and the starting mobile phase consisted of 20% B at a 0.65 mL/min flow rate, transitioned to 60% B by 0.6 minutes, 100% B by 0.75 minutes, and returned to 20% B by 3.10 minutes. Posaconazole was monitored by a Sciex API 5500 triple quadrapole MS/MS. The multiple reaction monitoring scan was conducted in positive polarity mode with a total scan time of 0.63 s and a dwell time of 100 ms. Fragment peaks were detected at 683.1 m/z (quantifying) and 614.1 m/z (confirmation) from the parent posaconazole 701.2 m/z. The internal standard d3-voriconazole (353.15 m/z) yielded fragments 284.2 m/z and 130.0 m/z. Multiquant software was used to quantify posaconazole concentration in serum based on a calibration curve generated from a ratio of the 683.1 m/z fragment over the 284.2 m/z internal standard peak. Performance of the LC-MS/MS method for detecting posaconazole levels in three control samples was compared with the manufacturer's reference values for the control material (RECIPE Chemicals).

Results: The LC-MS/MS method for posaconazole was linear over the analytical range of 0.1 to 5.54 µg/mL and R^2= 0.9988. This study established that the LC-MS/MS offered acceptable precisions with an intra- and inter-assays coefficients of variation of <5% and <6%, respectively. The correlation between samples (n=20) analyzed on the Sciex and the concentrations verified by RECIPE Chemicals samples was acceptable with R^2= 0.9662 (y= 0.9964x + 0.2634). The correlation with serum pool samples (n=10) spiked with posaconazole that were run by LC-MS/MS on the Sciex and by HPLC at an outside reference laboratory were also adequate with R^2= 0.9930 (y= 1.291x + 0.069).

Conclusions: The LC-MS/MS method offered a rapid and reliable method for monitoring posaconazole concentrations in serum samples from patients receiving posaconazole therapy.

A-345

Evaluation and Validation of LC-MS/MS for Quantification of 25-Hydroxyvitamin D2 and D3 Compared to a Chemiluminescent Immunoassay

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Background: The importance of monitoring 25-Hydroxyvitamin D2 and D3 concentrations (25(OH)D2 and D3) has increased recently as more physicians prescribe Vitamin D supplements because of their extra-skeletal roles in preventing cancer progression, autoimmune diseases, cardiovascular disease, hypertension, aging, diabetes, and infectious diseases, but high concentrations may be toxic. This study was designed to validate and compare the performance of an LC-MS/MS method to quantify 25(OH)D2 and D3 concentrations to a chemiluminescent immunoassay (CLIA) by DiaSorin.

Materials and Methods: Serum samples were analyzed by CLIA using DiaSorin Liaison XL analyzer and reagent. In lieu of utilizing an automated liquid handling station, an electronic pipette was used in the LC-MS/MS assay to improve the ergonomics, precision, and throughput of ≈135 samples-per-day. For the LC-MS/MS, an 80µL aliquot of patient sera, calibrators and controls from ChromSystems were deproteinized with 160µL of acetonitrile containing internal standard d6-25(OH)D3 (9.33 ng/mL) in a 96-well plate, sealed, rocked for 5 minutes, and centrifuged at 4,000 rcf for 6 minutes. The microplate was loaded onto a Shimadzu Nexera LC and the deproteinized supernatants (25µL) were injected onto heated (40°C) ThermoFisher "Cyclone" (50x0.5mm) and Thermo "Accucore" C18 (3x50mm) columns using a mobile phase of 18 ohm water (A) and methanol (B) both with 0.1% formic acid. The 25(OH)D2 and D3 fractions were eluted during a 4.1 minute gradient analysis starting with 30% B, transitioning to 95% B at 1.5 minutes, and re-equilibrating to 30% B at 3.1 minutes. 25(OH)D2 and D3 were detected and quantified on a Sciex API 5500 MS/MS in MRM APCI positive mode. Fragment peaks were detected for 25(OH) D2 (395.2 m/z) at 209.2 m/z (quantifying) and 269.2 m/z (qualifying), 25(OH)D3 (383.2 m/z) at 211.2 m/z (quantifying) and 229.2 m/z (qualifying), and d6-25(OH)D3 (389.2 m/z) at 371.3 m/z. Multiquant software was used to quantify 25(OH)D2 and D3 concentrations in serum based on internal standard-corrected calibration curves of the 25(OH)D2 and D3 quantification fragments and reported automatically to the hospital's LIS. LC-MS/MS and CLIA method performance were compared in patient serum samples. Method validation of the LC-MS/MS protocol was also conducted.

Results: There was a poor correlation between LC-MS/MS and CLIA (n=68) methods: standard error estimate (8.375), average error index (0.50), and regression equation (y=1.187x – 2.265; r^2= 0.7893). However, there was excellent correlation between LC-MS/MS results and CAP samples (n=6): standard error estimate (1.36), average error index (0.24), and regression equation (y=1.072x – 0.88; r^2= 0.9996). The summary of the LC-MS/MS method validation for total Vitamin D was as follows: linear range (1.0-68.0 ng/mL), intra-assay precision (<9.1%), inter-assay precision (3.7-7.2%), limit of detection (0.3 ng/mL), limit of quantitation (1.0 ng/mL), carry-over (0.3%), lipemia interference recovery (104%), hemolysis interference recovery (99%), icterus interference recovery (99%), ion suppression (95.3%). The average cost-per-billable-test was approximately 7-10 times lower than immunoassay methods.

Conclusions: The LC-MS/MS method using an electronic pipette and deproteinizing sample preparation is a rapid, accurate, and cost-effective method for measuring 25(OH)D2 and D3 concentrations given the excellent CAP sample correlation, 4.5 minute analysis, LIS instrument interfacing, and low cost-per-test.

A-346

A sensitive LC-MS/MS method for the quantification of urinary 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$) including pediatric reference values

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Background

Oxidative stress has been implicated in numerous diseases, including arthritis, atherosclerosis, Alzheimer's disease, cancer, diabetes, hypertension, and inflammation. Adducts generated from free radicals during oxidative stress could damage nucleic acids (DNA and RNA), proteins, and lipids, and contribute to disease initiation and acceleration. Therefore, early detection of oxidative stress is crucial in disease prevention and health management.

Mayo Clinic is currently offering a LC-MS/MS based clinical test to measure urinary 8-*iso*-$PGF_{2\alpha}$ with established reference interval for adults. Pediatric population represents a special group of people whose reference interval could be different from adult population.

We herein report the development and validation of an ultra-sensitive LC-MS/MS test for the measurement of urinary 8-*iso*-$PGF_{2\alpha}$ in infants and children. Reference interval for pediatric population was also established.

Method

Each urine sample was spiked with internal standard (8-*iso*-$PGF_{2\alpha}$-d_4) and subjected to solid phase extraction with Phenomenex Strata X-AW cartridge. The extracted sample was analyzed with a Thermo Ultimate 3000 UHPLC system coupled with a Thermo Quantiva triple quadruple mass spectrometer equipped with a HESI probe. Quantitation was performed with Multiple Reaction Monitoring mode under negative ionization mode. Calibrators were prepared by spiking various amount of 8-*iso*-$PGF_{2\alpha}$ into synthetic urine with no detectable 8-*iso*-$PGF_{2\alpha}$. Quality control samples were made from pooled pediatric urine samples. Left over urine samples (n=136) with normal urine analysis results from in- and outpatients from Children's Hospital Los Angeles were used to establish reference intervals for children age 2m - 18y. Tukey's method was used to exclude outliers, and EP evaluator was used to calculate reference interval.

Result

The liquid chromatography method is highly selective, separating 8-*iso*-PGF2α from other isomers. No peak was identified that could interfere with 8-*iso*-PGF2α quantitation in all the urine samples analyzed (n=136). The assay was linear from 0.024 nM to 20 nM (R^2 = 0.999). Recoveries were above 85% and matrix effects were below 5%. The variability (CVs) was determined at nM level: the intra-day variability ranged from 4.0 % to 4.5 % (n = 20); and the inter-day CVs ranged from 4.3 % to 5.7 % (n = 20). The accuracy of our laboratory developed test was evaluated with a clinical reference laboratory (n=40), and a correlation coefficient of 0.96 was observed. Reference interval for pediatric population was established to be < 0.5 ng 8-*iso*-PGF2α/mg creatinine, lower than the reference interval established by Mayo Clinic (<1 ng *iso*-PGF2α/mg creatinine) for adult population.

Conclusion

Overall, an ultra-sensitive LC-MS/MS assay was developed and validated to measure urinary 8-*iso*-$PGF_{2\alpha}$ for pediatric population with satisfactory selectivity, precision, and accuracy. The assay is very precise and accurate, and can be readily used for the assessment of oxidative stress for translational research and clinical usage in pediatric population.

A-347

A novel biomarker (UCN3) for sleep apnea measured by mass spectrometry using multiple reaction monitoring

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Background: Obstructive sleep apnea (OSA) is a common disorder affecting adults and children. Three percent of children (approximately 2.3 million) in the US are diagnosed annually with OSA. Polysomnography is the gold standard procedure for diagnosing this condition, but it is expensive ($1,500 to $2,000 per procedure), involves an overnight stay in a sleep laboratory, and requires the placement of numerous sensors. Having a rapid non-invasive urine-based assay able to assist in the diagnosis of OSA would be a major advance in respiratory medicine. Urocortin 3 (UCN3), a stress-induced 4.1 kD peptide, has been measured in the urine of children with OSA versus those with only primary snoring. A preliminary report based on ROC analysis showed a diagnostic sensitivity of 93.7% and specificity of 78.7% to predict OSA. Urine concentrations of UCN3 have been reported in the range of 1.0 to 1.2 ng/mL. Currently, there are no commercially available immunoassays for UCN3 which meet the limit of quantitation (LOQ) of <1.0 ng/mL and has been rigorously validated to meet CLIA '88 standard. We hypothesized that a mass spectrometry method using multiple reaction monitoring (MRM) and stable isotope dilution would enable quantification of UCN below 1.0 ng/mL.

Method: UCN3 precursor peptides generated from chymotrypsin, Asp-N, or trypsin were evaluated in-silico using Skyline and determined experimentally by nanospray-UPLC-MS using a Waters Synapt-G2-Si Q-TOF system. A heavy isotope form of a precursor peptide sequence (NIAK^AKNL) incorporating $^{13}C_6{}^{15}N_2$ L-lysine was synthesized (New England Peptide; Gardner MA) to serve as an internal standard for quantification; the concentration was determined by amino acid analysis. Carboxymethyl cellulose (CMC) resin was used to enrich UCN3 spiked in human urine; UCN3 concentration was determined using the bicinchoninic acid assay. Chymotrypsin digested peptides were desalted using solid phase extraction with a C18 loaded tip.

Results: Survey scans of precursor peptide from chymotrypsin digest showed three transition masses with strong intensities. The LOQ using a 1:1 mixture with a heavy isotope was 0.31 ng/mL (CV=5.3%, S/N>10), and linear up to 6 ng/mL. Ion quantifier/qualifier ratio was 3.76 (CV=9.88%) and 3.85 (CV=6.52%) for light and heavy precursor peptides respectively over the linear range. UCN3 bound to CMC at pH 8.5 is resistant to elution with sodium chloride up to 1.4 M; addition of 30% acetonitrile was required to elute the peptide. Recovery of spiked UCN3 from the CMC enrichment was 94-98% (n=3).

Conclusion: We have developed a mass spectrometry method for measuring UCN3. The method has a lower limit of detection <1.0 ng/mL and a dynamic range suitable for measuring the estimated normal reference range of UCN3 in human urine. We also developed a novel enrichment method for concentrating and purifying UCN3. Taken together, these results will enable more accurate quantitation of UCN3 in both adult and pediatric populations.

A-348

The Analysis of C3-Epimers of 25-Hydroxyvitamin D in Serum by LC-MS/MS

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Background: Vitamin D analysis has increased dramatically in clinical practice due to its association with multiple human diseases and the prevalence of vitamin D deficiency worldwide. Vitamin D exists in two forms, vitamin D2 and vitamin D3; each undergoes metabolism to form 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [25(OH)D3] which are used as the biomarkers for the assessment of vitamin D status. The epimeric forms of 25(OH)D, 3-epi-25-hydroxyvitamin D2 and D3, have been identified and may contribute to a large portion of the total 25(OH)D concentration, particularly in infant populations. Studies have shown that the C3 epimers have much lower bioactivity than the primary metabolites; therefore, a specific quantitation of these epimers is necessary for a proper clinical assessment of vitamin D status. Since these epimers are isobaric, chromatographic separation is necessary for accurate quantitation. In this study, the Raptor™ FluoroPhenyl column was used for chromatographic separation of 25(OH)D and their C3-epimers. The established chromatographic method was able to accurately quantitate the 3-epi-25-hydroxyvitamin D2 and D3 metabolites in fortified beagle serum. **Methods:** Serum was fortified with four analytes, 25(OH)D2, 25(OH)D3, 3-epi-25(OH)D2, and 3-epi-25(OH)D3, and extracted using a liquid-liquid extraction (LLE) method. Serum (400µL) was mixed with 15µL of internal standard solution (1µg/mL of d6-25-OH-D3 in methanol), 0.2 M $ZnSO_4$ (400µL) and methanol (400µL) in a 4-mL glass vial. A 2mL aliquot of hexane was added, mixed for 90 seconds, and then centrifuged for 10 minutes at 4300rpm. The hexane layer was removed and evaporated to dryness under nitrogen at 55°C. The dried extract was reconstituted with 100µL of a 50:50 water:methanol solution and injected (10 µL) for analysis on a Shimadzu Nexera XR UHPLC coupled to a Sciex API 4000™ mass spectrometer. **Results:**The calibration standards were prepared in synthetic human serum, SeraFlex LCMSMS, and subjected to the LLE procedure. Good linearity was obtained for all analytes with a concentration range of 1 to 100ng/mL (with 1/x weighting). Standard deviations were ≤10% (the lowest concentration was ≤20%) and R-squared values were 0.996-0.999 for all compounds. The quantitative results of 3 QC levels of fortified synthetic serum samples showed acceptable method accuracy with percent recovery within 10% of the nominal concentration for all QC levels. The %RSD values ranged from 0.9-6.6% and 2.2-4.5% for intra-day and inter-day analyses, respectively, indicating an acceptable method precision. The validated method was used to analyze the 8ng/mL fortified beagle serum which showed acceptable accuracy and precision. **Conclusion:**

It was demonstrated that the Raptor™ FluoroPhenyl column can provide unique selectivity for accurate and differential quantitation of 25-hydroxyvitamin D and C3-epimers in serum. The chromatographic analysis was performed using 0.1% formic acid in water and methanol as mobile phases with a 7-minute analysis time. The analytical method is applicable to the clinical analysis of total 25-hydorxyvitamin D concentration and provides the option to report the C3-epimer concentrations separately.

A-349

Maximizing the LC-MS Output by Using a New Four Channel HPLC with Multichannel Optimization

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Background: In a single channel LC-MS workflow, a mass spectrometer may only be utilized in a small portion of time during a HPLC run. Typically, the idle time of the mass spectrometer includes the elution time before the first analyte peak and the equilibration time after the last analyte peak. A new HPLC is designed to bring the productivity of up to four HPLC channels to a single mass spectrometer, four times the samples can be run, maximizing the LC-MS output. With built-in multichannel optimization software, up to four identical or different test methods can be run, and simplify workflow. We evaluated three units of prototype of a new four channels HPLC with a new Tandem-MS. **Methods:** This HPLC instrument has four LC channels, which are synchronized to a single mass spectrometer. Each channel operates independently, allowing four identical or different tests to run simultaneously. In addition, identical or different analytical columns and mobile phases can be used with each channel, providing method flexibility. Multichannel optimization ensures the maximum performance of the mass spectrometer with little to no idle time. When four channels run simultaneously, the data collection times are scheduled with staggered starts so that the data collections do not occur at the same time. In this

study, calibration standards, stable isotopically labeled internal standards, QC sample and test sample were spiked in synthetic urine. Tandem MS was operated in selected reaction monitor (SRM) mode with heated electrospray ionization (HESI) positive ion mode and specific SRM transitions of precursor ion to product ion were selected for identification and quantitation of each compound. For identical method, a test mixture of four example compounds (Atenolol, Warfarin, Lidocaine, Imipramine) in synthetic urine were analyzed (dilute and shoot) in four HPLC channels. Each HPLC channel used the same liquid chromatographic elution method, the same composition of binary mobile phases from its own solvent bottles, and the same type of analytical column. A total of 1976 samples were run unattended and continuously for about 60 hours. QC samples were inserted in every 30 samples for each channel. For different methods, a precision study was conducted with four different methods using this four channel HPLC (n=40 for each channel; 10 replicates per run, 4 runs per channel). In this study, identical mobile phases and identical analytical columns were used for each channel. Each HPLC channel ran with a different HPLC elution method and a different set of example compounds (Channel 1: Atenolol, Warfarin, Lidocaine, Imipramine; Channel 2: Amphetamine, Methamphetamine; Channel 3: Alprazolam, α-hydroxyalprazolam; Channel 4: Oxycodone, Noroxycodone). **Results and Conclusion:** Within-instrument precision (n=40), all test compounds showed the RSD's of less than 5% in both concentration and retention time; Between-instrument precision (n=40X3 units), showed RSD's of less than 10%. The test results demonstrated that this four channel HPLC can continuously run tests unattended for about 60 hours. In addition to pre-processed samples, samples of less complex matrices can be injected directly with a dilute-and-shoot process. For in vitro diagnostic use. Not available in all countries.

A-350

Development of Liquid Chromatography-Tandem Mass Spectrometry Method for Measuring Plasma Free Metanephrines

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Background: Pheochromocytomas are rare catecholamine-producing tumors of the chromaffin cells of the adrenal medulla. The secretion of catecholamines from pheochromocytomas are episodic, leading to sustained or paroxysmal symptoms, including hypertension, sweating, flushing, and tachycardia. Untreated pheochromocytomas are frequently lethal. The metanephrines (metanephrine, normetanephrine, and dopamine) are metabolites of catecholamines, and are continuously released from chromaffin granules, independent of the episodic secretion of catecholamines. This contributes to the higher sensitivity of metanephrines in screening for pheochromocytomas. Measurement of plasma free metanephrines are recommended as the first-line test in screening for pheochromocytomas. A negative result can exclude a pheochromocytoma. In this study, we aimed to develop a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for measuring free metanephrine and normetanephrine in plasma.

Methods: d3-metanephrine and d3-normetanephrine were used as the internal standards (IS). Calibrators and quality controls were prepared by spiking metanephrine and normetanephrine standards into blank serum. 20µL of IS solution containing 12 nmol/L of each IS and 1 mL distilled H_2O were added into 500 µL of calibrators, quality controls and samples, respectively. Mixed samples were subjected to solid phase extraction (SPE) using Phenomenex Strata X-CW polymeric weak cation cartridges. Eluent from the extraction was evaporated under nitrogen at 37°C, and the dried residues were dissolved in 150 µL of 90% acetonitrile/10% H_2O. Injected samples were separated on a Phenomenex Kinetex HILIC Column (50 x 2.1 mm, 2.6 µm) with a flow rate of 0.5 mL/min and a total run time of 7 min using the Shimadzu Nexera X2 UHPLC. The LC gradient started with 95% mobile phase B (Acetonitrile) and 5% mobile phase A (100 mM ammonium formate in H_2O with pH adjusted to 3.5 by formic acid). Mobile phase B was decreased to 85% over 4.25 min and the metanephrines eluted out with good separations in this gradient. Analytes and ISs were detected by the Sciex QTrap 5500 mass spectrometer in positive mode using the following transitions: Metanephrine: 180.2/148.0 (quantifier), 180.2/120.0 (qualifier), 183.1/151.1 (IS); Normetanephrine: 166.1/134.1 (quantifier), 166.1/121.1 (qualifier), 169.1/137.1 (IS).

Results: Our method demonstrated robust linearity for metanephrine and normetanephrine over a concentration range of 0.1 nmol/L – 5 nmol/L with R^2 > 0.999. The total precision displayed CVs < 10% for all QC levels. Limits of quantitation (LOQ) for both analytes were defined at 0.1 nmol/L with CVs < 20%. The SPE extraction recovery for metanephrine was 81-112%, and 43-90% for normetanephrine. About 50% ion suppressions were observed for both analytes due to matrix effect, but the addition of ISs completely compensated for the suppression. This method demonstrated good correlations with the LC-MS/MS method used in ARUP laboratories: Metanephrine: ARUP LC-MS = 1.065 HMH LCMS - 0.018, bias = 0.002 nmol/L, R^2 = 0.96; Normetanephrine: ARUP LC-MS = 0.930 HMH LCMS + 0.023, bias = -0.103 nmol/L, R^2 = 0.99. Assay interference, carryover, sample stability and post-extraction stability were also tested.

Conclusion: We developed a sensitive LC-MS/MS method for plasma free metanephrines measurement. This method showed good analytical performances and is ready for clinical use.

A-351

A Novel Dilute and Shoot LC-MS/MS Method for the Measurement of Nicotine, Cotinine, Nornicotine, and Anabasine in Human Urine.

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Background:

Nicotine from cigarette smoking rapidly diffuses into the blood stream and elicits its desired effect in the brain. The liver then metabolizes nicotine into multiple metabolites which have significance related to nicotine consumption. Cotinine is the primary metabolite of nicotine and is an indicator of exposure to tobacco smoke. If anabasine and nornicotine are present, this is a strong indicator that the patient recently smoked tobacco. Thus, testing for nicotine and its metabolites can be useful for monitoring tobacco usage and nicotine patch effectiveness for patients enrolled in tobacco cessation clinics. Herein, we have developed a simple yet robust dilute and shoot LC-MS/MS method for the quantitation of nicotine and its metabolites.

Methods:

A 25 µL aliquot of internal standards containing deuterated versions of nicotine, cotinine, anabasine, and nornicotine were added to a 100 µL aliquot of sample. This milieu was then diluted by 100 µL of mobile phase (85 % of 20 mM ammonium bicarbonate and 15 % acetonitrile). A 10 µL injection was carried by the mobile phase to the columns, 100 x 4.6 mm EVO C18 (Phenomenex, Torrance, CA) using an LC system from Thermo Fisher Scientific (Waltham, MA). The separated constituents were then analyzed by a 6500 triple quadrupole mass spectrometer (SCIEX, Framingham, MA). The MRM transitions for each analyte were as follows: nicotine 163.1/132.1, nicotine-d4 167.1/136.1, cotinine 177.1/80.1, cotinine-d3 180.1/80.1, anabasine 163.1/120.1, anabasine-d4 167.1/134.1, nornicotine 149.1/130.1, and nornicotine-d4 153.1/134.1. To prevent carryover, a second mobile phase which consisted of 45 % acetonitrile, 45 % isopropanol, 10 % acetone, and 0.1 % ammonium hydroxide flowed through the LC system lines and columns after each analysis.

Results:

The following metrics were used to evaluate the performance of the method: precision, linearity, recovery, and accuracy. Testing for interferences such as bilirubin, hemolysis, drugs of abuse, and common over the counter drugs was also performed. Precision was evaluated on an inter- and intra-run basis. Both series produced a coefficient of variation (% CV) of less than or equal to 10%. A total of 5 concentrations which spanned the analytical measurement range and run in triplicate were used for the linearity study. For each analyte, no measured value exceeded 15% from the expected value and all slopes for each analyte were all well within the 0.9 - 1.1 range. A total of 40 specimens previously analyzed at Mayo Clinic Rochester were used for reference for the accuracy study. All analytes had mean percent differences within ±10% and produced slopes well within the 0.9-1.1 range. Samples ranging from low levels to gross amounts of bilirubin and hemolysis as well as samples with low to high levels of drugs of abuse and common over the counter drugs did not interfere with the analysis.

Conclusion:

The dilute and shoot LC-MS/MS method for nicotine and its metabolites is a robust method with quick sample preparation and can be used clinically to evaluate samples from patients in need of nicotine replacement therapy.

A-352

Development of a Simplified Extraction and High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) Method for Plasma Propofol Quantitation

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Background: Propofol remains a key sedative and amnestic agent utilized in general anesthesia. Owing to its lipophilicity, propofol has a rapid onset allowing expedient induction of deep sedation in emergent procedures. Administration of propofol requires a delicate balance between depth of sedation and the primary side effects of hypotension and respiratory depression taken into the context of its short duration of action. A closed-loop infusion device that adjusts the dose through continuous monitoring of plasma propofol levels to achieve the appropriate depth of sedation has

been developed and tested in rabbits. In order to validate the quantitation accuracy of the device, we developed a liquid chromatography-tandem mass spectrometry assay to serve as a gold-standard for measurement of plasma propofol. Current applications for measuring propofol require significant sample pretreatment, derivatization, or both to achieve high sensitivity and low background interference.

Methods: A total of 6 calibrators and 3 levels of quality controls were prepared by spiking sterile filtered heparinized rabbit plasma with propofol over a concentration range of 0.1-20.0 µg/mL. Samples (500 µL) were combined with propofol-d17 internal standard and diluted 1:1 with de-ionized water before applying to Phenomenex Novum™ 6cc Simplified Liquid Extraction (SLE) cartridges. Analytes were eluted with 2 x 2.5 mL aliquots of methyl tertiary butyl ether. Prior to evaporating the samples to dryness under nitrogen at 25°C, eluents were spiked with 0.5% tetrabutylammonium hydroxide in methanol to prevent loss of propofol. Samples were reconstituted in 75 µL of 50:50 acetonitrile:DI water and injected onto a Nexera X2 UPLC coupled to a AB Sciex 5500 Q-Trap Mass Spectrometer with electrospray ionization in negative ion mode. Separation was achieved using a reversed-phase Hypersil Gold C18 column (100x2.1 mm i.d., 1.9 µm particle size) under isocratic flow of 80:20 acetonitrile:DI water at 0.500 mL/min. Analytes were detected in multiple reaction monitoring mode with the following ion transitions: propofol (177→161 m/z) and propofol-d17 (194→174 m/z).

Results: Propofol and propofol-d17 were eluted within 2.0 min. Propofol calibration curve was linear over the measuring range of 0.1-20.0 µg/mL (R^2>0.996). The LOQ was 0.1 µg/mL with a CV <15% and the LOD was 0.08 µg/mL. The intra- and inter-day precision of the 3.0, 8.5, and 17.0 µg/mL QC was 7.4, 9.5, and 14.2%, and 8.2, 9.4, and 15%, respectively. Accuracy was assessed using spike and recovery experiments at 6 concentrations over the measuring range with recovery ranging from 90-120%. Carryover for the assay was 0.17%. In post-column matrix effect infusion studies both analytes displayed a global reduction in signal of <10% compared to mobile phase alone. SLE cartridges provided significant increases to precision and recovery with a cleaner background in comparison to traditional liquid-liquid extraction. The addition of 0.5% tetrabutylammonium hydroxide to the eluents was essential to increasing sensitivity by raising the signal for both analytes by 3-fold.

Conclusion: We have developed a simple, accurate, and sensitive method for detecting propofol in plasma without the need for derivatization. This method will be used to assess the accuracy of a closed-loop infusion device for propofol during procedural sedation studies.

A-353

Optimization of derivatization reaction used in sample preparation method in analysis of methylmalonic acid in plasma for clinical research.

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Background: Methylmalonic acid (MMA) is small polar molecule that poses challenges for the development of quantitative LC-MS methods. Two analytical approaches have been described in the literature: analysis of methylmalonic acid in negative ionization mode and analysis of derivatized MMA in positive ionization mode. The product of the derivatization reaction with n-butanol shows improved retention to reverse phase chromatographic column and higher ionization efficiency than underivatized MMA, making this method a preferred solution for quantitative analysis. The derivatization reaction parameters described in the literature resulted in variable, up to 100 fold, reaction efficiency demonstrated by large variability in internal standard signal. Several reaction parameters were investigated and optimized to ensure reproducible and efficient butylation reaction of MMA.

Methods: The sample preparation method included protein precipitation followed by derivatization reaction, evaporation and reconstitution. The chromatographic separation was performed using a 2.8-minute isocratic LC method. Methylmalonic acid and its deuterated analog (internal standard) were detected on a hybrid quadrupole-orbitrap mass spectrometer using APCI ionization probe. The mass spectrometry method collected MS/MS spectra for each analyte in a PRM experiment at a resolution of 70K.The following derivatization reaction parameters were optimized: reaction time, temperature, n-butanol additives, and other additives which could catalyze the reaction.

Results: We found that reaction temperature does not have significant effect on reaction efficacy and reproducibility so we recommend that reaction is carried on at room temperature. We also found that extending the reaction time up to 1 hour did not improve reaction performance. The main reaction parameter found to play significant role in reaction efficacy and reproducibility was an additive. Addition of appropriate salt increased reaction efficacy and significantly improved reaction reproducibility, resulting in reproducible internal standard signal (within 10% difference). Implementation of new derivatization reaction parameters allowed us to develop a quantitative method with a limit of quantitation of of 25 nM, precision and accuracy within 10%, and negligible matrix effects.

Conclusion: We demonstrated improved performance of derivatization reaction allowing cost efficient sample preparation method for LC-MS analysis of methylmalonic acid in plasma.

A-355

Development and validation of riboflavin in human plasma by liquid chromatography electrospray ionization tandem mass spectrometry.

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Background: The Riboflavin (vitamin B2) serves as a precursor for coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes participate in a range of reactions of reduction and oxidation. Vitamin B2 is critical for metabolism and energy production. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has high analytical specificity and enables analysis in short period of time. **Objective**: Development a simple, rapid and sensitive LC-MS/MS method to determination of riboflavin in human plasma using deuterated internal standard (IS) and precipitation for prepare the samples. **Experimental**: 100 µL of plasma spiked with 75 µL of deuterated internal standard in acetonitrile was treated with zinc sulfate 0.1M. Chromatograph separation was obtained with a Poroshell 120 EC-CN column (100 mm x 2.1mm x 2.7µm) on an Agilent 1290 HPLC and 6460 Mass Spectrometer system in the positive-ion mode. The MS/MS detection was conducted by monitoring the fragmentation ions of 377.2→243.0 (*m/z*) for riboflavin quantifier, 377.2→172.0 (*m/z*) for riboflavin qualifier and 383.2→249 (*m/z*) for riboflavin-dioxopyrimidine-$^{13}C_4$, $^{15}N_2$ quantifier (internal standard). **Results**: The chromatographic run time was approximately 3.0 min. The linear range obtained for riboflavin was 0.5-50.0 ng.mL^{-1} and dilution was validated for samples that exceed the curve in 2 times. Limit of detection was 0.2 ng.mL^{-1}.The precision intra-day was less than 10% and inter-day was less than 11%. **Conclusion**: A rapid method has been developed successfully for the quantitative analysis of riboflavin in human plasma using a simple prepare of samples without derivatization and with a short run time.

A-356

Development and validation of pyridoxal 5′-phosphate in human plasma by liquid chromatography electrospray ionization tandem mass spectrometry.

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The Pyridoxal 5′-phosphate (PLP) is the active biologically form of vitamin B6. PLP is a cofactor in several enzyme-catalyzed reactions and its deficiency can cause neurological disorders. A rapid LC-MS/MS method was developed for quantitative determination of PLP in human plasma for clinical practice. Detection was obtained on a 6460 MS system (Agilent Technologies) and it was conducted by monitoring the fragmentation ions of protonated molecules of *m/z* 247.9→150.0 for PLP quantifier, 247.9→94.1 for PLP qualifier and 250.2→152.0 for PLP-d3. Chromatographic separation was performed on an Eclipse plus C18 RRHD column (100 mm x 2.1 mm, 1.8 µm) and isocratic mobile phase water:methanol (98:2, v/v) with 0.1% de formic acid at 350 µL/min. The analyte was measured within 2.1 minutes instrumental run time. The extraction procedure is a simple protein precipitation with trichloroacetic acid 5% using only 100 µL of sample and 25 µL of deuterated internal standard (PLP-d3). The linear range was achieved for PLP at 3.0 - 120.0 µg/L. The medium range of recovery was between 91 and 109% for PLP. Intra-day and inter-day precision ranged were between 1.6-9.6% and 6.6-9.5%. The tests of quantification limits, linearity, precision and recovery were adequate for clinical evaluation. In conclusion, the LC-MS/MS method has been developed and validated successful.

A-357

Matrix Effects Identified and Addressed for Urine Copper, Zinc, and Magnesium Performed by an Inductively Coupled Mass Spectrometry Method

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BACKGROUND Urine trace metal analysis is used for various purposes such as to help diagnose diseases, to monitor for malabsorption or dietary deficiencies, and to monitor exposure. Our laboratory runs an inductively coupled mass spectrometry (ICP-MS) method for quantifying copper, zinc, and magnesium in urine. Recently a significant bias was noticed via proficiency testing program. The investigation started with analyzing a certified NIST sample in nitric acid and no bias was observed. We then investigated whether matrix effect was the root cause for the bias and what modifications to the method could mitigate this problem. **METHODS** The assay was run on a Thermo Fisher X Series2 ICP-MS with a collision/reaction cell controlled by PlasmaLab (ver. 2.6.2.337). The original method employed a 1:10 dilution of patient specimens with 0.1% nitric acid prior to analysis. Additional experiments with dilutions at 1:15, 1:20, 1:25, 1:50, 1:100 were performed and analytical measurement range (AMR) was determined for each. Each AMR determination consisted of analyzing in triplicate a series of samples which included a spiked patient pool and serially diluted samples using 0.5% nitric acid. The acceptable criteria included a signal-to-noise ratio >10, accuracy 80-120%, and CV <20%. The purpose of these experiments was to identify the maximal sample dilution that would reduce the bias but still offer acceptable sensitivity. Statistics were calculated using Excel (Microsoft, Redmond WA, USA). **RESULTS** The 1:50 dilution allowed for a similar sensitivity compared to the original 1:10 dilution while significantly reducing the bias. The original average bias in the proficiency samples was -13% for magnesium, -11% for copper, and -15% for zinc. The average bias using 1:50 dilution was -6.9% for magnesium, 3.5% for copper, and -1% for zinc. **CONCLUSION** Matrix effect for ICP-MS methods could cause significant bias and increased dilution can be used to reduce the matrix effect while maintaining the sensitivity.

A-358

Determination of Whole Blood Selenium by an Inductively Coupled Plasma Mass Spectrometry Method

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BACKGROUND Selenium concentrations in human blood are variable, depending on the selenium content of foods consumed and the regional availability of the element. The richest sources of selenium include seafood, meat, cereals and grains. Both excessive and insufficient intake of selenium can have health implications. Low selenium intake may increase some forms of cancer, is implicated in increased incidence of cardiovascular disease (Keshan), weakening of the immune system, impaired growth, osteoarthritis in children, and fertility. Conversely, selenium toxicity includes gastrointestinal upsets, hair and nail loss, tooth decay, liver failure, skin lesions, fatigue and damage to the nervous system. The primary goal of this study was to develop a high-sensitivity inductively coupled plasma mass spectrometry (ICP-MS) method for quantification of Se in whole blood. **METHODS** This method was developed on a Thermo Fisher X Series2 ICP-MS with a collision/reaction cell controlled by PlasmaLab (ver. 2.6.2.337). Whole blood (1 mL) was added to 9 mL of 0.1% nitric acid, vortex mixed then centrifuged at 3400 g for 5 min. Statistics were calculated using Excel (Microsoft, Redmond WA, USA) and EP Evaluator Release 10 (Data Innovations, South Burlington, VT, USA). **RESULTS** Full technical validation was performed and the assay met the institutional requirements. The linearity of the assay was 13.3 to 563.3 µg/L with analytical recovery from 83.8 to 118.1%. Precision was evaluated based on EP10-A3 protocol. For spiked whole blood samples (N=30) with mean concentrations of 68 µg/L, 127 µg/L, and 190 µg/L, the within run coefficients of variation (CV) were 1.0%, 1.1%, and 1.6%, respectively and the total CV was 1.5%, 2.0%, and 2.5%, respectively. No significant carryover was observed from samples with concentrations up to 723 µg/L. Deming regression was performed using 30 patient samples ranging from 81 to 242 µg/L showed a slope of 0.977 (95%CI: 0.911-1.043), an intercept of 4.2 µg/L (-4.9 to 13.4) and an r of 0.9846 in comparison with a commercial ICP-MS method at NMS Labs (Willow Grove, PA). **CONCLUSION** The high sensitivity and accuracy make this ICP-MS methodology suitable for clinical monitoring of Se.

A-359

Quantification of Plasma Total Testosterone and Dehydroepiandrosterone by LC-Q-Exactive MS

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BACKGROUND: Total testosterone (TT) and dehydroepiandrosterone (DHEA) are measured in adults for androgen abnormalities and in pediatrics for cases of delayed or precocious puberty. The very low concentrations of TT in pediatrics and females made it essential to use mass spectrometry (MS) based methods for high specificity and sensitivity. The high resolution accurate mass (HRAM) capability of the quadrupole-Orbitrap (Q-Exactive) MS can be used to improve specificity, however; for some isobaric compounds separation must occur before the MS. This study aimed to develop a highly sensitive and specific assay using the LC-Q-Exactive MS system for the quantification of TT and DHEA at very low concentrations in plasma. **METHOD:** A Q Exactive quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher Scientific) coupled with Thermo Scientific Accela HPLC pump were used. TT and DHEA were extracted with methyl tert-butyl ether (MTBE) from plasma (500µL) after precipitation by mixing the plasma with 0.1M zinc sulphate (200µL) in water and testosterone-d3 (10µL; 225 ng/mL) in methanol. The supernatant was evaporated at 37°C under a stream of nitrogen then derivatized with hydroxylamine (100µL; 100 mg/mL) for 30 min. The resulting solution was extracted again with MTBE, dried down at 37°C under a stream of nitrogen and reconstituted with 125µL of (1:1 MeOH:H2O). The final solution (25µL) was injected onto an Accucore C18 (50 x 2.1mm, 2.6µm; Thermo Fisher Scientific, Waltham, MA). The mass spectrometer was set at positive heated electrospray ionization in the parallel reaction monitoring (PRM) mode. The MRM transitions (m/z) were 304.23>124.07 and 304.23>112.07 for TT, 307.23>124.07 and 307.23>112.07 for d3-Te, and 304.23>253.19 for DHEA. Statistics were calculated using EP Evaluator v10 and Microsoft Excel. **RESULT**: The LC run time was 5.5 minutes per injection. Testosterone and DHEA were separated both chromatographically and with unique transitions post-derivatization. TT was linear from 50-15240 pg/mL using transition 307.3>124 and DHEA was linear from 0.11-60.70 ng/mL using transition 304.2>253 with analytical recovery ranging from 86-118% for all compounds. Within-run and total CVs were < 5.5% and 7.5%, respectively for TT and < 26% and 21%, respectively for DHEA. Method comparison with an immunoassay was completed for TT. The Deming regression statistics for the comparison were as follows: range 11.80-980.80 ng/dL, slope 1.090, intercept 26.39, SEE 62.77, and correlation coefficient 0.9820. **CONCLUSION**: An accurate and precise LC-Q-Exactive method for total testosterone and DHEA was developed.

A-360

High-Throughput LC-MS/MS Measurement of Pregnenolone in Human Blood Serum for Research Purposes

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Background: Pregnenolone is a biosynthetic precursor to other steroids such as corticosteroids, androgens, and estrogens. It is converted to progesterone by 3-beta-hydroxysteroid dehydrogenase or to 17-OH-pregnenolone by 17-alpha-hydroxylase. Researchers investigating how these enzymes function need to quantify pregnenolone within an analytical range of 10 to 500 ng/dL (0.3 to 1.5 nmol/L) in blood serum. Since pregnenolone does not ionize well by either atmospheric-pressure chemical-ionization (APCI) or electrospray ionization (ESI), derivatization with hydroxyl amine was necessary to reliably achieve the desired analytical range.

Methods: Pregnenolone was measured in blood serum using a multi-channel ultra high-performance liquid chromatography (UHPLC) coupled to a triple-quadrupole mass spectrometer (MS) with heated electro-spray ionization (HESI). Sample preparation involved extracting specimens with methyl-t-butyl ether after spiking them with pregnenolone-D_4 internal standard (IS). The extracts were evaporated and the residues were reacted with hydroxyl amine to form positive-ion oxime derivatives. The preparations were dried and reconstituted with water and methanol (1:1). Injections were made into a 4-channel UHPLC system. A 4.5-minute water-to-methanol gradient eluted the analyte and IS through a heated column, packed with solid-core silica with phenyl groups bonded to its surfaces, into the HESI source. Selective-reaction monitoring (SRM) within a 1-minute data window produced quantitation and conformation chromatographic peaks.

Results: Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. Method precision, assessed as percent coefficient of variation (%CV) of peak areas from 20 replicate injections of two pools of test specimens, were better than 5% and 8% for intra- and inter-batches, respectively. Carryover, measured in blanks immediately following injections of the highest calibrator, never exceeded 0.2%. Specimen IS peak areas averaged 65% relative to the averaged IS peak areas in calibrators and QCs, indicating moderate ion-suppression by matrix. However, the IS in each sample successfully compensated for matrix effects as proved by method comparison results. The desired analytical range from 10 to 500 ng/dL (0.3 to 1.5 nmol/L) was achieved and was consistently linear ($r^2 \geq 0.999$ with 1/X weighting). For method comparison, 40 donor samples were analyzed and results were compared with those from a reference lab. Pregnenolone values among these samples ranged from 13 to 130 ng/dL and the percent difference between two analytical methods did not exceed 20% for 93% of the samples. Sample throughputs were 13, 26, 39 or 52 injections per hour when multi-channeled across 1, 2, 3 or 4 channels, respectively.

Conclusion: We developed a sensitive, robust, high-throughput quantitation assay for pregnenolone which can measure 10 to 500 ng/dL (0.3 to 1.5 nmol/L) in blood serum. The LC-MS/MS method can be multi-channeled with other HESI-MS/MS methods.

A-361

High-Throughput LC-MS/MS Measurements of Estrone (E1) and Estradiol (E2) in Human Blood Serum for Research Purposes

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Background: Estrone (E1) and estradiol (17-β-estradiol or E2) are two steroid hormones involved in the development and function of female anatomical and physiological characteristics and processes such as the menstrual cycle. Researchers studying the effects of E1 and E2 on such things need to quantify them within an analytical range of 5 to 500 pg/mL (18.5 to 1,850 pmol/L) in blood serum. E1 and E2 form negative ions by deprotonation in both electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) sources of mass spectrometers with low efficiency. In order to robustly achieve the needed quantitation limits, most researchers use dansyl chloride to form positively charged derivatives of these and other estrogens.

Methods: E1 and E2 were measured in blood serum using a multi-channel ultra high-performance liquid chromatography (UHPLC) coupled to a triple-quadrupole mass spectrometer (MS) with heated electro-spray ionization (HESI). Sample preparation involved extracting specimens with methyl-t-butyl ether after spiking them with estradiol-D_5 internal standard (IS). The extracts were evaporated and the residues were reacted with dansyl chloride to form positive-ion dansylated derivatives. The preparations were dried and reconstituted with water and acetonitrile (1:1). Injections were made into a 4-channel UHPLC system. A 5.5-minute water-to-methanol gradient eluted the analyte and IS through a heated column, packed with solid-core silica with hydrocarbon groups bonded to its surfaces, into the HESI source. Selective-reaction monitoring (SRM) within a 1-minute data window produced quantitation and conformation chromatographic peaks.

Results: Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. Method precision, assessed as percent coefficient of variation (%CV) of peak areas from 20 replicate injections of two pools of test specimens, were better than 6% and 7% for intra- and inter-batches, respectively. Carryover, measured in blanks immediately following injections of the highest calibrator, never exceeded 0.5%. Specimen IS peak areas averaged 37% relative to the averaged IS peak areas in calibrators and QCs, indicating high ion-suppression by matrix. However, the IS in each sample successfully compensated for matrix effects as proved by method comparison results. The desired analytical range from 5 to 500 pg/mL (18.5 to 1,850 pmol/L) was achieved and was consistently linear ($r^2 \geq 0.995$ with 1/X weighting). For method comparison, 40 donor samples were analyzed and results were compared with those from a reference lab. Values among these samples ranged from 16 to 156 pg/mL for E1 and 11 to 356 pg/mL for E2 and the percent difference between two analytical methods did not exceed 20% for 95% of the samples. Sample throughputs were 10, 21, 32 or 43 injections per hour when multi-channeled across 1, 2, 3 or 4 channels, respectively.

Conclusion: We developed a sensitive, robust, high-throughput quantitation assay for estrone and estradiol which can measure 5 to 500 pg/mL (18.5 to 1,850 pmol/L) in blood serum. The LC-MS/MS method can be multi-channeled with other HESI methods.

A-362

Analytical and preanalytical validation of simultaneous measurement of estrone and 17beta-estradiol in serum by LC-MS/MS

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Background:MALDI-TOF MS is increasingly used for routine bacterial and fungal identification in Japan, whereas application of LC-MS/MS in clinical chemistry laboratories has remained very limited. Although immunoassays are often used for measurement of serum estradiol (E2) when fast turnaround time is required, more sensitive and specific measurements are needed for determination of menopausal status, estrogen deficiency and in the diagnosis of sex hormone related disorders. Furthermore, simultaneous measurement of estrone (E1) and E2 is often requested particularly from gynecologic oncologists. Indeed, increased risk of endometrial cancers has been shown in subjects with high serum estrogen levels. The aim of this study is to develop and validate liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous measurement of E1 and E2 in human serum.

Methods:For sample preparation, 100μL of calibration solution, QC sample or patient serum were diluted, spiked by 20μL of internal standard (10ng/mL) and were loaded on the supported liquid extraction (SLE) plate and were then extracted by using 1.8 mL of extraction solution. The extracted samples were dried under nitrogen and were derivatized by dansyl chloride acetone solution. An aliquot for 40μL was then subjected to LC-MS/MS.The analytes were separated on a CAPCELL CORE C18 column (Shiseido) that was attached to the Bruker UPLC. A water/acetonitrile solvent gradient was used to achieve chromatographic separation of the E1 and E2 in 10 minutes.The selected reaction monitoring (SRM) was performed with a Bruker EVOQ Elite in electrospray ionization (ESI) and positive ion mode. The SRM transitions were m/z 504>171 for E1 and 506> 171 for E2. Serum E2 levels were also determined by immunoassay routinely used in our clinical laboratory (Architect CLIA, Abbott).

Results:The lower limit of quantifications of E1 and E2 were 6.2pg/mL and 7.3pg/mL, respectively. The analytical mesurement range for E1 was 6.2-1200pg/mL and 7.3-1600pg/mL for E2. Intra-assay CVs (n=20) were 2.7% (at 52.6pg/mL) for E1 and 6.4% (at 33.5pg/mL) for E2 , and inter-assay CVs (n=20) were 5.5% (at 52.6pg/mL) for E1 and 6.2% (at 33.5pg/mL) for E2. The recoveries were 98.6-100.8% for E1, 99.7-99.8% for E2. The accuracy was found to be within specified limits of BCR576, BCR577 and BCR578. There were no significant interference by the marked hyperbilirubinemia, hemolysis and chye. There were no effects by the up to 10 times of freeze-thaw cycles and there were no differences among the E1 and E2 values obtained using 6 different types of blood collection tubes.Method comparison studies for samples with E2 300pg/mL showed: [CLIA E2] = 0.8598[LC-MS E2] +9.9988 (n= 19).

Conclusion:We have developed and validated simultaneous measurement of estrone and 17beta-estradiol in serum by LC-MS/MS. We are now planning to use this method for E1 and E2 measurement on a routine basis in our university hospital.

A-363

Noninvasive prenatal diagnosis of fetal RhD status from RhD negative pregnant women using MALDI-TOF Mass Spectrometry

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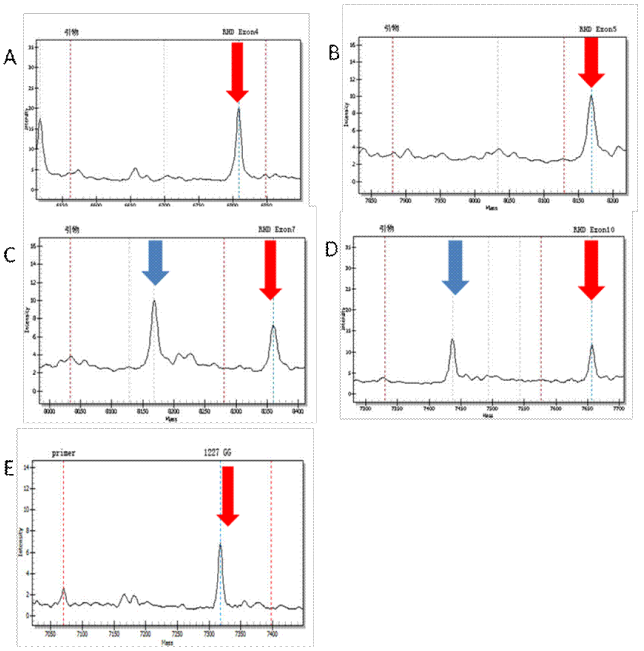
Background: Noninvasive prenatal genotyping of fetal RHD can prevent the unnecessary administration of prophylactic anti-D to women carrying RhD-negative fetuses. We establish a method for noninvasive prenatal diagnosis of fetal RHD genotyping from Chinese RhD-negative women based on matrix-assisted laser ionization time of flight mass spectrometry(MALDI-TOF MS).

Methods: RhD negative pregnant women with single fetus (20~40 gestational weeks) were recruited from August 2013 to March 2015. The existence of fetal DNA was confirmed by SNPs. Fetal RhD genotype was detected by MALDI-TOF MS targeting exon 4, 5, 7, 10 and RHD 1227A to predict the fetal RhD status. A double blind trial was carried out to compare the results of fetal RhD detected by MALDI-TOF MS and serological tests on cord blood.**Results:** A total of 40 plasma samples were collected. Fetal RhD genotype was detected by MALDI-TOF MS targeting RHD exon 4, 5, 7, 10 and RHD 1227A. 38 cases were identified RhD positive when one case was typed RhDel and one was RhD-negative. Five neonatal blood samples could not be obtained

due to loss to follow up, and the remaining 35 genotypings were in concordance with newborn D phenotypes at delivery.

Conclusion: These preliminary results demonstrate the feasibility of noninvasive prenatal diagnosis of fetal RhD status from RhD-negative maternal plasma in Chinese population using MALDI-TOF MS.



A-366

IntrinsiX™ - A novel calibration approach for performing quantitative LC-MS/MS analysis of serum methotrexate for clinical research

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Background: Batch mode analysis has limited the utility and throughput of quantitative LC-MS/MS assays. Here we describe the novel IntrinsiX™ workflow applied to the analysis of methotrexate in serum using ^{13}C-labeled analogs of methotrexate as internal calibrators. An accurate and precise quantitative result is generated in a single injection, eliminating the need to analyse a traditional set of external calibrators.

Methods: Cerilliant (Round Rock, Texas, USA) supplied four ^{13}C-labeled analogs of methotrexate that were designed to minimise isotopic interference. The analogs were used to prepare a 4-point IntrinsiX calibration curve over the range 0.025 - 10 µmol/L. IntrinsiX calibrators were added to each serum sample (50µL) and proteins precipitated using methanol. Following centrifugation, the supernatant was diluted and injected onto a Waters HSS-SB C18 UPLC column (2.1x30mm, 1.8µm) using a Waters ACQUITY UPLC® I-Class and quantified with a Xevo® TQD mass spectrometer.

EQA samples supplied by NEQAS (Nottingham, UK; n=14) and WEQAS (Cardiff, UK; n=9) were analyzed using an in-house conventional LC-MS/MS method, in which six non-zero calibrators are used for quantification, and the results compared with the new IntrinsiX approach.

Results: Following CLSI EP6-A the calibration range was shown to be linear from 0.0175 - 13.0µmol/L, with no detectable carryover up to 100 µmol/L. Coefficients of variation for inter- and intra-method imprecision for 0.1µmol/L, 1.0µmol/L, 2.5µmol/L and 7.5µmol/L samples were all ≤ 6.8% (n=25, days=5).

The agreement between the new IntrinsiX approach and the conventional LC-MS/MS method for the analysis of the EQA samples was described by the Deming equation y=-0.99x-0.02 (n=23, range 0.025 –2.18µmol/L), demonstrating significant constant bias with no proportional bias (p>0.05). The correlation between the new IntrinsiX approach and the all laboratory trimmed mean (ALTM) for the EQA results was described by the Deming equation y=0.94x+0.03 (n=14, range 0.030 - 2.14µmol/L), again demonstrating significant constant bias with no proportional bias (p>0.05).

Interference testing demonstrated a mean recovery of 101% for both endogenous compounds and metabolites tested. Following CLSI EP7-A2, recovery of samples containing 0.1 and 1.0µmol/L methotrexate (n=3) were unaffected (mean 101.0%, range 95.5 - 106.3%) when co-spiking with high concentrations of endogenous compounds (albumin, bilirubin, cholesterol, triglycerides and uric acid) and Intralipid®. Similarly, recovery was unaffected (mean 101.1%, range 98.2 - 104.9%) when methotrexate pools were supplemented with 5 and 50µmol/L 7-OH methotrexate (n=3) and 4-deoxy-4-amino-N^{10}-methylpteroic acid (DAMPA; n=3), showing absence of interference from these metabolites.

Conclusions: We have successfully implemented a novel calibration approach for performing quantitative LC-MS/MS analysis of serum methotrexate for clinical research. Incorporating the calibrators into each test sample allows improved throughput, shorter time to first result and the possibility of a workflow that does not require samples to be grouped into batches. Additionally, each sample is perfectly matrix-matched as demonstrated by the excellent results of the interference testing (mean bias 101%).

For Research Use Only. Not for use in diagnostic procedures.

A-367

Determination of Estetrol in Human Plasma by a Validated LC-MS/MS Method

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Background: Estetrol (E_4), or 15α-hydroxyestriol, is an estrogen steroid hormone, found in detectable levels in maternal plasma at around week 20. It is produced by the fetal liver during pregnancy and reaches the maternal circulation through the placenta. E_4 is detected from the 9-th week of pregnancy in maternal urine and after the second trimester levels in maternal plasma rise steadily with concentrations of unconjugated E_4 to about 1 ng/mL towards the end of pregnancy. So far the physiological function of E_4 has not been studied and is unknown. The possible use of E_4 as a marker for fetal well-being has been studied quite extensively. This paper presents the development and validation of LC-MS/MS determination of E_4 with the aim to be applied in the course of a food effect study.

Methods: E_4 and Estetrol-d_4, internal standard (d_4-E_4) were extracted from human plasma with Ethyl acetate and derivatized with dansyl chloride. Chromatographic separation was performed on C18 analytical column with gradient elution utilizing mobile phases consisting of acetonitrile (different proportions), water and formic acid Positive electrospray ionization and multiple reaction monitoring were used to follow the predominant transitions: collision energy 36, m/z 538→171 for E_4, and m/z 542→171 for d_4-E_4. Raw data of mass chromatograms were collected and processed by specialized software, and weighted ($1/X^2$) linear regression was performed to determine the concentration of E_4. Validation strategy was strictly adhered to current industrial guidance.

Results: Selectivity was assessed with 8 individual sources of human plasma (including one lipemic and one hemolyzed) and confirmed with matrix effect (ME) averaging 94-102% for E_4, 93-105% for d_4-E_4, and relative ME of 97-102%. Accuracy ranged from -3.90 to 1.84 % within runs and from 1.04 to 3.86 % between runs. Precision was up to 6.84 % within-runs, and up to 9.99% between-runs. Linearity was assured with 8 point calibration curve in the range 0.0259 ÷ 25.9480 ng/mL, $R^2 > 0.99$, y=3.906x + 0.021. Lower limit of quantification was set at 0.0259 ng/mL with accuracy and precision of less than 10% for both within runs and between runs. Freeze-thaw stability was determined for five cycles each lasting 24 h, post-preparative stability was documented for 48 h at 10ºC, short-term stability at room temperature was proven for 4h at daylight; stock solution stability and long term stability in plasma were documented for 154 days at -20ºC. With run time of 2.0 min, a throughput of over 170 samples per working day was achieved.

Conclusion: The method was validated according to current industrial requirements and allows the accurate and precise determination of E_4 in human plasma.

Key words: Estetrol, LC-MS/MS

Word count: 427

A-368

A Rapid LC-MS/MS Method for the Quantification of Lacosamide, Desmethyl Lacosamide, Gabapentin, Clozapine, and Topiramate

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Background: Lacosamide (LCM), Gabapentin (GAB), and Topiramate (TOP) are antiepileptic drugs approved by the FDA for treatment of epilepsy. Lacosamide is metabolized to the O-desmethyl lacosamide (ODL), the major metabolite in human. Clozapine (CLZ) is an atypical antipsychotic medication primary used for the treatment of schizophrenia. Measurement of these drugs has been performed by liquid chromatography-ultraviolet (HPLC-UV), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and in some cases enzyme immunoassay. However HPLC-UV methods can suffer from longer run times and potential interferences while enzyme immunoassays can be relatively expensive. LCM, ODL, GAB, and CLZ have a high ionization efficiency in positive electrospray ionization mode, while TOP has a poor ionization efficiency in positive mode which necessitates negative electrospray ionization. Our objective was to develop a rapid, accurate, and sensitive LC-MS/MS assay for the quantification of LCM, ODL, GAB, CLZ, and TOP. **Methods:** Serum (25µL) and IS solution (150µL; lacosamide-$^{13}C,D_3$, Gabapentin-D_{10}, Clozapine-D_4, and Topiramate-D_{12} in methanol) were vortex mixed and centrifuged. Supernatant (10µL) was added to 1000µL of 0.1% formic acid in water and vortex mixed then 3µL was analyzed on Accucore C18 column in an LC-MS/MS system. Total chromatographic time was 1.90 minutes with a ionization polarity switch at 0.90 minutes. A quantifier and a qualifier transition were monitored for all analytes. **Results:** No differential matrix effect or interferences were observed. Analytical Measurement Range (AMR) date for all 5 analytes is presented in Table 1. The total coefficient of variation was <4.7% for LCM, <5.6% for ODL, <4.4% for GAB, <5.5% for CLZ, and <7.2% for TOP at three levels tested. **Conclusion:** This rapid and sensitive LC-MS/MS assay meets the sensitivity, accuracy, and precision requirements for clinical use.

Table 1: AMR Data

Analyte (Units)	Analytical Measurement Range	% Recovery
Lacosamide (µg/mL)	0.5 - 48.1	84.4 - 100.8
Desmethyl Lacosamide (µg/mL)	0.6 - 52.3	87.5 - 106.1
Gabapentin (µg/mL)	0.5 - 47.6	87.1 - 99.5
Clozapine (ng/mL)	20.7 - 2113.2	82.7 - 105.7
Topiramate (µg/mL)	1.0 - 50.8	80.8 - 101.6

A-369

Determination of Monosialogangliosides in Human Plasma by a Novel UPLC/MS/MS Assay in Combination with Chemical Derivatization

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Objective To develop a LC/MS/MS method and quantitatively monitor the plasma level of monosialogangliosides in patients affected by GM3 Synthase Deficiency (GSD), an inherited neurological disorder characterized by seizure and profound developmental stagnation, for clinical diagnosis and therapeutic evaluation of an ongoing clinical trial. **Clinical Relevance** Gangliosides are a large subfamily of glycosphingolipids that present abundantly on the plasma membrane of neuronal and glial cells of vertebrates. These molecules are structurally characterized by a distinctive oligosaccharide moiety being attached to a ceramide portion with variable length and saturation degree on the fatty acid chains. Physiologically, they play critical roles in the regulation of various receptor-mediated cell signaling pathways and essential cellular events. Disruption in their metabolic pathways is pathologically implicated in the development of numerous neurodegenerative disorders, such as Parkinson disease, Alzheimer disease, and ganglioside GM3 synthase deficiency (GSD). In order to more comprehensively understand the disease etiologies, a reliable LC/MS/MS method with enhanced sensitivity is urgently demanded for relevant biomedical studies. **Methodology** In this study, a novel reverse phase UPLC/MS/MS method for determination of monosialogangliosides, GM1, GM2, and GM3, in human plasma has been developed and validated. This assay employed DMTMM & PAEA chemical derivatization for signal enhancement and deuterium-labeled monosialogangliosides as internal standards (IS). The analytes and ISs were extracted from plasma using protein precipitation procedure, cleaned up with liquid-liquid partition, dried under nitrogen purging, and derivatized with 2-(2-Pyridilamino)-ethylamine (PAEA) & 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). Thereafter, the samples were injected into a Shimadzu Nexera UHPLC system interfaced to an AB Scix Qtrap 5500 mass spectrometer that operated in ESI positive and Multiple Reaction Monitoring (MRM) mode to achieve detection with superior sensitivity and specificity. **Validation** Considering that the m/z from singly charged molecular ions of monosialogangliosides were mostly beyond the detection range of our Qtrap 5500 mass spectrometer, and they showed low preference to be doubly charged by both positive and negative ESI, we introduced a novel DMTMM & PAEA chemical derivatization method to increase the abundance of their doubly charged molecular ions in positive ESI. The sensitivity of monosialogangliosides in positive ESI was observed to undergo a 15-20 fold enhancement after derivatization. In addition, more than 15 different components were chromatographically resolved from each other within an 11min run. Moreover, calibration curves ranging from 10~2000, 10~2000, and 80~16000 ng/ml with correlation coefficients of 0.9981, 0.9989, and 0.9977 were established for plasma measurements of monosialogangliosides GM1, GM2, and GM3, respectively. Thereafter, we validated this assay based on the FDA guideline for bioanalytical method validation on precision, accuracy, stability, and extraction recovery. The relative percent error (R.E.) and coefficient of variation (CV) from measurements were below 11 and 11% for each monosialoganglioside species. The extraction recovery was found to be above 80% for each monosialoganglioside species. The loss of derivatized analytes from storage was found insignificant (<10%) under studied conditions. **Conclusion** In summary, we developed and validated a novel quantitative assay for determination of monosialogangliosides in human plasma using LC/MS/MS, which has been successfully applied to the ongoing clinical study.

A-370

Development, Optimization, and Evaluation of an Ultra Performance Liquid Chromatographic-Tandem Mass Spectrometric (UPLC-MS/MS) Method for the Quantification of the Anti-Malarial Atovaquone in Plasma

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Background: Malaria is a life-threatening parasitic disease primarily spread through the bites of infected mosquitoes. Malarial parasites are released into the bloodstream, resulting in infection of both liver cells and erythrocytes. A primary modality in disease treatment is the administration of anti-malarial agents. One prophylactic drug, atovaquone (ATQ), has been used both in single- and multi-drug applications for disease treatment. Early pharmacokinetic studies have demonstrated high inter-individual drug variability. Thus, there is a significant need to monitor plasma drug concentrations to fill in pharmacological gaps. With the scarcity of analytical methodologies available in the literature, we have developed and optimized an ultra-performance liquid chromatographic-tandem mass spectrometric (UPLC-MS/MS) method for the robust quantification of ATQ in human plasma.

Methods: ATQ and its deuterated standard, atovaquone-d5 (ATQ-d5), were acquired from Toronto Research Chemicals. Calibrators and quality control solutions were prepared by spiking both compounds into drug-free K_2EDTA human plasma (Biological Specialty Corporation). Following protein precipitation, samples were evaporated to dryness and reconstituted in 20:80 water:acetonitrile containing 0.1% formic acid. Samples were separated on a Synergi 2.5 µm Polar-RP 100A (100 x 2 mm) column (Phenomenex). ATQ was detected over 1.3 minutes on an API 4000 mass analyzer (SCIEX) using an ESI source operated in negative ionization and selected reaction monitoring (SRM) modes. The method was validated in accordance with the FDA Guidance for Industry Bioanalytical Method Validation recommendations. Validation metrics included the assessment of precision and accuracy, linearity, stability, and matrix effects.

Results: Parent to product ion transitions for both ATQ and ATQ-d5 were identified through the direct infusion of the compounds into the mass analyzer. Transitions identified for monitoring in SRM mode for ATQ and ATQ-d5 were 365.1-198.9 and 370.0-204.1 *m/z*, respectively. Due to pharmacokinetic parameters associated with ATQ, two calibration curves were generated to quantify the drug at both lower and higher (therapeutic) concentrations. Thus, two analytical measuring ranges were established at 5-1000 ng/mL and 1000-25000 ng/mL, respectively. Linearity was assessed from the slope of $1/x^2$ (sub-therapeutic range) and 1/x (therapeutic range) weighted least squares-fitted linear regression analysis. Representative calibration curves yielded regression equations with r^2 values of 0.9971 and 0.9978, respectively. Each curve required 0.05 mL samples of calibrator, quality control (QC) or unknown. For higher concentrations, samples were diluted 10-fold with drug-free plasma prior to protein precipitation. QC materials for both lower and higher ranges were prepared at low (15 ng/mL, 3000 ng/mL), mid (150 ng/mL, 8750 ng/mL) and high (850 ng/mL, 22500 ng/mL) concentrations, respectively. Pre-validation intra-assay precision and accuracy studies demonstrated values within expected thresholds defined by regulatory guidelines.

Conclusion: This work describes the development, optimization, and analytical evaluation of a UPLC-MS/MS method for ATQ quantification in plasma. The assay can be run in a reflexed fashion by which samples with ATQ concentrations not detectable by the higher, therapeutic range calibration would be subsequently run using the lower, sub-therapeutic calibration. In contrast to previous methods, the presented method requires low sample volumes, has a limit of quantitation of 5 ng/mL, and an analytical run time of 1.3 min.

A-371

Validation of an LCMS Method for Chiral Determination of Methamphetamine

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Background: Methamphetamine is a highly addictive and abused stimulant drug due to its potent stimulation of the central neural system. Several prescription formulations of d-methamphetamine are available. Patients who are on a drug monitoring program may test positive for methamphetamine during their drug confirmation. Physicians need to ensure their patient is not using over-the-counter inhaler formulations which contain l-methamphetamine. l-methamphetamine is a vasoconstrictor intended as a decongestant. d-methamphetamine is a major drug trafficking concern due to illegal illicit drug sales. There is a need for robust methodology with simple sample preparation in the clinical laboratory to support care-provider decisions. Isomeric chirality determination by separation of "l" and "d" stereoisomers may support putative decisions regarding licit or illicit drug. While GCMS is known for its high chromatographic resolution and ability to separate isomers through a derivatization process, labs may not have access to this instrumentation. Furthermore, a simple sample preparation process is desired for routine testing. **Methods:** An LCMS method was validated using an Agilent (Santa Clara, CA) 1200 liquid chromatograph coupled with a 6400 tandem mass spectrometer using electrospray ionization in positive mode. Our laboratory has evaluated several methodologies for isomeric determination of methamphetamine. With that experience, it has obviated the need for simple sample preparation, along with a rugged analytical testing methodology. To that end, we developed a crash-dilute-shoot (CDS) sample preparation method compatible with a chiral stationary phase analytical column. Sample preparation involved a 50 microliter aliquot of patient urine with 50 microliters of internal standard solution. This was homogenized with equal parts methanol for precipitation, followed by 30 minute centrifugation. The final dilution step resulted in an overall 1:20 sample:dilution. The column is an Astec Chirobiotic Supelco (Sigma-Aldrich, St. Louis, MO) dimensions of 150 mm x 4.6 mm with 5 µm particle size. A 5 mm C18 guard column (Agilent) was installed to preserve the column from patient specimen testing. An isocratic chromatograph (flow rate 1 milliliter/minute) resulted in elution of both isomers under 9 minutes. Mobile phase was 95% methanol with 0.1% acetic acid and 0.04% ammonium hydroxide. Mass spectrometric conditions were in multiple reaction monitoring for methamphetamine precursor 150.1 *m/z* and transitions 119.1, 91.1 *m/z*. The methamphetamine internal standard precursor was 155.1 *m/z* with transition 121.1 *m/z*. The dwell time was set to 200 milliseconds for each transition. A five level calibration curve was prepped in the same manner as patient samples for quantitation. **Results:** The calibration yielded linear quantitation curves for both isomers of methamphetamine. Controls were indicated at 50% below LOQ for both isomers, along with a +50% control for d methamphetamine and +50% for l methamphetamine. A negative urine control is included with each batch. Internal validation results gave excellent correlation versus the online/validated method of isomer separation by chiral selective cyclodextrin by capillary electrophoresis tandem mass spectrometry. The method has been qualified for clinical testing of patient urine specimens for isomeric determination of methamphetamine. **Conclusion:** This method is a robust and practical assay for the determination of methamphetamine isomer in the clinical laboratory.

A-372

Development and Validation of a Novel LC/MS/MS Method for the Quantification of Red Cells Methotrexate Polyglutamates

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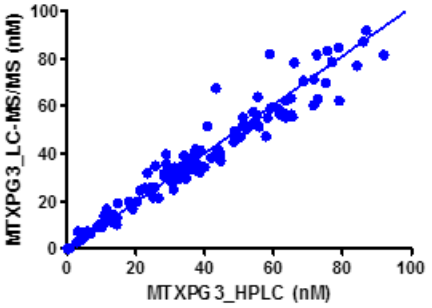
Objective: Therapeutic drug monitoring of red blood cells (RBC) methotrexate polyglutamates (MTXPG) is recognized as a valuable tool to optimize exposure to methotrexate in autoimmune rheumatic diseases. Our objective was to develop and validate a novel LC/MS/MS and establish its concordance with our reference method that uses HPLC with post column photo-oxidation and fluorimetric detection.

Methods: The LC/MS-MS consisted of a TSQ Quantiva (Thermofisher, Uppsala, Sweden) system. The chromatographic method used a pentafluorophenyl 2.1x50mm, 2.6 µm particle column, with mobile phase consisting of 0.1% formic acid and 0.01% trimethylamine with acetonitrile gradient. The sample treatment procedure used 100 µl RBC and consisted of a deproteinization step with perchloric acid in the presence of deuterated MTXPG3 as the internal standard. A 10 µl perchloric acid extract was injected onto the LC/MS/MS system following centrifugation. Sample analysis was performed in positive ionization with an m/z 713→308 transition for MTXPG3, the preponderant MTXPG in RBC. The run time was 6 minutes (vs 30 minutes for the reference method). The LC/MS/MS method was compared to our reference using RBC lysates obtained for routine testing in our clinical laboratory. Analysis consisted of Deming's regression slope and linear regression correlation coefficient.

Results: The analytical performance of the LC/MS/MS method consisted of intra-day and inter-day coefficient of variation below 15% at three different concentrations ranging from 5 to 100 nmol/L RBC. Detection limit was 1 nmol/L RBC. Using a total of 130 RBC lysates, there was a good concordance between MTXPG3 levels determined using the LC/MS/MS when compared to those determined using the reference method (Deming's slope = 1.026; Regression coefficient = 0.9525).

Conclusion: The LC/MS/MS method developed is equivalent to our reference method and can be used in clinical practice to optimize MTX dosing.



A-373

Development and Validation of Simultaneous Measurements of Four Vitamin D Metabolites in Serum by LC-MS/MS for Clinical Laboratory

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Background: Mass spectrometry (MS) is a powerful analytical tool in clinical laboratories. MALDI-TOF MS is used for bacterial and fungal identification on a routine basis in an increasing number of microbiology laboratories in Japan, whereas application of LC-MS/MS in clinical chemistry has remained very limited. The measurement of serum 25-hydroxyvitamin D [25(OH)D] as an indicator of vitamin D status is still widely conducted by immunoassays. Compared to other techniques, the advantages of LC-MS/MS include ability to simultaneously measure multiple analytes and the need for small sample volume for analysis, which in turn enhances the diagnostic capabilities of LC-MS/MS. The aim of this study is to develop and validate LC-MS/MS method to simultaneously measure four vitamin D metabolites in serum for application in clinical laboratories.

Methods: For sample preparation, 280µL of internal standard solution was added to 20 µL of calibration solution, QC sample or serum and were vortexed for one minute. The samples were applied to 96 well supported liquid extraction plate and were incubated for five minutes. The analytes were eluted three times with 700uL of ethyl acetate:hexane mixture (50:50, v/v). After elution, solvent was evaporated to dryness under nitrogen at 45 °C and was derivatized by the Cookson-type reagent (DAPTAD) that rapidly and quantitatively reacts with the s-cis-diene structure of vitamin D metabolites, and can markedly enhance the ionization efficiency. The derivatized samples were evaporated to dryness and then, the residue was dissolved in 23 µL of 30% v/v CH3CN containing 0.1% v/v HCOOH, and 20 µL sample was injected to LC-MS/MS.

Reconstituted samples were introduced from Shiseido HPLC to Thermo Fisher TSQ Vantage. Vitamin D metabolites were separated on a Shiseido core shell column

(CAPCELL CORE C18, 2.1×75mm). The column temperature was maintained at 40 °C and run time was 5.5-min. Samples were ionized using an electrospray ionization (positive-ion mode) and ions were detected by selected reaction monitoring.

Results: The LLOQs were 0.091, 0.020, 0.013, 0.024 ng/mL and the analytical measurement range was up to 59.4, 25.8, 66.1, 200 ng/mL for 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2 and 24,25(OH)2D3, respectively. The accuracy was found to be within specified limits of NIST SRM 972a. The intra-assay CVs were 0.9%, 3.0%, 4.4% and 3.8% for the low level 25(OH)D3 samples (7.16 ng/mL) and 1.9%, 3.1%, 3.5% and 1.6% for the medium level 25(OH)D3 samples (18.3 ng/mL) for 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2 and 24,25(OH)2D3. The inter-assay CVs for 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2 and 24,25(OH)2D3 were 2.7%, 5.7%, 5.6% and 6.6% for the low and 2.6%, 5.8%, 6.1% and 3.2% for the medium, respectively. The extraction recoveries were 97.5%-99.0%, 93.3%-103.9% and 92.8%-100.5% for 25(OH)D3, 3-epi-25(OH)D3 and 25(OH)D2, respectively.

Conclusion: We have developed and validated simultaneous measurements of the four vitamin D metabolites 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2 and 24,25(OH)2D3 in serum by LC-MS/MS. This method will be useful to obtain accurate quantitative data of these four vitamin D metabolites for clinical samples.

A-376

Amino acid analysis by mass spectrometry for concentration determination of C-reactive protein in candidate reference material SRM 2924

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Background: The National Institute of Standards and Technology (NIST) is developing a standard reference material (SRM) - SRM 2924, C-Reactive Protein Solution. This material consists of recombinant C-reactive protein (CRP) in aqueous buffer intended to serve as a "pure substance" reference material to provide traceability to SI units when used as a calibrant in the analysis of future reference materials containing CRP in biological matrices such as serum. The objective of this study was to determine the concentration of CRP using a higher order method. Analysis of this candidate material will include concentration assignment by amino acid analysis (AAA) using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS).

Methods: The material was received from the manufacturer in 12 boxes each containing 100 vials with 1 mL of aqueous buffer with a concentration of 0.49 g/L of CRP as measured by ultraviolet absorption with an extinction coefficient of 1.70 at 280 nm (A_{280}). A stratified random sampling plan was executed whereby two vials were selected from each box and further divided into four analysis groups to be performed in different weeks using independently prepared calibration and LC buffers. AAA was performed by vapor phase hydrochloric acid hydrolysis (118 °C for 48 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 calibration curve. A control material, NMIJ CRM 6201-b, C-reactive Protein Solution, was included in duplicate to assess accuracy and reproducibility. The control material was diluted 2-fold with equivalent buffer to match the expected levels in SRM 2924. The five calibrants, six samples and two controls were hydrolyzed within the same hydrolysis vessel for each separate analysis group.

Results: Linear regression conducted for each of the five individual amino acids within a sample yielded similar slopes, intercepts and regression coefficients with values close to 1, 0 and greater than 0.999, respectively. The five values obtained for each amino acid in each sample were likewise very similar and were averaged to obtain a single result for each sample. The values determined for each analysis group were 20.7 µmol/kg, 20.3 µmol/kg, 19.8 µmol/kg, 21.1 µmol/kg with coefficient of variation being 2.4%, 1.9%, 4.4% and 1.8%, respectively. The value of CRP in NMIJ CRM 6201-b was found to be 38.9 µmol/kg (cv = 0.9%) comparing well to the certified level of 40.0 µmol/kg (uncertainty = 1.6 µmol/kg) which was also value assigned via AAA. The combined average concentration was 20.5 µmol/kg (cv = 2.7%) which corresponds to a value of 0.47 g/L, within 4% of the A_{280} value.

Conclusion: The result of the AAA on SRM 2924 show that the analysis is repeatable within each separate run and across multiple analysis groups spanning several weeks in time indicating a robust method. Assignment of the CRP level in SRM 2924 will be based on this study but will also include an estimate of uncertainty. Release of this material is anticipated during 2016.

A-378

Development of a high throughput hemoglobinopathies workflow using high resolution accurate mass analysis

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Background: Hemoglobin profiling research focuses on detecting, characterizing, and performing relative quantitation of all hemoglobin chains, including sequence variants and PTMs. Recently, mass spectral detection has emerged as a suitable method to perform hemoglobinopathies due to the advances in resolution at fast mass spectral acquisition rates for both full scan and tandem mass spectrometry while maintaining accurate mass determination over large dynamic ranges. To satisfy the analytical requirements for a high-throughput assay, routine sample preparation is needed along with automated data processing capabilities are needed.

Methods: All experiments were performed on a Q Exactive Focus mass spectrometer. Hemoglobin samples were created directly from whole blood or dried blood spots and diluted with 90:10:0.1 (H_2O/MeOH/acetic acid) and loaded into a separate well of a cation exchange plate. Bovine hemoglobin was spiked into some samples to increase the complexity. The plate was washed to remove salts and small molecules, and then targeted MW range was displaced using an ammonium formate solution. Each sample was loaded directly onto an SEC column for on-column washing and elution into the mass spectrometer. HR/AM MS and tandem MS was acquired using a 70,000 resolution setting and all subsequent data was processed using the Pinnacle software for automated qualitative and quantitative analysis.

Results: The primary challenge associated with hemoglobinopathies is to detect and confirm hemoglobin sequence variants with mass shifts as small as 1 Da in a high throughput method. The MW range of both hemoglobin chains makes intact profiling feasible with mass resolution around 50,000. The approach presented here combines rapid sample preparation to isolate the targeted MW range covering hemoglobin (ca. 4 minutes per plate), with on-line clean up and introduction into the mass spectrometer for subsequent profiling. Multiple data acquisition methods were used, including alternating HR/AM MS and all ion fragmentation (AIF), as well as modified data dependent acquisition (DDA) on selected mass ranges. Data acquisition was performed in two minutes and the injection cycle time was six minuntes. Automated data processing was performed using known base sequences for human alpha and beta hemoglobin chains, and targeted searching routines in the Pinnacle software. To increase the complexity of the sample, bovine hemoglobin was spiked into some samples at known ratio covering 7:3, 5:5, and 3:7 (human:bovine) as well as neat samples for each species. Reproducibility and robustness was evaluated by preparing all at least 25 replicates per hemoglobin mixture.

Conclusion: Research results were able to demonstrate accurate MW determination and sequencing from reported hemoglobin chains, identifying sequence truncations as compared to the Uniprot sequences. In addition, PTMs such as deamidation, deoxydation, and oxidation was evaluated for both human and bovine hemoglobin chains. The measured variance for each chain in all samples was less than 20% despite using a SEC for introduction into the mass spectrometer and the ratios between species was less than 15%.

A-379

A high-resolution accurate mass (HRAM) mass spectrometry method to assist in identification of hemoglobin variants

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Background:

Hemoglobinopathies are some of the most common hereditary disorders in the world. Depending on hemoglobin mutation, patients may present with anemia, polycythemia, or persistent cyanosis. Correct variant identification aids in treatment choice and understanding disease severity as well as possible consequences of family planning. Typical screening methodology is based on HPLC (high performance liquid chromatography) and CZE (capillary zone electrophoresis). While of great utility, these methods cannot separate some clinically significant rare variants. Since mass spectrometry does not depend on differential mobility of hemoglobins, variants that are otherwise silent can be detected if they exhibit sufficient mass difference from normal subunits. With high-resolution instruments more accessible to clinical laboratories, even variants with subtle mass shifts are able to be identified. An HRAM MS method was developed to investigate the ability to detect abnormal hemoglobin variants in order to aid hemoglobinopathy diagnosis.

Methods:

10 uL of EDTA whole blood samples were lysed in 20% acetonitrile followed by solid phase extraction (SPE). The samples were then injected onto a nanoLC column (EasySpray C18 Pepmap) coupled to a quadrupole-orbitrap hybrid mass spectrometer (ThermoScientific Q-Exactive). An isolation window of 920-1200 m/z range ensured that hemoglobin was the major observed compound reducing potential interferences from other proteins. After LC-MS analysis, samples were deconvoluted using an automated method in Protein Deconvolution 3.0 (ThermoScientific) utilizing Xtract mass algorithm to produce monoisotopic masses. Selection criteria of a minimum of 3 charge states and 10 signal-to-noise (S/N) cut-off reduced interference from low abundance proteins. In order to screen mass difference with possible variants, an update to HbVar database was implemented allowing for difference in mass searches.

Results:

Normal masses for alpha (15116.898±0.004 Da) and beta (15856.258±0.007 Da) subunits were verified in our patient population. Analysis of over 40 hemoglobin samples included Hb D (Δm (*beta*)= -0.991±0.006 Da), Hb E(Δm (*beta*)= -0.987±0.011 Da), Hb SC(Δm (*beta*)= -30.771±0.453 Da), and Hb-Philadelphia (Δm (*alpha*)=14.04 Da) variants. Despite known difficulty deconvoluting hemoglobin subunits with a small mass change, we demonstrated that it is possible to resolve 1 Da subunit mass differences and even extend the method to heterozygous samples without chromatographic separation as a result of high resolution and Xtract deconvolution algorithm.

Conclusion:

The combination of monoisotopic mass reports for patient hemoglobin subunits with a newly-updated HbVar database allows for straightforward variant searching based on reported mass difference. This methodology should lead to better identification of hemoglobin variants when used in combination with conventional hemoglobin identification techniques.

A-380

Advantages of Standardized/Kitted Methods for Protein Digestion; a Monoclonal Antibody Example

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Background: Monoclonal antibodies (mAb) are becoming a more common place treatment for cancers, autoimmune diseases, and other diseases thought to be untreatable in the past. Therapeutic drug monitoring of these biologics is starting to be considered of clinical significance, especially as these therapeutics are lifelong treatments with great expense. Method development to monitor drug concentrations in serum encompasses immunoassays or mass spectrometry pathways; including the intact light chain quantitation or the use of tryptic peptides unique to the mAb. The latter is a highly complex assay with sample clean-up, reduction, alkylation, and trypsin digestion. Many of these steps are manual, with long incubation times and can be prone to error in a clinical laboratory. As future mAb peptide methods are implemented into the clinical laboratory setting, the standardization and improved traceability of pre-analytical steps for parts or the entire assay could be very advantageous. **Objective:** The goal of this study was to perform a method comparison between a laboratory developed manual sample preparation protocol using in-house reagents and a kitted digest method for the analysis of tryptic peptides from infliximab. **Methods:** Residual waste serum samples with physician-ordered infliximab testing were obtained from the clinical laboratory (n=39). Samples were processed per standard operating procedure. Briefly, a protein level standard is added to serum, and the mixture undergoes a protein crash using saturated ammonium sulfate (SAS). The reconstituted pellet is then denatured, reduced, alkylated, and digested as previously published; with DTT, IAA and trypsin weighed out and reconstituted daily. Side by side, the SAS mixture was prepared using a kitted digest (Waters ProteinWorks Digest Kit, Waters Inc.) that incorporates denaturation, reduction, alkylation, and digestion steps, following manufacturer's directions. Analysis for both methods was performed by LC-MS/MS on an API 5000 triple quadrupole mass spectrometer (ABSciex). **Results:** Sample processing using the standard operating procedure has a turn-around-time (TAT) >10h, from the initial denaturation to the digestion quenching. The kitted digest method allowed completion of the protocol within 4 hours, which would allow sample preparation to be performed in one single shift. The accuracy of infliximab using Waters' ProteinWorks Digest Kit was measured using ordinary linear regression; y=1.02x+1.10; R^2=0.99. Additionally, quantitation using tryptic peptides *m/z* unique to the light chain or the heavy chain of infliximab yielded similar results; y=0.97x-0.21, R^2=0.96, and may be used as a different measure of accuracy and complete digestion. **Conclusions:** The kitted method is amiable to any upstream enhancement/enrichment steps that are employed with mAb workflow, and results in equivalent quantitation of infliximab in residual serum samples. Although precision and cost still need to be fully evaluated, this proof-of-concept initial comparison showed important advantages such as lot traceability, ready-to-use reagents and a significant improvement in TAT compared to the current method, what can allow better follow up and accommodate an eventual increase in test volumes without a substantial method change and adding efficiency to the sample pre-analytical processes.

A-381

A Single-Injection LC-MS/MS Method for the Assessment of Impaired Glucose Tolerance

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Background: Early diagnosis of prediabetes is crucial for delaying and preventing its progress to type 2 diabetes. Millions of people are diagnosed with type 2 diabetes every year, and the total costs of diagnosed diabetes in the US is more than $245 billion per year. Impaired glucose tolerance (IGT) can be an important indicator of prediabetes. Current tests to diagnose IGT, such as the OGTT, are cumbersome and undesirable to the patient. We developed and clinically validated a test for impaired glucose tolerance in 2014 (Quantose™ IGT), utilizing biomarkers discovered on our metabolomics platform. Due to the varying polarity of the analytes, the method was performed in two LC-MS/MS injections, one traditional reverse-phase and one ion-pair injection for the more polar analytes. Because using a two-injection method significantly limited throughput, it was desired to develop a single-injection LC-MS/MS test for IGT.

Methods: Plasma samples are mixed with isotopically labeled internal standards and extracted via protein precipitation. The extracts are analyzed by a single reverse-phase, negative MRM LC-MS/MS method for the quantitation of 2-hydroxybutyric acid (0.500 to 40.0 µg/mL), 3-hydroxybutyric acid (1.00 to 80.0 µg/mL), 4-methyl-2-oxopentanoic acid (0.500 to 20.0 µg/mL), 1-linoleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (2.50 to 100 µg/mL), oleic acid (10.0 to 400 µg/mL), pantothenic acid (0.0100 to 0.800 µg/mL), and serine (2.50 to 100 µg/mL). An IGT risk score is calculated from the analyte results using a multivariate algorithm that also incorporates glucose.

Results: Reverse-phase chromatography was chosen to accommodate all seven biomarkers, which showed significant differences in polarity and had previously been analyzed in two separate LC-MS/MS injections. Analytical method validation was performed on four identical LC-MS/MS systems with five runs on each instrument for five separate days. Acceptable linearity (R^2>0.99) was observed for all the analytes over the ranges. Inter-assay imprecision of all analytes (n=100 per level: low, mid, and high levels) was less than 5.8%. Inter-assay imprecision of the IGT scores was less than 2%. Inter-assay accuracy at the lower limit of quantitation was within +/-10% of the nominal value and imprecision at the LLOQ was less than 9%, for all analytes (n=100). Relative analytical recovery was determined to be between 96.3% and 103% for all analytes. Short term stability evaluation indicated that plasma samples were stable after 6 hours at room temperature, 4 days in a refrigerator, or 5 cycles of freeze/thaw. Specificity and potential interference were assessed during the validation, and their impacts reasonably addressed. Method comparison to the two previously-used clinically validated methods was performed and the correlation between the old and new methods was greater than 0.95 for all analytes, with most analytes correlating above 0.99. The overall IGT score correlation was >0.999 with less than 1% average bias.

Conclusions: The new method reduces the time per test and cost by about 50% compared to the previously used two methods. The assay shows excellent precision, linearity, specificity, and accurate comparison to previously-used methods. This method for assessing IGT has now been used on over 3000 patients, and has performed robustly.

A-382

Automated mass spectrometric method for identification and quantitation of wild-type and familial variants of amyloid-beta peptides in cerebrospinal fluid

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Background: Until recently, quantitative analysis of amyloid-beta (Aβ) peptides in CSF has relied almost exclusively on the use of immunometric assays. LC-MS/MS methods to date have focused on wild-type Aβ peptides; however, known familial

mutations that occur within the Aβ42 region could result in a falsely low Aβ42 by LC-MS/MS and subsequently a disease risk misclassification. To account for specimens containing familial variants, we sought to develop a strategy to detect and identify variants along with wild-type Aβ peptides.

Methods: After incubation of CSF with denaturant, peptides were concentrated by solid phase extraction; extracts were then analyzed by high performance LC coupled to a triple quadrupole mass spectrometer. ^{15}N-labeled synthetic Aβ42 and Aβ40 peptides were used as internal standards. Sample processing steps were automated on a liquid handler. The method was evaluated following clinical guidelines including assessment of sensitivity, selectivity, linearity, precision, accuracy, stability and interferences. In addition to wild-type peptides, representative mutant Aβ42 synthetic peptides D23N (Iowa), A21G (Flemish), E22G (Artic), E22Q (Dutch), and E22K (Italian), were studied. A method comparison between the Innogenetics ELISA and the LC-MS/MS method was completed using 155 CSF specimens from the biobank at the University of British Columbia's Clinic for Alzheimer's Disease and Related Disorders.

Results: The analytical measurement range was 100-3000 ng/L for Aβ42 and 100-20000 ng/L for Aβ40. For the diagnostically relevant Aβ42 peptide, the total coefficient of variation near the medical decision limit was 7.9%. Acceptable recovery (85-115%) was observed for up to 5% whole blood contamination and no significant interference was observed with bilirubin or intralipid. The method comparison yielded the following regression: LC-MS/MS = 2.64*ELISA - 247.4, r^2 = 0.63. All peptide variants studied were resolved chromatographically from wild-type using our LC method. Linear regression analysis between CSF specimens (n=40) processed by manual and automated sample prep yielded r^2 = 0.97 for both Aβ42 and Aβ40.

Conclusion: Using instrumentation common to hospital labs, we developed an automated method for quantitation of Aβ peptides in CSF, inclusive of familial Aβ variants. The method was validated following established clinical guidelines and a data analysis procedure was established to identify the presence of variant Aβ peptide sequences without a priori knowledge of the isoform(s) present in specimens submitted for analysis.

A-383

Ethyl Glucuronide and Ethyl Sulfate in Urine: LC-MS/MS Method Evaluation and Assessment of Total Excretion Levels Post Alcohol Intake

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Background: Ethyl glucuronide (ETG) and ethyl sulfate (ETS) are metabolites of ethanol that are excreted in urine and considered specific biomarkers for alcohol consumption.

Objectives: To evaluate the performance characteristics of Q-Exactive mass spectrometer coupled with liquid chromatography for the quantification of ETG and ETS in urine and to assess the total levels of ETG and ETS excreted in the urine following low and moderate alcohol consumption.

Methods: Linearity, precision, and functional sensitivity were assessed using synthetic urine spiked with ETG and ETS. For ETG and ETS excretion study, 36 healthy volunteers (18 males and 18 females) consumed either 0.2g or 0.4g of ethanol per kg body weight. Nine timed urine specimens were then collected over the next 72 hours. Urine (20 µL) was diluted in 380 µL of diluent containing ETG-D5 and ETS-D5 before 10 µL of which was injected. The analysis was carried out on an LC-Q-Exactive system using a Kinetex C18 column (100 x 2.1 mm, 2.6 µm); total run time was 5 minutes. Additionally, urine creatinine (creat) concentrations were measured using Jaffe reaction on Beckman Olympus to calculate the ETG/creat and ETS/creat ratios (µg/g). All values are expressed as mean±SD.

Results: Assay was linear between 50 and 50,000 ng/mL with R^2 values of 0.9984 and 0.9997 for ETG and ETS, respectively. Functional sensitivities at a CV of 10% were 23 ng/mL and 35 ng/mL for ETG and ETS, respectively. Between-day imprecisions (CV) ranged between 3 and 5% at low, medium and high levels of ETG and ETS. Concentration of ETG and ETS peaked in urine either 6 hours or 18 hours following alcohol intake. At 0.2g/kg intake level, average peak ETG and ETS concentrations were 4485±8550 ng/mL and 992.0±1546 ng/mL, respectively. At 0.4g/kg intake level, average peak ETG and ETS concentrations were 8195±5543 ng/mL and 1647±1093 ng/mL, respectively. In the first 24 hours following 0.2g/kg ethanol intake (day 1), 4482±5423 µg of ETG (ETG/creat =35±52) and 1244±1199 µg of ETS (ETS/creat =10±11) were excreted. In the second 24 hours (day 2), excretion dropped to 174.0±146.0 µg of ETG (ETG/creat =1.2±1.1) and 77.00±51.00 µg of ETS (ETS/creat =0.47±0.35). Day 3 levels were 91.00±75.00 µg of ETG (ETG/creat = 0.60±0.42) and 64.00±53.00 µg of ETS (ETS/creat =0.38±0.15). At 0.4g/kg intake level, day 1 excretions were 12129±6039 µg of ETG (ETG/creat =78±40) and 2740±1064 µg of ETS (ETS/creat =18±6.8). Excretions on day 2 dropped to 118.0±64.00 µg of ETG (ETG/creat =0.82±0.47) and 82.00±45.00 µg of ETS (ETS/creat =0.52±0.16). On day 3, 65.00±42.00 µg of ETG (ETG/creat =0.54±0.37) and 52.00±33.00 µg of ETS (ETS/creat =0.38±0.23) were excreted.

Conclusions: The validated LC-MS/MS method is highly precise and offers sensitive quantification of ETG and ETS in urine. 24-hour ETG and ETS excretion levels following low and moderate ethanol intake are reported. Total levels and peak concentrations of excreted ETG and ETS are highly variable with no significant differences between males and females but with significant differences between the two levels of alcohol intake tested.

A-384

Agreement and validation of reliable scores for identification of microorganisms using MALDI-TOF

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Background: Identification of microorganisms using MALDI-TOF (MT) changed substantially standard procedures and workflows in clinical microbiology laboratories. Rapid identification, high accuracy and reduced costs and workload figure among the main benefits of this technology. However, as all new method, some improvements can only be reached along expertise and practice. The aim of this study is to evaluate the accuracy of the identification by MT using the standard protocol for protein extraction and direct identification (without extraction). We also intended to compare and validate the lower reliable score at genus and species level using the direct identification. **Methods:** We performed the identification using the Microflex LT® (Bruker Daltonics, Bremen, Germany) platform and the software Biotyper 3.2. For the interpretation of results we followed the manufacture's recommendations as following: 2.300 – 3.00 (highly probable species identification); 2.000-2.999 (secure genus identification and probable species identification); 1.700-1.999 (probable genus identification) and 0-1.699 (no reliable identification). **Results:** We performed the study in two steps. At the first step we selected 160 isolates previously identified in the routine and we processed at the MT with and without tube extraction to compare the agreement between the results. Twenty strains of the following isolates were selected: *S. aureus*, *S. pyogenes*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Gardenerella vaginalis* and *C. albicans*. We found 100% of agreement between both protocols despite lower scores according to direct protocol as expected. At second part of our study we selected more 160 strains randomly to compare not only the accuracy but also the lowest reliable scores obtained with the identification without extraction. Different species were tested, among them *Staphylococcus* spp. *Streptococcus*, *Enterococcus* spp., *E. coli*, *Proteus* spp., *Pseudomonas* spp. *Klebsiella* spp. and *Gardnerella vaginalis*. A 100% of agreement was observed at specie level. Comparing the scores obtained we noted that using direct identification, a break point of 1.750 is the lowest score for a reliable identification for all isolates tested at genus and species level. **Conclusion:** we conclude in our study that despite recommendations of manufacturers, scores under the established may be reliable for releasing genus and species identification in the routine laboratory using the protocol without tube extraction.

A-385

Mass spectrometry imaging workflow in clinical research

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Cancer incidence in Europe was recently estimated at 3.45 million cases, with 1.75 million deaths, and costing the EU 124 billion Euros every year. With such incidence rates, fundamental research and understanding of cancer biology is required to prevent (prognosis), identify (diagnosis) and treat cancer. Mass spectrometry imaging (MSI) is now increasingly used for clinical research applications due to significant technological improvements that have made the technique more accessible. Matrix-assisted laser desorption/ionization (MALDI), initially introduced by Caprioli et. al [1], is the dominant MSI technique used today, due to the ability of MALDI to analyse intact proteins directly from tissue. Furthermore MALDI is widely available and has been commercially developed by a number of vendors.

In the last few years, several alternative ambient ionization techniques have been developed that can ionize clinically important molecules, such as lipids, directly from tissue. One of these techniques, desorption electrospray ionization (DESI),

is a surface analysis technique incorporating an electrospray probe, that can be utilized as a spatially resolved imaging technique by rastering a surface under the spray using a high precision X,Y stage. As the electrospray droplets impact upon a surface, chemical constituents are desorbed and transferred into the atmospheric inlet of the mass spectrometer source. Ionization occurs due to the charge imparted onto the droplets. No modification to the sample such as matrix addition is necessary and therefore minimum sample preparation is required to run a DESI imaging experiment, making this technique more compatible within a clinical research environment

Here we describe the workflow for clinical research where MALDI and DESI imaging techniques were employed to characterize the molecular profiles which were significantly different between the normal and the tumour part of the tissues. The tissue sections analyzed were frozen clinical human tissue sections used for research puroposes, from liver and colorectal biopsies. For the MALDI MSI experiments, matrix solution was sprayed automatically using a nebulizing spray device, making the sample preparation step more reproducible than manual spraying. Consecutive or similar tissue sections were also analysed by DESI imaging. However in these experiments, no sample preparation step was required. Solvent solutions mainly used for the desorption and ionisation step were a mixture of Methanol-Water, which proved ideal for lipids and/or small molecule metabolite detection. By managing solvent and gas flow rates appropriately, in combination with the optimised voltages, the DESI technique resulted in negligible destruction of the tissue surface and therefore the same tissue sample can be histologically stained. In this case the molecular distribution was compared with the tissue's microscopic structure obtained from the H&E stained image.

A-386

Quantitative Testing for Polychlorinated Biphenyls (PCBs) in Human Serum Utilizing Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS)

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Background:

PCBs consist of a class of compounds produced for commercial use from 1930 to 1977 that were ultimately banned in 1979. Aroclors, named by the manufacturer consists of mixtures of PCB congeners in certain ratios that contain desirable characteristics with applications in plasticizers, adhesives, sealants, electrical transformers, capacitors, and wiring, among others. Although banned almost 40 years ago, PCBs continue to pose serious health risks to humans through diet and other environmental exposures. Varying volatility, fluctuating environmental decomposition rates, varying metabolic rates once adsorbed, and possible exposure to multiple Aroclors with overlapping congeners often make Aroclor testing in humans difficult to interpret. The objective of this study is to present a method for measuring 11 individual PCB congeners in human serum by GC-MS/MS.

Methods:

Sample preparation involves adding a mixture of 8 fluorinated analogue internal standards to 0.5mL of specimen, followed by the addition of urea, 2-propanol, water, methanol and petroleum ether. Samples were then mixed, centrifuged and the organic supernatant brought to dryness. Next, the extracts were reconstituted in hexane, then poured into solid phase extraction cartridges containing Florisil® and sodium sulfate. Additional hexane was added to each cartridge for elution, which was collected, concentrated and transferred to autosampler vials for analysis. Agilent's GC-MS/MS platform utilizing Retention Time Locking (RTL) and Multiple Reaction Monitoring (MRM) time segments were used for acquisition to enhance selectivity and sensitivity. Analysis was performed on a DB-5MS capillary column (15mx0.25umx0.25mm) using an oven ramp ranging from 80°C to 270°C, over a 10 minute run time.

Results:

The scope contains reporting limits ranging from 10 pcg/mL to 80 pcg/mL and a linear dynamic range spanning two and a half orders of magnitude for quantitation. Precision data were obtained by calculating the %CV from target for both the high and low QC tested five times per batch over the course of three batches. With-in run precision ranged from 2.8% to 7.0% and total precision ranged from 2.6% to 6.1% for all target compounds. Accuracy was determined using five replicate samples spanning the linear range. The slope values ranged from 0.992 to 1.051, y-intercept values ranged from -5.3 to 2.41, and systematic error values ranged from 3.7% to 6.2% for all target compounds. The correlation coefficient for calibration curves ranged from 0.999 to 1.000 for all target compounds.

Conclusions:

In conclusion, we were able to accomplish all of the project goals by successfully developing, validating and implementing a congener specific PCB assay by GC-MS/MS. The method is robust meeting all validation acceptance criteria including, precision, accuracy, selectivity, sensitivity and linearity. Other notable aspects include small volume of samples required for analysis, simple and cost effective sample preparation, quick run time and easily interpretable data.

A-387

Automating Liquid Chromatography-Tandem Mass Spectrometry (LC-MSMS) testosterone analysis for the small (or large) clinical laboratory - Tecan® AC Extraction Plate™ (AC Plate) used on a Tecan Freedom EVO® 100 automated liquid handler (ALH).

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LC-MSMS is recommended for quantifying serum testosterone in female and pediatric samples. We sought to implement a robust, highly automated LC-MSMS method also suitable for testosterone in adult male samples.

The proposed simplicity of the AC Plate is a desirable feature. Pipetting and orbital shaking are the only functions required for AC Plate extraction. Traditional sample preparation for testosterone requires additional ALH accessories or manual steps. We evaluated extraction reagent pH, solvent, and metal salt content and then validated the optimized method.

An ALH with an orbital mixer performed all extraction steps. The LC-MSMS was a Waters Acquity LC-XEVO TQS in positive ESI mode. The column was a Waters HSS C18, 2.1x150mm, 2.5 µm. Mobile phases A and B (MP-A/B) were 2 mmol/L ammonium acetate, 0.1% formic acid and acetonitrile, 0.1% formic acid. The LC gradient was 90:10 to 10:90 MP-A:MP-B, flow rate 0.4/0.6 mL/min, run time 6.3 min. MRMs acquired were 289/97, 289/109, 292/100, 294/97. The $^{13}C_3/^{2}H_5$-mixed working internal standard (I.S.) was 50/800 ng/dL. Injection volume was 3-10 µL.

Reagents, samples and barcoded plates are placed on the ALH deck. The user enters the number of samples to be tested and the starting well in the plate. Barcodes on sample tubes and plates are scanned by the robot. The plate map is transferred by MS VBScript into a Sample Table template on a shared directory. The sample table is imported to MassLynx by scanning the barcode on the injection plate.

The EVO robot extraction protocol is: add 100 µL serum, 25 µL I.S., and 175 µL of protein releasing reagent (0.33 mmol/L LiCl, 24% acetonitrile, 0.1% NH_4OH), shake for 10 min, discard the residue. Wash twice with 250 µL of 0.1% NH_4OH, shake for 5 min and discard the residue after each wash. Elute with 100 µL of 35:65 H_2O:acetonitrile, shake for 5 min and transfer to a 700 µL glass insert plate.

Mean recovery and decrease from matrix effect were 46% and 24% respectively. No interference was observed from epi-testosterone, DHEA, or other drugs and steroids tested. No interfering peaks were found from BD SST tubes and the mean bias was -4% between samples collected with or without SST gel. The validated analytical measurement range (AMR) was 2-1,000 ng/dL. S:N at the lower limit of quantitation ranged from 23-48. Within and between run precision (n=5) using BioRad LiquiChek Immunoassay-Plus and CAP Accuracy Based survey materials at means of 18, 87, 279, 508 and 987 ng/dL had CVs <5% and <12% respectively. No carryover was observed between 3,000 ng/dL and < AMR samples. Mean bias for CAP ABS samples was -4%. Deming regression

statistics for patient samples ranging between 12 and 566 ng/dL versus a CDC-HoST certified reference laboratory were slope 0.92, intercept 2.4, SEE 5.9, bias -6.9%.

We conclude that the AC Plate meets the vendor's claims for ease of use and implementation with good analytical performance across the desired AMR. This extraction consumable appears suitable for small or large laboratories desiring to simplify and automate sample preparation for serum testosterone.

A-389

Glycated hemoglobin in normal (AA) and variant hemoglobins (AC, AE, AD) using mass spectrometry: comparison with boronate affinity HPLC

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Background:

HbA1c is a well-established marker of glycemic control and an aid to diabetes diagnosis. There are many methods for measuring HbA1c based on a variety of principles including immunoassay, capillary electrophoresis, ion-exchange and boronate affinity chromatography (BAC). Hemoglobin variants can affect HbA1c results from some methods, which can negatively impact clinical interpretation of

results. BAC is generally considered to be immune to interference from Hb variants due to the fact that separation is based on binding specifically with cis-diol groups of glucose bound to hemoglobin and not on the structure of the hemoglobin chain. We evaluated the relationship between the glycated and non-glycated beta chains of normal and variant hemoglobins using LC/MS.

Methods:

Hemolysates from non-diabetic and diabetic subjects with normal HbAA or with three common heterozygous variants (HbAE, HbAC, or HbAD) were prepared. Measurements of the percentage of glycated globin chains were done using an LC-MS system operated in positive ESI mode. The quantitation of glycated forms was based on comparison of the areas under the curves in the extracted-ion chromatogram of the 17+ charged forms of glycated β vs. non-glycated β and the 16+ charged forms of glycated α vs. non-glycated α globin chains. The percentages of glycation of intact α and β globin chains were calculated. The ions β17+ and α16+ were chosen for quantitation because of their high signal intensities and absence of interferences in MS. Due to the fact that glycated and non-glycated globin chains may have different ionization efficiencies, we checked the validity of the method by analyzing mixtures of purified glycated and non-glycated normal globin chains in different known ratios.
Results:

Results from this LC/MS method showed a strong linear correlation of measured β glycated ratios with β glycated values from IFCC Reference Material ($y = 1.075x - 0.016$, $R^2 = 0.9998$). Results obtained by LC-MS for normal hemoglobin and three common heterozygous variants (HbAE, HbAC, HbAD) showed very similar linear correlations (i.e. comparable slope and intercepts) with Trinity Ultra^2 boronate affinity HPLC.

Conclusions:

The fact that all variants produce results that are comparable to that observed for normal HbAA suggests that the evaluated variant globin chains have glycation rates similar to that of HbAA. These results support the use of boronate affinity as a comparison method in Hb variant interference studies.

A-390

Validation of a Sensitive Method for the Simultaneous Quantification of Six Endogenous Anabolic Steroids Using GC-MS/MS

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Background: Endogenous anabolic androgenic steroids are listed by World Anti-Doping Agency (WADA) as prohibited performance enhancing substances. Accurate measurement of anabolic steroids is required for the detection of doping abuse. **Objective:** To validate a GC-MS/MS method for the quantification of androsterone (A), etiocholanolone (Etio), 5α-androstane-3α,17β-diol (5αAdiol), 5β-androstane-3α,17β-diol (5βAdiol), testosterone (T), and epitestosterone (E) in urine. **Methods:** For linearity and functional sensitivity, synthetic urine spiked with anabolic steroids was used. For precision, three urine samples containing low, medium or high levels of anabolic steroids were tested in triplicates for five days. Samples were spiked with deuterated internal standards and treated with β-glucuronidase followed by solid-phase extraction of steroids using 3M Empore C18 columns. Samples were derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide. Analysis was carried out on Thermo Trace 1310 gas chromatographer coupled with TSQ Quantum XLS Ultra mass spectrometer. **Results:** Assay was linear between 200 ng/mL and 7500 ng/mL for A and Etio, between 1 ng/mL and 200 ng/mL for 5αAdiol, between 1 ng/mL and 500 ng/mL for 5βAdiol, and between 0.25 ng/mL and 200 ng/mL for T and E. R^2 values were >0.99 for all steroids. Assay imprecision (CV) ranged from 3% to 11% for A and Etio, from 1% to 3% for 5αAdiol and 5βAdiol, and from 2% to 7% for T and E for all three levels of steroids tested. Functional sensitivities at 10% CV was 10 ng/mL for A and Etio, 1.5 ng/mL for 5αAdiol, 1.7 ng/mL for 5βAdiol, 0.25 ng/mL for T, and 0.23 ng/mL for E. **Conclusion:** The validated method for endogenous anabolic androgenic steroids quantification in urine using GC-MS/MS is sensitive and demonstrates excellent analytical performance.

Tuesday, August 2, 2016

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

Nutrition/Trace Metals/Vitamins

A-392

Evaluation of Serum Vitamin B12 Level in Type 1 Diabetes Mellitus

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Background:

Type 1 Diabetes Mellitus an autoimmune condition is known to be associated with multiple co-morbidities. Vitamin B12 deficiency is a potential co- morbidity that is often overlooked in these patients, despite the fact that many diabetic patients are at risk for this specific disorder. Studies done on the other population have demonstrated the presence of vitamin B12 deficiency or low vitamin B12 level in Type 1 diabetes. Defining the prevalence of low or deficient serum vitamin B12 levels in the diabetic population in this part of world may aid physicians to consider screening for vitamin B12 levels in Type 1 diabetic patients and carry out further evaluations.

Methods:

The cross sectional study was done by selecting 40 Type 1 Diabetes Mellitus patients from outpatient department (OPD) visiting endocrinology unit in Kanti children's Hospital. 30 healthy control groups were also selected based on inclusion/ exclusion criteria. Serum C-peptide, vitamin B12, creatinine, blood sugar level were assessed along with glycosylated hemoglobin in both groups. SPSS ver. 22 was used to analyze the data; t-test and one way ANOVA were used to find mean differences and Pearson's correlation was used to establish the correlation.

Results:

The mean age of Type 1 Diabetic patients was 10.44 ± 3.68 years, which included 21 male and 19 female patients. Among 40 patients, 47% were diagnosed before 1 year and 53% of them were diagnosed for more than 1 year. A total of 30 controls were also included in the study, males and females being equally represented. The mean age of control group was 4.87 ± 3.53 years. The age ranged from 1 year to 14 years. The case and the control group did not differ in biochemical and demographic characteristics except in their age, the difference in age was statistically significant. The mean serum vitamin B12 level of the case was 280.37 ± 111.34 pg/ml. Among the population 40.0% i.e. 16/40 were found to be deficient and 37.5 % i.e. 15/40 were sub clinically deficient. Whereas the mean serum vitamin B12 level of the control group was 462.67 ± 184.32 pg/ml. 2 out of 30(6.7%) were deficient, 8 out of 30 (26.75%) were found to be sub clinically deficient. Significant difference was noticed in the mean serum level of vitamin B12 between two groups.

Conclusion:

This study demonstrated the presence of low serum vitamin B12 levels in Type 1 Diabetics. The routine screening for this condition along with confirmatory test and detail clinical examination could benefit the Type 1 diabetic patients. However, further studies on a larger population using additional markers to investigate the actual cause of deficiency are must to strengthen this statement.

A-393

Quantification of human urine < serum iodine by inductively coupled plasma mass spectrometry

S. Yu, L. Qiu, Q. Cheng, J. Han. *Peking Union Medical College Hospital, Beijing, China*

Background: Thyroid diseases in China are prevalent, and whether that was associated with iodine salt in their food was controversy because of lacking efficient method for the measurement of iodine. This paper aims at establishing a method for quantification of iodine in human urine and serum by inductively coupled plasma mass spectrometry (ICP-MS), and providing an assay that can be used to the evaluation of the level of iodine in routine clinical laboratory. **Method** This study was method establishment and evaluation. Ammonia, isopropanol and ultrapure water was mixed at certain ratio, and used to dilute samples in the ratio of 1:10, and then the diluted samples were analyzed by ICP-MS. Re was used as the internal standard. And linearity, lower limit of detection, recovery, precision, accuracy, carryover and stability was evaluated thoroughly. Results of iodine of pregnant women who required iodine tests were retrospectively analyzed to evaluate the status of iodine. **Results** The method only needs 30s for analysis of one sample. It was sensitive with a lower limit detection of 0.87 ug/L, the correlation coefficient was higher than 0.9999 in ten measurements. The recovery in both serum and urine was approximately 100%. Compared with NIST standard reference material 3668, the bias was less than 5% which showed that the method had good accuracy. The inter-coefficient variation (CV) for serum iodine and urine iodine was 1.2%~3.0%, 2.0%~2.9%, respectively; and total CV for serum iodine and urine iodine were 3.0%~3.8%, 4.1%~4.9%, respectively. The mean carryover of this method was 0.03% and iodine was stable for at least one month at -20°C and 4°C. The urine and serum iodine for pregnant women was 154.8±89.7 μg/L (mean±SD),75.8±21.4 ug/L, respectively. The correlation between urine and serum iodine was 0.21.**Conclusion** A rapid and simple ICP-MS method for urine and serum iodine measurement has been established. It is accurate and precise and can be used in the evaluation of iodine in routine clinical laboratory.

A-394

Evaluation of Serum Vitamin B12 Reference Intervals According to Different Age Groups on 2 Immunoassay Platforms

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Background: Vitamin B12 deficiency is common in the older population due to gastrointestinal malabsorption, inadequate nutritional intake, increased cobalamin demand or the use of certain medications, and may be related to cognitive impairment, mood and psychotic disorders. The cut-off limits of 350 or 400 pg/mL for sufficiency were proposed by different studies. Raising clinician awareness in order to accurately diagnose and treat vitamin B12 deficiency can prevent irreversible structural damage and reduce morbidity among elderly patients.

Methods: In our study, we aimed to determine the age specific parametric reference intervals for DXI (competitive binding immunoenzymatic assay; Beckman Coulter, USA) and modular E (electrochemiluminescence immunoassay; Roche Diagnostics, Germany) for the evaluation of method performances in detecting insufficiency and the number of patients requiring treatment, using hospital data (Beckman-Coulter data; between January-July 2015 and Roche data; between July 2013-June 2014; n=75136) of Marmara University Pendik E&R Hospital.

Results: Data were analyzed in accordance with CLSI EP28-A3C guideline on Defining, Establishing, and Verifying Reference Intervals In The Clinical Laboratory. The reference ranges were significantly different in all age groups for the two instruments (Independent Samples T-test; $P<0.05$) (Table 1). In our adult population, 72% of patients analyzed by Beckman-Coulter and 46% of patients analyzed by Roche were below the 300 pg/mL limit of insufficiency and 87% of patients analyzed by Beckman-Coulter and 70% of patients analyzed by Roche were below the 400 pg/mL threshold of treatment.

Conclusion: Clinicians should be aware that reference ranges might change considerably between different platforms and affect the number of patients determined as insufficient. Reliance on reference ranges which have a low sensitivity and specificity in diagnosing deficiency states may lead to miss this treatable condition.

Table 1. Serum vitamin B12 reference ranges on Beckman-Coulter and Roche Immunoassay platforms

DXI (Beckman Coulter)			Modular E (Roche)		
Age interval (n)	Range (5-95%)	Median (pg/mL)	Age interval (n)	Range (5-95%)	Median (pg/mL)
0-1y (n=778)	101-727	339	0-1 y (n=720)	119-917	418
0-1 y-female (n=390)	106-693	347	0-1 y-female (n=341)	104-918	422
0-1 y-male (n=388)	94-790	331	0-1 y-male (n=379)	124-911	415
1-12 y-female (n=3645)	147-663	336	1-12 y-female (n=2371)	209-947	487
1-12 y-male (n=3837)	145-651	336	1-12 y-male (n=2598)	205-964	490
13-18 y-female (n=2001)	109-457	231	13-18 y-female (n=1230)	142-656	342
13-18 y-male (n=1184)	110-447	228	13-18 y male (n=722)	146-613	324
19-70 yrs (n=34995)	116-566	269	19-70 y (n=21005)	149-774	364

A-395

Direct validation of an analytical procedure for determination of β-carotene in human serum by UPLC-UV

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The validation of analytical methods is a procedure aimed at ensuring the quality and reliability of a test. Also aims to ensure compliance with the requirements nationally and/or internationally accepted and verifying the adequacy of the method it intends to use. β-carotene is a carotenoid that, once ingested, can be converted into Vitamin A (retinol) or act as an antioxidant to help protect cells from the damaging effects of free radicals. The aim of this study was toperform direct validation of procedure for determination of β-carotene in human serum by UPLC -UV. Initially, there was prepared a standard solution of β-carotene in dichloromethane with a concentration of 500.0 mg/mL. An intermediate solution was prepared in ethanol with a concentration of 20.0 mg/mL, to be tested points related to the analyte calibration curve. The points tested had the following concentrations: 50.0ng/mL, 100.0ng/mL, 300.0ng/mL, 500.0ng/mL, 850.0ng/mL, 1000.0ng/mL, 2000.0ng/mL and 3000.0ng/mL. Apart from this curve, a serum pool was contaminated to obtain the same concentrations above. The extraction of β-carotene is based on protein precipitation using organic solvents simultaneously with a simple liquid-liquid extraction. Thus, 450 µL of a solution of ethanol / n-butanol (1-butanol) 50:50 (precipitant solution) is added to 100 µL of serum and, after that, are mixed for 30 seconds using a mixer vortex. Then, this mixture is centrifuged at 14000 RPM for 10 minutes. Finally, the supernatant is transferred to the vial. To quantify the β-carotene in UPLC,10 µLof sample are injectedin the equipment. The column used was an Acquity UPLC BEH C18 1,7µm 2,1x50 mm. The wavelength was 453 nm and the mobile phase flow was 0.4 mL/min. The results were statistically processed for evaluation of some essential parameters for a validation. Among these parameters, linearity, accuracy, limits of detection and quantification and matrix effects were evaluated for consolidation of the procedure.The calibration curves for all compounds were linear with $r^2 > 0.9996$. The linear analytical range of the procedure was between 50.0 and 2000.0ng/mL. Accuracy (92.87-111.71%), intra-assay precision (0.75-10.77%) and inter-assay precision (4.26-16.45%) were acceptable.The determination limit was 10.0ng/mL and the quantification limit was 50.0ng/mL. Based on the slopes of the aqueous and biological matrix curves, F-test and T-test, it was concluded that the matrix effect is nonexistent in the present study. In conclusion, the UPLC-UV method has been developed successfully for quantitative analysis of β-carotene in clinical routine.

A-396

Comparison of a LOCI Vitamin D Total Assay* on the Dimension EXL System to CDC-certified Assays

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Background: Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The Vitamin D Standardization Program (VDSP) was created to establish a standard for accurate and comparable results for the detection of 25(OH)vitamin D across laboratories. As part of the VDSP, the CDC Vitamin D Standardization-Certification Program (VDSCP) enables laboratories to earn certification based on passing four consecutive quarterly sample challenges that assess bias and imprecision. The goal of this study was to investigate the relationship between a LOCI® Vitamin D Total assay* on the Dimension® EXL™ Integrated Chemistry System under development by Siemens Healthcare and current VDSCP-certified vitamin D assays.

Methods: 101 samples purchased from various specimen vendors and sent to the (University of Ghent) to have values assigned from the ID-LC/MS/MS reference method were tested using the Dimension EXL, ADVIA Centaur® XP, Roche cobas e 411, and DiaSorin LIAISON vitamin D assays. The range of the reference method values for the samples was 9.3 to 70.2 ng/mL. Results that were outside the analytical measurement range of one or more assays tested or the VDSCP measurement range of 9 to 110 ng/mL were excluded. Passing-Bablok regression analysis was performed to assess the relationship between each method and the Dimension EXL assay, and all assays to the reference method, as recommended by CLSI EP-9A3.

Results: The Dimension EXL Vitamin D Total assay demonstrated slope within ±10% of the Roche, ADVIA Centaur, and DiaSorin vitamin D assays. Additionally, all assays demonstrated slope within ±5% to the reference method, as shown in the table.

Comparison to Dimension EXL Assay		
Platform	**Slope**	**Intercept (ng/mL)**
Roche cobas e 411	1.10	0.04
DiaSorin LIAISON	1.00	1.00
ADVIA Centaur XP	1.09	2.50
Comparison to ID-LC/MS/MS		
Platform	**Slope**	**Intercept (ng/mL)**
Roche cobas e 411	0.95	0.47
DiaSorin LIAISON	1.05	-0.13
ADVIA Centaur XP	0.95	-0.36
Dimension EXL	1.05	0.51

Conclusion: The Dimension EXL Vitamin D Total assay demonstrates acceptable correlation to the vitamin D reference method procedure and methods that have obtained VDSP certification.

*Under development. Not available for sale. Due to local regulations, not all products will become available in all countries.

A-397

Serum Transferrin Receptor- Ferritin Index as a Marker of Iron Deficiency Anemia in Active IBD patients in Indian Population

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Background: Inflammatory bowel diseases (IBDs)are Crohns disease(CD) and Ulcerativecolitis (UC).Anemia in IBD is most common systemic complication. Currently available tests are serum ferritin ,Transferrin saturation index , Serum soluble transferrin receptor(sTfR) levels have limitations in inflammatory conditions and differentiating Iron Deficiency Anaemia (IDA) from Anaemia of Chronic Disease (ACD). The aim of the study was to assess the sensitivity and specificity of Serum Transferrin Receptor-Ferritin Index(sTfR-F) as the positive marker of iron deficiency anemia (IDA) caused by IBD

Methods: This is a Retrospective study of 480 patients of IBD, attending OPD in Asian Institute Of Gastroenterology Hyderabad ,India from Dec 2013-Dec 2015 Group I consists of CD{n=260;M;F160:100},UC {n=220;M:F120:100}, age groups (0-70)years,which were divided into Subgroup-A(0-12) Children,Subgroup-B(12-18) Adolescents,Subgroup-C(18-70) Adults were, diagnosed on Clinical,,Endoscopical, Histological, Radiological and Biochemical findings,were compared with age and sex matched Group II healthy controls (n=200 M: F 120:80).For all investigations of HS crp, Stool for fecal calprotectin, Hemoglobin, S.Ferritin(SF), Vit B12,B9, LDH, Transferrin Saturation index(Iron/TIBC),and sTfR were done .sTfR levels were measured by Immunoturbidimetry On Cobas e501.S.Ferritn(SF) were measured by Electrochemiluminesce on Cobas e601Roche Diagnostics. sTfR-F index was calculated on the ratio:sTfR/Log Ferritn. Criteria for IDA was ferritin<30ng/ml,transferrin saturation <15 %and sTfR- F>2.0 in the presence of inflammation.

Results: In study I: Group I patients were compared with Group II controls which showed 320 (66.5%)patients had active disease which were assessed by inflammatory markers, in that 280 (58.3%) patients had CD(n=182) 65.0% and UC(n=90)32.1%,other colitis(n=8) 2.9 % diagnosed as anemic.In Study II :Anemia patients are subgrouped as Subgroup I: 158 (56.4%) patients had IDA .Subgroup II: 46 (7.3%) patients had ferritin between 30-100ng/mL(mixed IDA/ACD) SubgroupIII:66 patients (23.3%)had ferritin >100ng/mL(ACD) and Subgroup IV:10 (5.6%)patients had Vit B12 and B9 deficiency excluding sTfR-F analysis. In Study III Patients in Subgroup II again subdivided in the presence of inflammation, to identify IDA with sTfR-F index as Group A: 28 of 46 patients (60.8%) had sTfR-F index >2, Group B: 15 patients (32.6%) had sTfR-F index =1- 2, and Group C: 3 patients (6.2%) had sTfR-F index <1.Intially only Subgroup I was diagnosed as IDA(56.4%), but with sTfR Index additional patients in GroupA, has increased IDA by 66.5%.so Overall IDA was diagnosed in 186 of 280patients (66.5%),but 64 (23.3 %) of 158 diagnosed with IDA did not have anemia.In Study IV: IDA cases, sensitivity of sTfR-F index was100%, sTfR 89% and SF 85%.Specificity of sTfR and sTfR-F Index were 80.60% > SF which has low specificity 73.90%. In ACD sensitivity sTfR-F index & sTfR is 89.80% SF 81.80%. So specificity sTfR is 100%, sTfR-F index 97.20%, SF 77.80%. In Study V: In IDA A statistical significance ($p < 0.0001$) was seen in female compared to male, and in children when compared to adolescence and adults with sTfR-F index

Conclusion: Our study suggests sTfR-F index to be very efficient and positive marker ,So can be an early diagnostic marker, to improve the diagnosis of IDA in IBD patients

A-398

A Lumipulse *G* Assay for Quantitation of 25-OH Vitamin D in Human Serum and Plasma

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Background: Circulating 25-OH vitamin D is the indicator of vitamin D status in human body and the major storage form of vitamin D in blood (Holick MF, 2009). 25-OH vitamin D assays are required to measure both 25-OH vitamin D2 and 25-OH vitamin D3.

Methods: The Lumipulse G 25-OH Vitamin D assay is a Chemiluminescent Enzyme Immunoassay for the quantitative determination of 25-OH Vitamin D2 and 25-OH Vitamin D3 in human serum and plasma on the Lumipulse G1200 System via a two-step sandwich immunoassay method using two monoclonal antibodies against 25-OH vitamin D. The amounts of 25-OH vitamin D in specimens are obtained from the luminescence signals derived from the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt).

Results: The Lumipulse G 25-OH Vitamin D assay demonstrated linearity from 6.9 to 150.0 ng/mL for serum and plasma, and an analytical sensitivity with the LoQ (Limit of Quantitation) ≤ 4 ng/mL. The precision study of 3 controls and 5 sera (n = 120 for each sample) revealed a total %CV ≤ 8.4% at 3 testing sites using 3 lots of reagents. There was no High Dose Hook effect with up to 3,000 ng/mL of 25-OH vitamin D2 and 25-OH vitamin D3 in samples. Equimolarity study showed a recovery within 100 ± 10% for the pooled serum samples with varying amounts of 25-OH vitamin D2 and 25-OH vitamin D3 in 7 different ratios, and with a targeting final concentration of 50 ng/mL. Interference studies demonstrated an average percent difference ≤ 10% between control and test samples for potential interferents, including 13 endogenous substances (human anti-mouse antibody, rheumatoid factor, conjugated bilirubin, unconjugated bilirubin, human gamma globulin, biotin, triglycerides, hemoglobin, human serum albumin, uric acid, cholesterol, L-ascorbic acid and human vitamin D binding protein) and 22 drugs, which were spiked individually into sera (test samples). Cross-reactivity study revealed a ≤ 2% cross-reactivity with each 100 ng/mL 3-epi-25(OH) vitamin D2, 100 ng/mL 3-epi-25(OH) vitamin D3, 20,000 ng/mL vitamin D2, 20,000 ng/mL vitamin D3, 8,000 ng/mL 1αOH Vitamin D3 and 25 ng/mL Paricalcitol, and ≥ 21% cross-reactivity with each 1,25(OH)2 vitamin D2, 1,25(OH)2 vitamin D3 and 24,25(OH)2 vitamin D3 at 100 ng/mL, respectively. A comparison of Lumipulse G 25-OH Vitamin D with the predicate device, LIASON 25 OH Vitamin D TOTAL, was analyzed using weighted Deming regression. The slope and correlation coefficient (r) obtained were 1.09 and 0.9476, respectively, for the tested specimens (n = 137) which ranged from 4.0 to 107.3 ng/mL. A comparison of Lumipulse G 25-OH Vitamin D with the CDC Reference Method, ID-HPLC-MS/MS 25-OH Vitamin D was also similarly analyzed. The slope and correlation coefficient (r) obtained were 0.97 and 0.9986, respectively, for the tested specimens (n = 119) which ranged from 5.8 to 149.0 ng/mL.

Conclusion: The Lumipulse G 25-OH Vitamin D assay has demonstrated to be accurate, precise, and sensitive for the quantitative and equimolar determination of 25-OH vitamin D in human serum and plasma.

A-399

Development of a One-Step HPLC Method for Simultaneous Quantitation of Vitamin B6 and Its Metabolite 4-Pyridoxic Acid in Plasma and Serum

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Background: Vitamin B6 deficiency is associated with a wide spectrum of symptoms and can cause seizure in infants. Pyridoxal 5-phosphate (PLP), the biologically active form, is a valid indicator of vitamin B6 status. Concurrent measurement of the final metabolite 4-pyrodoxic acid (PA) provides additional information regarding supplement intake and hypophosphatasia. The aim of this study is to develop a simple method that simultaneously detects PLP and PA.

Methods: A reverse phase HPLC method with fluorescence detection was optimized by comparing different derivatization, columns, mobile phases, and calibrations. The optimized method was evaluated and data was analyzed using EP Evaluator software.

Results: Procedure and typical chromatogram were shown in figure 1. Pre-column derivatization using semicarbazide showed best performance in terms of signal to noise ratio, retention time and peak shape when compared to pre- or post-column derivatization with chlorite, pre-column or in-mobile phase derivatization using sodium bisulfite. C18 50mm-columns with 2.7um core-shell and 1.8um particles achieved baseline separation for both PLP and PA, while 4.6um particle could not resolve PA with its adjacent peaks. When compared to calibration using 5-level external standards, adding 4-deoxypyridoxine as internal standard did not improve precision or accuracy. The method became less robust to column pressure variation because of a timely switch in excitation and emission wavelength between the closely eluting 4-deoxypyridoxine and PLP peaks. The analytical measurement range was 7.7-300 nmol/L and 3.7-300 nmol/L for PLP and PA respectively. The total imprecision was below 15% for PLP and 5% for PA. The spike recovery of PA was 94.2±4.6%. Method comparison of PLP with a reference laboratory showed correlation coefficient 0.9845, slope 1.070, intercept -3.54 and mean bias 2.39nmol/L (n=43).

Conclusion: This method combines derivatization and protein precipitation in one step. It is simple and reliable for routine evaluation of vitamin B6 status.

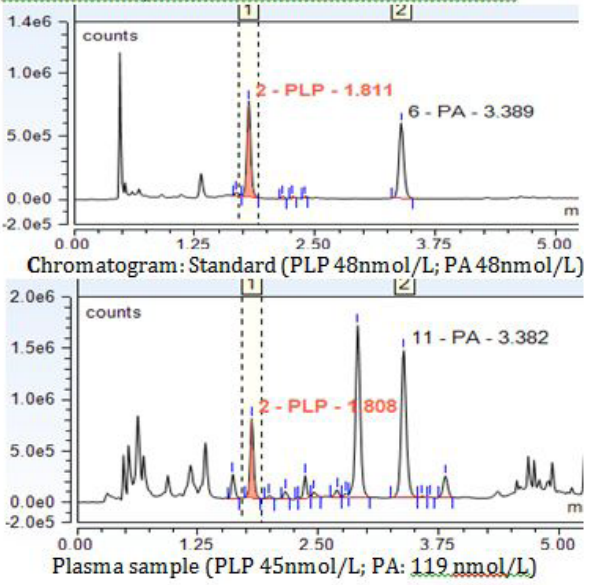
Fig.1 Procedure and Typical Chromatogram

Sample Preparation

500µL of plasma or serum mixed with 40 µL of 125mg/ml semicarbazide/glycine solution and 40µL of 70% perchloric acid. Vertex and incubated at 4°C for 30minutes. Supernatant was stabilized by adding 25% NaOH 20:1 ratio before injection.

HPLC Conditions

Column: Poroshell 120 EC-C18 3.0X50mm 2.7 micron
Mobile phase: A 25mM Na2HPO4, pH7; B Methanol
Injection: 20µL; Flow rate: 0.6ml/min. Run time: 7.5 min
Ex 367 EM478, after 2.6 min switch to EX325EM425.



A-400

Efforts to Control Physician Ordering of Serum 25-Hydroxy-Vitamin D in a Community Setting Have Failed to Prevent Unnecessary Testing

A. Ptolemy, M. Godwin, G. Waite, D. Bailey, H. Li. *Dynacare, London, ON, Canada*

Background: In 2010, the Ontario Health Technology Advisory Committee (OHTAC) in Ontario Canada recommended that routine serum 25-hydroxy-vitamin D (vitamin D) testing not be performed, except for patients with osteoporosis, rickets, osteopenia, malabsorption syndromes, and renal disease or those taking drugs that affect vitamin D metabolism. If a patient meets one of these eligibility requirements, the Ontario Health Insurance Plan (OHIP) will pay for this testing. If they do not, the patient is

responsible for payment. On December 1, 2010 a new OHIP laboratory requisition was issued requiring clinicians to classify the patient as insured or uninsured for the ordered vitamin D test. In the fiscal year 2011/2012 OHIP insured vitamin D test volume decreased relative to the previous year. The OHTAC noted that they expected this trend to continue, however, this expectation has not yet been verified in a published follow-up study. **Objective:** Assess the impact of the OHTAC recommendations and OHIP laboratory requisition changes on the frequency of vitamin D tests performed by our regional reference laboratory and characterize the vitamin D status of our patient population. **Methods:** All vitamin D test orders from July 2005 to July 2015 were extracted from our laboratory information system. Patient vitamin D status was determined using the respective definitions: deficiency, <25 nmol/L; insufficiency, 25 to 75 nmol/L; sufficiency, 75 to 250 nmol/L; and toxicity, >250 nmol/L. **Results:** *N*=1,137,526 (*N*=338,350 male, *N*=799,176 female; median age 56 y; age range <1 to 101 y) insured vitamin D tests were performed from July 2005 to July 2015. Monthly volume increased exponentially from *N*=884 tests in July 2005 to a peak of *N*=30,533 tests in March 2010. The new OHIP laboratory requisition was introduced in December 2010 and that month's insured vitamin D testing volume (*N*=2,143) was 89% less than the test volume of November 2010 (*N*=19,117). Following this requisition launch, insured vitamin D test volume grew an average of 3.8% per month. The prevalence of vitamin D insufficiency consistently trended downward from 62% of the patient population in July 2005 to 49% in July 2015. Vitamin D sufficiency prevalence, however, consistently trended upward from 36% in July 2005 to 50% in July 2015. Since April 2012, the monthly prevalence of vitamin D deficiency in our patient population was <1.5%. The monthly prevalence of vitamin D toxicity did not exceed 1% over the studied time period. **Conclusion:** Mandating ordering physicians to classify vitamin D insurance status on the laboratory requisition in December 2010 did initially reduce insured test volume; however, subsequently insured test ordering has consistently increased month-to-month. The OHTAC vitamin D test utilization recommendations have not changed and this relative increase is not justified by the observed trends in vitamin D sufficiency and insufficiency levels within our patient population. The OHTAC and other laboratories may use this study to review their institutions current polices on vitamin D test utilization and potentially mitigate undue financial burden to their health-care systems through inappropriate ordering practices.

Wednesday, August 3, 2016

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

Animal Clinical Chemistry

B-002

Histopathological Changes in Some Organs of Diabetic Rats Administered Aqueous or Ethanolic Root Extract of Uvaria Chamae

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Background: *Uvaria chamae* is a medicinal plant that is used in many parts of the world in the treatment of diabetes, and other diseases. The chemical constituents of *Uvaria chamae*, include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. Traditionally, the root extracts are 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 evaluated the histopathological changes in the heart, liver and pancreas of normal and diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* roots. **Methods:** Thirty six Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20 g] as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal 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. Normal and diabetic rats were then administered the aqueous or ethanolic extract (300 mg/kg body weight per day) for 35 days. Animals were euthanized by decapitation and blood collected for glucose assay. Organs were collected and preserved in buffered formalin for histopathological evaluation. **Results**: There was a significant ($p<0.05$) decrease in blood glucose levels in the diabetic groups treated with aqueous or ethanolic extract compared to the diabetic control. We observed intimal ulceration, medial thickening and luminal stenosis of the coronary artery in the heart of diabetic control rats compared to the normal control. Diabetic rats treated with ethanolic extract showed good luminal patency. We also observed mild vascular congestion and dilatation in the diabetic rats treated with the aqueous extract with well delineated luminal patency. The liver of the diabetic control group showed portal congestion and infiltrates of chronic inflammatory cells(portal hepatitis) when compared to the normal control. However, treatment of the diabetic groups with aqueous or ethanolic extract showed dilatation of blood vessels and activation of the kupffer cells (local immune system) when compared to the diabetic control. The normal control groups treated with aqueous or ethanolic extract showed exuberant pancreatic islet cells compared to the untreated normal control. Similarly, the diabetic groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the diabetic control group. **Conclusion:** The observed activation of hepatic kupffer cells and the resurgence of pancreatic islet cells due to aqueous or ethanolic extract consumption are indicative of the potential benefits of each extract in the effective management of diabetes. The observed good luminal patency of the coronary artery by either extract supplementation may prevent lipid deposition in the arteries that is often associated with the development of diabetic complications.

B-003

Anti-diabetic properties of combined inositol hexakisphosphate and inositol in streptozotocin-induced type 2 diabetes mellitus Sprague-Dawley rats

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Background: Diabetes mellitus is ranked among the major causes of morbidity and mortality associated with non-communicable diseases worldwide. Type 2 diabetic patients struggle with classic early symptoms of increased body weight, dyslipidemia, polyphagia, polydipsia and polyuria. Inositol hexakisphosphate (IP6) is a plant constituent found in appreciable quantities especially in grains and seeds while inositol is a carbohydrate found in plants and animals. Inositol and IP6 are thought to individually exhibit hypoglycemic activities but the full mechanism is not yet known. Therefore the anti-diabetic effects of an IP6 and inositol combination in a type 2 diabetes mellitus (T2DM) rat model was evaluated in this study. **Methods:** Thirty male Sprague-Dawley rats with average body weight of 168 ± 5.9 g were used in this 8 week study. Type 2 diabetes mellitus was induced in 18 of these rats by feeding them high-fat diet for 4 weeks followed by intravenous administration of a low dose of streptozotocin (Sigma-Aldrich, 35 mg/kg/body weight in 0.1 M-citrate buffer, pH 4.5) after two weeks. Type 2 diabetes mellitus was confirmed by hyperglycemia (blood glucose ≥ 300 mg/dL) and a positive response to an anti-diabetic drug response test. Diabetic rats were placed into three groups (6 rats per group) namely; IP6 and inositol combination (IP6+INO; 650 mg/kg body weight/day), glibenclamide (Glib; 10 mg/kg body weight/day) and diabetic control (DC). In addition, two groups of non-diabetic control rats were fed normal diet (NC) and high fat diet (HFC) during the initial 4 weeks of the experiment. However, for the final four weeks, all rats were fed normal diet and given their respective treatment regimes. The rats were fasted overnight, euthanized by decapitation and blood samples were collected at the end of the 8 week period. **Results**: Treatment with IP6 & inositol combination significantly reduced blood glucose concentration (306 ± 53 mg/dL) and insulin resistance score (1.93 ± 0.45) compared to the diabetic control group (522 ± 24 mg/dL and 5.10 ± 0.69 respectively; $p < 0.05$). A similar effect was observed with Glibenclamide. However, the combined supplement was more effective in lowering serum total cholesterol and triglycerides by 26% and 31% respectively compared to the glibenclamide treated group ($p < 0.05$). Body weight, fluid intake and food consumption were also significantly reduced in the IP6+INO group by 8%, 16% and 10% respectively compared to the Glibenclamide treated group ($p < 0.05$). Food and fluid intake were reduced by 52% and 28% respectively, whereas serum leptin concentration was increased by 34% in rats treated with the combined supplement compared to the diabetic control group ($p < 0.05$).**Conclusion:** Treatment of T2DM rats withIP6 and inositol combination significantly improved blood glucose concentration and ameliorated insulin resistance, dyslipidemia, polyphagia and polydipsia. This study shows that a combined IP6 and inositol supplementation may be effective in the management of T2DM and associated metabolic disorders.

B-004

Evaluation of Hematology and Flow Cytometry Parameter Stability in Non-Human Primate, Canine, and Rodent K_2EDTA Whole Blood Samples to support Pre-clinical Toxicology Studies

S. Wildeboer, C. Phanthalangsy, C. Drupa, R. Giovanelli, S. Sokolowski. *Pfizer, Groton, CT*

Background: Historically, our laboratory has determined K_2EDTA sample stability to be 24-30 hours post collection when stored at 2-8°C for all hematology parameters. Based on literature, there are several variables that may affect pre-analytical stability, such as processing delays and sample storage conditions. Therefore, an evaluation of stability beyond 30 hours was needed to support multi-site collection and evaluation of hematology and flow cytometry parameters. Extended pre-analytical stability of various species in K_2EDTA whole blood provided our laboratory with a more robust methodology and consistency in eliminating variation and bias from reported results of whole blood samples.

Methods: At least 10 samples from normal colony animals (both male and female) of non-human primate (NHP) Cynomolgus monkey, Beagle canine, Sprague-Dawley(SD) and Wistar rat, and CD1 and C57BL/6 mouse K_2EDTA whole blood samples were analyzed for either hematology or flow cytometry parameters. Samples for hematology or flow cytometry were analyzed within 2 hours of collection and at various time points between 6 and 168 hours post collection to establish stability. Samples were stored at 2-8°C between analysis time points and were allowed to come to room temperature prior to analysis. Hematology whole blood samples were analyzed for a complete blood count (CBC), automated white cell differential (Auto DIFF), and reticulocyte (RETIC) using the Siemens Advia 2120 hematology analyzer. Additionally, lymphocyte subset stability was assessed using the FACSCanto flow cytometer and FACSDiva software. Total T cell, helper T cell, cytotoxic T cell, B cell and natural killer (NK) cell populations were measured using lineage specific antibodies and reported as a percentage of lymphocytes. Stability values showing acceptable recovery or actual difference were considered stable.

Results: NHP blood was stable up to 96 hours for CBC, RETIC, total T cells, helper T cells, cytotoxic T cells, B cells and NK cells. However, the automated differential was only stable up to 72 hours for NHP. Canine blood was stable up to 48 hours for CBC, 96 hours for Auto DIFF, RETIC, white blood cell count (WBC), and platelet

count (PLT), and 168 hours for all flow cytometry parameters assessed. SD rat blood was stable up to 28 hours for CBC, 32 hours for RETIC, 72 hours for Auto DIFF and WBC, and 54 hours for all flow cytometry parameters. Lymphocyte subsets were stable in Wistar rat blood for 30 hours. CD-1 and C57BL/6 mouse blood was stable for 96 and 72 hours, respectively, for lymphocyte subsets.

Conclusion: All outlined criteria for the validation of extended sample stability in NHP, canine, rat and mouse whole blood samples for hematology as well flow cytometry parameters were met and have been used to support discovery and multispecies pre-clinical studies. Therefore, with the evaluation of extended sample stability we have been able to provide support across multiple sites, and offer a more robust methodology and confidence in reported results of whole blood samples.

B-006

Evaluation of the Meso Scale Discovery Rat Skeletal Troponin I Assay in Rat and Mouse

T. Lambert, K. Lynch, N. Chau, T. Sellers. *GlaxoSmithKline, King of Prussia, PA*

Skeletal troponin I (sTnI) is a member of the troponin complex of regulatory proteins required for muscle contraction. Recent evidence suggests that skeletal muscle injury results in leakage of sTnI into blood and measurement of serum or plasma levels of sTnI may provide a noninvasive biomarker of muscle injury. Our objective was to evaluate the Meso Scale Discovery® (MSD) Rat Skeletal Troponin I Assay for measurement of skeletal troponin I in rat serum. Acceptable precision (CV ≤10%) and accuracy (RE ±13%) of standard curve values were observed across 10 analytical runs. Precision of sTnI values in rat serum samples was excellent (CVs ≤10%) at concentrations in the range of 5-150µg/L; less precision was observed (CV=21%) in the area of the LLOQ (0.27µg/L). Dilutional linearity ($y=1114.8x - 4782.2$; $r^2=0.99$) and recovery (101-106%) of sTnI in rat serum was demonstrated. Serum sTnI was stable for up to 6 months at -80ºC. Serum sTnI values in clinically healthy Wistar rats (n=20/sex) were below LLOQ to 11.4µg/L for males and at or below LLOQ for females. In two nonclinical safety studies, sTnI increases (up to 1984µg/L) correlated with histologic evidence of myofiber degeneration and/or necrosis and increases in AST and aldolase. In mice, sTnI increases (474 to 1859µg/L) were observed in plasma of muscular dystrophy mutant mice (C57BL/10ScSn-mdx/J) compared to wild-type C57BL mice (0.38-3.09µg/L). In conclusion, the MSD rat skeletal troponin I assay performed well and sTnI increases correlated with skeletal muscle injury in rats and mice.

Wednesday, August 3, 2016

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

Automation/Computer Applications

B-007

Standardization of Ask At Order Entry Questions: A Prudent Question is One-Half of Wisdom

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Background: The United States federal mandates for achieving Meaningful Use (MU) cause numerous work groups in the industry to discuss how to best represent actual content with standardized terminology. During the development of the Laboratory Orders Interface (LOI) and the electronic Directory of Services (eDOS) implementation guides in the Standards and Interoperability Framework's (S&I Framework) laboratory related initiatives, the need for guidance, expansion and harmonization of Ask at Order Entry (AOE) questions became evident. The primary goal is to provide a suite of harmonized standards for electronic messaging of laboratory data for the US realm for inclusion in MU regulations. A secondary goal is the reduction of variations in how the same AOE question is asked. Use cases considered for this project were ambulatory laboratory test ordering as well as Public Health reporting, such as pediatric lead level reporting. Engagement of subject

matter experts who help with consolidating duplicate AOE questions into a standard format for representation as a single concept provides further harmonization and enhanced interoperability. In addition, this project will establish a review process prior to submission for standard codes when new AOE questions are needed.

Methods: During the course of LOI and eDOS implementation guide development, commonly used AOE questions were collected from several national laboratories, Public Health laboratories and Public Health agencies. AOE questions were consolidated and standard codes were assigned from the Logical Observation Identifiers Names and Codes (LOINC®) database, maintained by the Regenstrief Institute, where appropriate codes existed. AOE questions where the required information could be communicated in other parts of the HL7 message were also identified. Several of the AOE questions were asking the same questions in different ways and this duplication creates confusion and hinders semantic interoperability. The entire AOE collection was shared with the Laboratory Messaging Community of Practice (LMCoP), a forum comprised of laboratory and standards experts from state and federal Public Health laboratories, national clinical laboratories, the National Library of Medicine and professional organizations, whose purpose is to resolve lab related issues from a laboratorian's viewpoint. The LMCoP, acting as a conduit to lab related professional organizations, provided its recommendations for subsequent review by appropriate laboratory domain content experts from the American Society for Clinical Pathology (ASCP), the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP) for completeness and proper LOINC® code assignment, as well as identification of a preferred single concept representation where overlap existed.

Results: The current iteration of AOE questions contains 131 questions, a 37% reduction from the 210 originally collected. Fifty eight (58) new terms have been submitted to the Regenstrief Institute; in addition twenty three (23) existing LOINC® terms were revised, removing trial status or survey-specific method information as result of this review.

Conclusion: The curated list of AOE questions, properly mapped to LOINC® terminology, has been published in the eDOS Implementation Guide and is available to laboratories when implementing electronic data exchange.

B-008

iRICELL® fully automated and integrated platform - correlations between chemistry and microscopy system, comparison with manual microscopy and the clinical diagnostic significance

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Objective: The main goal of our study was to compare the automated microscopy with manual microscopy, evaluation of the performance of the **iRICELL®** system in the clinical significance, correlations between chemistry and microscopy system. In our study, we emphasize the clinical performance of the **iRICELL** system and their diagnostic importance. ***Relevance:*** The analysers use reactive test strips for analysing and providing volumetric determination of the chemical parameters of the urine; density, pH, nitrites, proteins, glucose, ketone bodies, urobilinogen, bilirubin. The urinary erythrocytes and leukocytes, their investigation being useful for the localization and the source of haematuria and leukocyturia. The analyser is a fully automatic instrument, it is fast (it processes 100 samples/hour), it automatically recognizes and analyses quantitatively 2 elements, indirectly (without human intervention). The role of the automated analysis systems for the urinary sediment is that of eliminating the error sources described below: the centrifugation of the samples can cause a variable degree of loss of the urinary sediment elements, the placement of the urinary sediment between the slide and cover glass can also induce various errors through the lysis of the elements, the non-uniform distribution of the sediment elements under the cover glass, as well as their embedment in the mass of mucus, the counting of elements between the slide and cover glass is subjective. ***Methodology:*** The samples were first screened using the ***iRICELL*** fully automated and after that the selected pathological samples were examined using (for comparation) the manual microscopy at a higher power (x 400). Initially, we utilised on a normal microscope but with reduced light with the condenser placed at a lower position to increase the contrast. We selected 50 photos, representing the most important cases. The investigation was performed by using different microscopic techniques (bright field, phase contrast and interference contrast) in the observation of the sediment elements in both unstained and stained samples (May-Grünwald-Giemsa stain, Sternheimer-Malbin stain). ***Validation:*** Our results are based on the study of 800 patients, who were admitted to our hospital between January 2012 and September 2015. The patients were diagnosed with various types of renal diseases and were hospitalized in either the Nephrology or Dialysis Departments. Each photograph belongs to one patient. For urinalysis we used either the first or the second morning urine specimen. The microscopic examination revealed the presence of the same pathological urinary sediment element like the the ***iRICELL*** system. ***Conclusions:*** Our study shows that the ***iRICELL*** system compared to the classic examination of urine has greater relative sensitivity, increases the analytical accuracy and the credibility of the results, thus decreasing the workload of the microscopist and supporting the physicians. The relatively high incidence of chronic kidney disease is explained by the fact that in our laboratory we examine a relative high number of samples received from the Department of Nephrology. The benefits of use of ***iRICELL*** system are the following: it is an accurate non-invasive technique, it eliminates the risk associated with invasive methods and decreases costs.

B-009

Computational Approaches for Inpatient Mortality Predictive Modeling in a Swiss Cohort

C. T. Nakas, N. Schütz, M. Werners, A. B. Leichtle. *Inselspital - Bern University Hospital, Bern, Switzerland*

Background: Decision support systems that apply electronic health record (EHR) data for calculating of the risk of inpatient mortality are proposed as a "big data" computational approach for efficient patient care. Scoring systems like the Acute Laboratory Risk of Mortality Score (ALaRMS) as well as statistical learning techniques are competing approaches. We assessed predictive accuracy and model calibration of different approaches by applying them to a big biomedical dataset in a large Swiss University Hospital.

Methods: We used the complete hospital admissions database of the Inselspital Bern from 2012 to 2015, including more than 100'000 entries. Admission laboratory profiles, age, and sex were included, the outcome was inpatient mortality. The decision-support systems were used to assess mortality risk for a specific patient based on baseline data and clinical laboratory results at admission. We compared the ALaRMS score, generalized linear modeling (GLM) procedures, and non-linear and tree-based methods, and provide robust statistical models for inpatient mortality predictive modeling.

Results: ALaRMS AUC was comparable to the expected accuracy (AUC=0.858). A bias-corrected ALaRMS score yielded results comparable to a simplified 3-parameter logistic regression model (AUC=0.819 vs 0.801). Logistic regression methodology with penalization provided a robust model (AUC= 0.872). Artificial neural networks and random forest methodologies showed similar accuracy.

Conclusion: Todays GLM procedures provide calibrated unbiased models that can be used as efficient decision support tools for inpatient mortality prediction. Electronic Health Record (EHR) data is a pivotal resource for decision support in clinical practice and might aid preemptive patient triage.

B-010

Web-based Method Comparison for Clinical Chemistry

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Background: Method comparison and bias estimation are daily activities for Clinical Chemists and Pathologists. Software required for compliance with guidelines, such as CLSI EP09 A3, are commercially available but are sometimes expensive. Among the open-source alternatives for method comparison studies are those employing the R programming language. The additional R package mcr, written by Roche Diagnostics, is based on CLSI EP09-A3 but using it requires some basic knowledge of programming to use. Recognizing this as a limitation to its use, we have developed a dashboard-style web-interface to mcr requiring no programming knowledge.

Methods: In our application, Shiny, Shiny Dashboard, rhandsontable and dt packages are used for the construction of a graphical user interface and the Rmarkdown package produces the output documents: PDF, MS Word or HTML. The mcr package—the work-horse of the website—processes the user-defined data to generate both Bland-Altman and scatter plots and provides a statistical report. The dashboard allows manual data entry cutting and pasting from spreadsheet applications. The user selects the desired regression procedure from drop-down menus (Table 1), calculations are performed by a server, and the results appear interactively.

Results: Any web browser can be used to access the website and generate graphics and statistics. https://bahar.shinyapps.io/method_compare

Conclusion: In summary, we have developed a website for method comparison studies using R and various R packages. The site offers a simple, yet inexpensive way to evaluate the relative analytical performance of two analytical methods.

Table 1. Options provided for plots and statistics.

Bland-Altman	**CI**
X vs. Y-X	Analytical
X vs. (Y-X)/X	Jacknife
0.5*(X+Y) vs. Y-X	Bootstrap
0.5*(X+Y) vs. (Y-X)/X	Nested Bootstrap
rank(X) vs. Y-X	
rank(X) vs. (Y-X)/X	
sqrt(X*Y) vs. Y/X	**Bootstrap CI**
0.5*(X+Y) vs. (Y-X) / (0.5*(X+Y))	Quantile
	Student
	Bias Corrected and Accelerated
Regression	Bootstrap-t
Ordinary Least Square	
Weighted Ordinary Least Square	
Deming	**Correlation**
Weighted Deming	Pearson
Passing-Bablok	Kendall
Passing-Bablok Large Data	Spearman

Abbreviations: CI = confidence interval, X = method 1 (reference method), Y = method 2 (test method), sqrt = square root

B-011

Use of patient registry and automated notifications to improve genetic test utilization

R. Schifman, D. R. Luevano. *Southern AZ VA Healthcare System, Tucson, AZ*

Background: Repeat testing for the same germline mutation or allele is unnecessary. However, previous results may be unavailable or overlooked causing redundant testing and delay in diagnostic evaluations. This study evaluated the use of a multi-institutional patient registry and automated notification system to improve genetic test utilization.

Methods: A national genetic test registry was created for patients enrolled in the Veteran Affairs (VA) healthcare system that contained 15 years of test results from the VA Corporate Data Warehouse, which was updated daily thereafter. Tests included hemochromatosis (HFE), factor V Leiden (FVL), prothrombin G20210A gene mutation (PT G20210A), HLA-B27 and *HLA-B*57:01*. An automated system performed daily searches for registry patients having new orders that triggered an email notification to designated laboratory personnel at specific VA facilities where testing was requested. Alerts contained patient identification, date, location and results of previous test(s). Test cancellation rates after notifications were compared to a control group of VA facilities that did not receive alerts.

Results: Between February, 2015 and January 2016, 22 VA laboratories received 232 notifications for duplicate orders over 1 to 11 months, depending on date of entry into program. This included 39 HFE, 53 FVL, 14 PT G20210A, 56 HLA-B27 and 70 *HLA-B*57:01* tests. Previous testing was performed at a different facility in 87 (37.5%) cases. A total of 142 (61.2%) tests were cancelled that included 30 (76.9%) HFE, 35 (66.0%) FVL, 9 (64.3%) PT G20210A, 23 (51.8%) HLA-B27 and 39 (55.7%) *HLA-B*57:01* tests. The median laboratory cancellation rate and 90th percentile range was 66.7% (22.8%-100%). A total of 949 duplicate orders were observed among 101 facilities in the control group which included 313 HFE, 202 FVL, 94 PT G20210A, 164 HLA-B27 and 176 HLA-B 5701 tests. Previous testing was performed at a different facility in 280 (29.5%) cases. A total of 32 (3.4%) orders in the control group were cancelled as duplicates that included 3 (1.0%) HFE, 12 (8.3%) FVL, 10 (10.6%) PT G20210A, 3 (1.8%) HLA-B27 and 4 (2.3%) *HLA-B*57:01* tests. The median laboratory cancellation rate and 90th percentile range in the control group was 0.0% (0.0%-14.2%). **Conclusion:** A national patient registry with automated notification system was found to be an effective strategy for improving utilization of genetic tests. This intervention reduced unnecessary retesting and provided more rapid information for diagnostic evaluations. However, cancellation rates for laboratories in the intervention group varied widely. This was not evaluated but may have been due to local practices or how alerts were administratively managed by the laboratory. Finally, interoperability of the VA laboratory information system enhanced the effectiveness of this intervention since over one-third of notifications involved results reported from another facility.

B-012

Analytical performance evaluation of newly developed immunoassay analyzer "LUMIPULSE® L2400"

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Background: For efficient operations in clinical laboratories, Immunoassay analyzer intends to have random-access system and also implement space-saving, short-time of assay and the module system with clinical chemistry analyzers. This time, we developed fully chemiluminescent enzyme Immunoassay (CLEIA) system "LUMIPULSE L2400" and, we report the evaluation results of the basic performances on this system. The features of L2400 are as follows. The processing capability is up to 240 tests per hour as maximum. It is able to access 24 analytes with full random-access, and immunoreaction time is approximately 20 minutes. Also, measurement in a short-time that is about 12 minutes is available to shorten the reporting time. (Reagent for short time assay are in development).Regarding its extensibility, it can be connected with clinical chemistry analyzer and it also implements flexible system connection by adopting external sampling method.

Methods: Fully automated CLEIA system LUMIPULSE L2400 was used for the measurement and LUMIPULSE PrestoII (FUJIREBIO INC.) was used for the system comparison. Dedicated regents used for this study were Lumipulse Presto AFP, CA19-9, BNP, HBsAg-HQ, and TP (FUJIREBIO INC., Japan). The reproducibility tests (N=6) of the above five analytes were executed to calculate coefficient of variation. (C.V.) The correlation tests of the above five analytes were carried out using more than 30 specimens for the instrument comparison of L2400 versus PrestoII.

Results: Basic evaluation for Lumipulse Presto AFP, CA19-9, BNP, HBsAg-HQ, TP was performed on L2400. The results are as follows. Reproducibility: C.V. (%): AFP: 0.9-1.9%, CA19-9: 1.1-1.8%, BNP: 0.7-1.8%, HBsAg-HQ: 1.2-3.0%, and TP: 0-1.0%. Correlation between L2400 and PrestoII is that AFP: regression y=1.00x, correlation coefficient r=1.000, CA-19-9: regression y=0.92x-0.23, correlation coefficient r=1.000, BNP: regression y=0.94x-1.30, correlation coefficient r=0.998, HBsAg-HQ: regression y=1.04x-0.26, correlation coefficient r=0.998, and TP: regression y=0.92x-0.57, correlation coefficient r=0.997.

Conclusion: The results of basic evaluation for AFP, CA19-9, BNP, HBsAg-HQ, and TP on LUMIPULSE L2400 were excellent. These results demonstrated that LUMIPULSE L2400 is sufficiently applicable for routine laboratory tests. *note:

LUMIPULSE L2400 and the reagents were approved by PMDA in Japan. Not approved by US-FDA, CE-IVDD.

B-013

Automated IFA methods compare well with established manual IFA screening and titration for ANA HEp-2

T. Matthias[1], J. Blecken[1], A. Frey[1], M. Daves[2], A. Joos[2], S. Platzgummer[2]. *[1]AESKU. Diagnostics GmbH&Co KG, Wendelsheim, Germany, [2]Labor f. Clin.Chem, Meran, Italy*

Background: Often, only basic nuclear patterns like homogenous, speckled, nucleolar, centromere or cytoplasmic are reported by laboratories. The detection of other patterns requires well trained readers. As a result, different systems have been developed which automate part of or the complete IFA method and reading process. Methods: This study compares 2 commercially available HEp-2 antinuclear antibody (ANA) indirect fluorescent antibody (IFA) assays using a sensitivity panel (120 clinically determined patients) and a specificity panel consisting of 80 clinically confirmed negative patients. We compared the NOVA View® system from INOVA with the HELIOS® IFA Processor from AESKU.Systems/AESKU.Diagnostics to assess their capability for screening and titration of these samples. The automated method was directly compared to manual reading of the same processed slides on respective microscopes and also compared with the known clinical information.

Results: The results of the two automated methods were in good agreement. The HELIOS® system detected 188 samples correctly from negative and positive samples (versus 187 detected by the NOVA View® system). The falsely detected positive samples were all of low titer (1:80). The HELIOS® system found 157 patterns in agreement to the target pattern (NOVA View® 156). From 80 negative samples AESKU detected 73 correctly (NOVA View® 71). Conclusion: Both systems resulted in an overall sensitivity >95% and a specificity of 91.25 and 88.75 (for AESKU HELIOS® versus NOVA View®). The pattern recognition also showed only minor aberrant findings resulting in a slightly better detection of cytoplasmic and nuclear membrane patterns by the Helios-system while NOVA View detected slightly better the centromeric pattern.

B-014

Comparison between the performance of the HELMED® Blot Module and the HELIA® using the *AESKUBLOTS*® ANA-17 Pro

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Background: Immunoblotting is a common method for efficient profile testing of autoimmune and infectious diseases. Automation offers higher throughput testing, therefore AESKU.SYSTEMS developed two solutions to facilitate automated immunoblot testing.To compare the performance of the HELMED® Blot Module that is a fully automated Blot processor, and the HELIA®, an automated analyzer for line immunoassays.

Methods: 39 routine samples were tested on the *AESKUBLOTS*® ANA-17 Pro (AESKU.DIAGNOSTICS) utilizing in parallel the HELMED® Blot Module and the HELIA® system (both AESKU.SYSTEMS, Wendelsheim). By performing samples with the HELMED® Blot Module the *AESKUBLOTS*® were analyzed by the AESKU. SCAN® software

Results: 28 samples were found to be positive for one or more parameters. 2 samples showed equivocal results and 9 were completely negative for all ANA antigens. Overall agreement (concordance correlation coefficient) between the HELMED® Blot Module and the HELIA® system was 0.9476 (95% CI: 0.9216 to 0.9652). Notably, all discordant samples were characterized by very borderline signal. Comparing the level of immunoreactivity of the different coated antigens and sample diversity the Pearson precision (ρ) was 0.9718 (95% CI: 0.9547 to 0.9825; $p<0.0001$).

Conclusion: The HELMED® Blot Module and the HELIA® system are able to identify the ANA positive samples with the same level of band intensity of the coated antigens. Both approaches are able to reduce inter-laboratory variability and time required to perform ANA testing, especially in high throughput laboratories.

B-015

Multicenter evaluation of a new high-throughput HbA1c testing platform

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Background: This non-interventional, multicenter study with anonymized leftover patient samples was performed to evaluate the reliability and analytical performance of the novel HbA1c cobas c 513 analyzer. Methods: A performance evaluation was carried out at three European study centers to validate the overall system functionality, user interaction and analytical performance of the new cobas c 513 analyzer using the Tina-quant® HbA1c Gen. 3 assay. This established assay is standardized against the approved IFCC reference method for measurement of HbA1c in human blood and is approved for monitoring of long-term blood glucose control in individuals with diabetes mellitus, as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. The HbA1c determination is based on the turbidimetric inhibition immunoassay for hemolyzed whole blood. The novel analyzer has the capacity to process up to 400 closed whole blood or hemolysate samples for HbA1c testing per hour. Results are reported in mmol/mol hemoglobin A1c (IFCC) and %HbA1c (DCCT/NGSP). Method comparisons were performed with commercially available dedicated HbA1c analyzers COBAS INTEGRA 800 CTS, Tosoh G8 and Menarini HA-8180V using fresh and frozen anonymized residual samples from routine. The evaluation also covered usability testing and practicability assessment. **Results:** HbA1c applications for both whole blood and hemolysate samples show a very stable analyte recovery of assigned target values ±1 SD (~5.5 % ± 0.34 and ~10 % ± 0.6) and high precision using both quality control materials and different concentrations of whole blood pools or hemolysates. The repeatability and intermediate precision for the whole blood and hemolysate applications in %HbA1c was 0.4 - 0.7 % and 0.8 - 1.5 % respectively. The comparison of HbA1c Gen. 3 on cobas c 513 to HbA1c Gen. 2 on COBAS INTEGRA® 800 CTS using 10052 whole blood samples from two labs combined shows high concordance (slope (95%CI) = 1.00 (1.00, 1.01); intercept (95%CI) = -0.15 (-0.13, -0.18)). Moreover analyte concentrations as measured by the cobas c 513 and Tosoh G8 (slope (95%CI) = 0.94 (0.94, 0.95); intercept (95%CI) = 0.21 (0.16, 0.26); n=500) and Menarini HA-8180V (slope (95%CI) = 0.96 (0.94, 0.97); intercept (95%CI) = 0.29 (0.19, 0.40); n = 249) are comparable. The cobas c 513 also proved to reveal reliable results with system handling provocations as they can occur during routine use. Recovery rates of 98.2 to 102.6% were obtained with IFCC reference materials. The HbA1c Gen. 3 whole blood application on cobas c 513 moreover exhibited linearity in the tested range of 4.8 - 14.0 % HbA1c. The quantification of HbA1c Gen. 3 on cobas c 513 was not influenced by common Hb variants HbAS, HbAC, HbAD, HbAE and HbA2. The cobas c 513 system was rated with "exceeds expectations" in 92.3% of questions by operators with regard to practicability and usability. **Conclusion:** The cobas c 513 has proven to be a reliable system that yields excellent analytical performance of the Tina-quant® HbA1c Gen. 3 assay in high throughput laboratories. Additionally, operators have rated the system usability as exceeding expectations.

B-016

New protocol for quantification of alpha1-antitrypsin stool using a commercial kit for human serum

M. C. M. Freire[1], C. C. Silva[2], R. P. Souza[2], L. M. Oliveira[2], D. J. Araujo[2], D. C. Silva[2], E. Mateo[1], A. C. S. Ferreira[1]. *[1]Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil, [2]Hermes Pardini Institute, Vespasiano, Brazil*

Background: Alpha1-antitrypsin (A1A) is an alpha1-A globulin that circulates in the blood and protects the tissues of the body from being damaged by substances contained in white blood cells (e.g., trypsin, elastase, collagenase and other proteolytic enzymes). The synthesis of alpha1-antitrypsin is controlled by a pair of genes at the proteinase inhibitor locus that is inherited as co-dominant alleles. Since A1A is resistant to degradation by digestive enzymes, it is used as an endogenous marker for the presence of blood proteins in the intestinal tract. An elevated A1A clearance suggests excessive gastrointestinal protein loss and an A1A deficiency states often have a genetic cause. Gastrointestinal protein enteropathy has been associated with regional enteritis, sprue, Whipple intestinal lipodystrophy, gastric carcinoma, allergic gastroenteropathy, intestinal lymphangiectasia, constrictive pericarditis, congenital hypogammaglobulinemia and iron deficiency anemia associated with intolerance

to cow's milk. Although there are some commercial kits available to quantify A1A in stool, none has *registration* at BrazilianHealth Surveillance Agency (ANVISA), making difficult the implementation of this test on Brazilian market. **Objective**: On this project, we aimed to validate the N Antiserum to Human α1-Antitrypsin Kit (Siemens Healthcare Diagnostics) to quantify A1A instool by means of immunonephelometry on the BN Systems (Siemens). This kit is an *in vitro* diagnostic reagent used for the quantitative determination of α1-antitrypsin (α1-proteinase inhibitor) in human serum. **Methods**: A different method of sample preparation and some modifications of manufacturer's instructions were necessary to validate the utilization of this kit for stool samples. Fresh stool samples were collected from 40 healthy patients. The samples were homogenized and separated in two aliquots of 0.5 g each: an aliquot was maintained in an incubator at 37°C for three hours and the other one was diluted in a solution of NaCl 0.9%.The homogenate was centrifuged at 5,000 rpm for 20 minutes. The supernatantwas centrifuged at 13,200 rpm, for 20 minutes and used for analysis. An aliquot was performedimmediately and other was frozen and sent to a reference laboratory to compare the results. **Results**: Two samples were selected for precision analysis. The intra-assay variation of the test was calculated from 20 replicate determinations on each one of two samples. The inter-assay variation was calculated from data on two samples obtained in 20 different assays over a period of ten days. To the precision test, the following coefficients of variation (CV) were obtained: 3.24% (intra-assay); 7.32% and 7.69% (inter-assay). Comparison of the results between the two laboratories yielded a coefficient of correlation 0.816 (CI = 95%; ρ= 0.0012, Spearman). **Conclusion:** These results reveal that the N Antiserum to Human α1-Antitrypsin Kit was efficient for quantitative determination of α1-antitrypsin in stool after the necessary preparation, unfolding a good alternative to the execution of this test.

B-017

Process Management Opportunities for Lab IT Solutions

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Background: Laboratories are turning to information technology (IT) for automation and software solutions to help manage increasing cost pressure and improve the percentage of timely, accurate, and reliable test results. While many IT solutions currently used in the laboratory offer streamlined solutions that manage data from the medical devices and middleware (data management), laboratory personnel are missing opportunities for adopting IT solutions that optimize their laboratory's overall efficiency (process management).

Objective: Obtain laboratory-management feedback on opportunities for IT solutions that would improve the overall efficiency of the laboratory. Use this feedback to determine feature sets for future IT solutions.

Methods: Online surveys and field interviews were conducted with laboratory managers from five countries (USA, Germany, UK, Italy, and Spain). Four specific feature opportunities were tested: workflow intelligence, increased productivity, centralized control, and centralized visibility. The collected feedback was used to determine if the:

- Overall appeal of the feature opportunity matches their laboratory's needs
- Feature opportunity is unique
- Laboratory manager is motivated to learn more about a solution that addresses this feature opportunity

Results: 95 laboratory managers were interviewed. The percentages of those who believed that the feature opportunity is extremely likely or very likely to meet the needs of their laboratory are listed in the table below:

	Overall Appeal		Uniqueness		Ability to Motivate	
	%	Rank	%	Rank	%	Rank
Workflow intelligence	89	2	57	1	57	1
Increased productivity	90	1	47	2	47	2
Centralized control	81	4	39	3	39	3
Centralized visibility	82	3	34	4	34	4

Conclusion: Based on the high overall appeal of the four feature opportunities, the following features were identified as having high value for improving the laboratory's efficiency and overall quality:

- Workflow intelligence and increased productivity: Advanced reporting for turnaround times, samples, tests, and automation utilization; real-time information about priority samples
- Centralized control and visibility: Consolidated inventory and alert management; ability to remotely control the medical devices within the laboratory

B-018

Optimized Handling of Every Tube through Machine-vision-guided Automation

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Background: Sample-container variation poses a significant challenge to the reliability and performance of automated in vitro diagnostic equipment. Historically, manufacturers have chosen to respond by restricting the variety of tube types supported by each instrument. However, modern clinical laboratories receive patient samples from an ever-increasing array of sources and often have minimal influence over the containers they must process. This forces them to expend considerable time and resources on the error-prone task of transferring samples from one container to another to overcome the different limitations of each device.

For its [product name]*, [company name] has invested in the development of a machine-vision system that fully characterizes each sample container as soon as it is loaded onto the instrument. This allows the platform to support more than 30 tube types, including 5 varieties of capillary tubes and a tube-top sample cup (TTSC) that can be placed in any supported vessel.

Methods: The Drawer Vision System (DVS) images every tube while the operator is closing the drawer. STAT samples are recognized and prioritized in less than 10 seconds. Within 30 seconds, every tube in the drawer is characterized as capped, uncapped, or uncapped with a TTSC. Tubes with a TTSC are moved more gently and handled with special care through all stages of processing. Capped tubes that are accidently placed on systems are sorted into user-configurable exception trays. For all tubes, the sample-transfer robot dynamically adjusts the pick location in the drawer based on the measured center of the top of the tube, ensuring that tube tilt is minimized and jostling reduced. Empty slots in the tray are automatically detected and skipped, improving system throughput and eliminating the need to load tubes in a specific pattern.

Results: More than 10,000 sample vessels were evaluated during the development of the DVS. Images of each of these tubes are maintained in an image library, and any algorithm change is validated against all of them before it is released. Routine tubes were correctly identified 99.96% of the time. In the remaining 0.04% of cases, irregularities such as severely peeling barcode labels and tubes leaning in tray slots prevented the tube from being classified with a high degree of certainty. After manual intervention to correct these anomalies, all tubes were correctly identified. Empty tray slots, capped tubes, and tubes with TTSCs were correctly identified in all evaluated cases. The absolute mean error for tube diameter measurement was 0.40 mm, with a standard deviation of 0.37 mm. The maximum error for 99.6% of tubes was 1.99 mm.

Conclusions: Using its custom-developed machine-vision system, the [product name] is capable of optimizing the handling of every tube. This frees the operator from the burden of presorting samples, because loading the instrument is as simple as placing any supported tube in any location. Laboratories no longer need to adjust their workflows in order to overcome the limitations of their equipment.

*Under development. Not available for sale.

B-019

Multivariable Statistical QC Techniques for Detecting Unnatural Behavior of a Method Performed on Several Instruments. A Practical Example with Direct Bilirubin Performed on Two Cobas c311® and two Cobas c501®.

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Background: In two laboratories, one associated with an emergency room, the other associated with a hospital, two cobas c311® and two cobas c501® analyzers are used interchangeably to assay direct bilirubin. Consequently, the same reagent lot and the same QC material lot are used on the four analyzers to ensure interchangeability of the results. This study illustrates the usefulness of multivariable QC statistical techniques to detect an unnatural behavior of the analytical method. ***Methods*:** Instruments, two Cobas c311 and two cobas 501 (Roche). Reagent, ROCHE D Bili® lot# 610028 exp.8/31/2016 (Roche) was prepared and maintained on the instruments for 14 days according to manufacturer's instructions. QC material, Liquichek® pediatric control level 2 lot# 21632 exp. 8/31/2017 (Bio-Rad) was assayed once every shift of eight

hours; the QC values were stored and analyzed with Unity Real Time® 2.0 (Bio-Rad). The quality control values collected in a month were electronically transferred to Minitab® (Version 17, Minitab Inc.) statistical software for numerical and graphic multivariable data analysis. ***Results:*** While for the Unity Real Time QC monthly summary statistics (z score < 2.5, CV ratio <1.5) were acceptable and the L-J chart for all four instruments did not display any abnormal behavior of the method, the T-squared chart, as obtained with the values of all four instruments, clearly showed parallelism (Otelling's T-square P <<0.05 for 20 of 31 of the comparisons). The parallel boxplots by day and the L-J chart, as generated by Minitab, gave for all four instruments an immediate visualization of two parallel down trends of seven days period. These trends were clearly shown by the locally weighted scatterplot smoother applied to the L-J charts. Interestingly, the newly reconstituted reagent brought back the QC values around the mean performance for only seven days. The reagent's stability was suspected as the assignable cause and it was decided to use the reagent for only seven days. This corrected the behavior of the method. ***Conclusions:*** Since the mean function of a QC process is an arbitrary function of time, sometimes the detection of a trend departing from the white noise is not an easy task. This practical example showed that the use of multivariable statistics and their graphic representations gave a warning that prompted further studies. These indicated that the instability of the reagent was the most probable assignable cause . The discrepancy between visual impressions obtained with the L-J charts of Unity Real Time and Minitab is most probably explained with the ratio of the length of y axis to the length of x axis. While the y/x ratio for the three types of L-J charts, as produced by Unity Real Time, is 0.11, 0.2 and 0.3, that for L-J chart as produced by Minitab is 0.55. In general, it is more difficult to visualize some parallel down trends using narrow, stretched charts. In conclusion, the use of multivariable statistical techniques and their graphic representations may be useful for monitoring the behavior of a process. The availability of statistical software, like Minitab, is obviously of paramount importance.

B-020

Multicenter Study of the Sysmex CS-2100i and CS-5100 Systems Compared to the Sysmex CA-1500 System Using Siemens Healthcare Reagents

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Background: The objective of this study was to compare the performance of two automated coagulation analyzers, the Sysmex® CS-2100*i* and Sysmex CS-5100 Systems (CS-2100*i* and CS-5100), to the Sysmex CA-1500 System (CA-1500) using Siemens reagents. Performance characteristics of the systems for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, antithrombin (AT), and D-dimer were compared.

Methods: Three U.S. laboratories participated in method comparison (MC) studies. Result comparability was investigated using leftover samples.* MC of CS-5100 and CS-2100*i* versus CA-1500 was based on a total of 2510 and 2423 results respectively (sum of results over all parameters). In addition, reference intervals were determined at the three U.S. sites in accordance with CLSI guideline EP28-A3c. Determination of the reference intervals was based on at least n = 60 samples per site, with a controlled ethnical distribution representing the ethnical distribution among the U.S. population.

Results: Analysis of MC data was done by Passing-Bablok regression and difference plot and revealed very good agreement to CA-1500, showing slopes between 0.957 and 1.083 and correlation coefficients ≥0.993 (depending on instrument and application). Reference intervals showed good comparability between the predicate device, CA-1500, and the two test devices, CS-2100*i* and CS-5100.

Conclusion: Results of both systems were in good agreement with CA-1500. Based on the data collected during these studies in combination with improved functionality, CS-2100*i* and CS-5100 provide high performance, quality, and efficiency to mid- to high-volume coagulation laboratories.

Product availability may vary from country to country and is subject to varying regulatory requirements.

*Donors gave informed consent and review boards were involved.

B-021

The Average of Deltas: Detection of Systematic Error Using the Average of Intra-Patient Differences

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Background: Traditional quality control (QC) procedures only provide a momentary glimpse of assay performance. Many laboratories employ alternate QC strategies designed to monitor assay performance for the development of systematic error (SE). One strategy, the delta check, compares a patient's most recent chemistry results to historical values but delta checks are limited in that they are best suited to detect large SE. Another strategy gaining in popularity is patient moving averages (MA). With MA the mean patient analyte value is monitored to detect development of SE. The limitation of MA is the inability to equitably detect SE in skewed patient populations. Given that delta checks and MA both have weaknesses; the objective of our study was to develop an Average of Deltas (AoD) monitoring strategy that relies on monitoring the mean difference between pairs of consecutive, intra-patient results.

Methods: From a database of 4.2 million results spanning 638 days we generated arrays containing pairs of patient results collected within 18-26 hours of each other for each assay in our study. To develop sensitive AoD protocols that detect SE equal to the reference change value for each assay we employed an simulated annealing algorithm in Matlab (Mathworks, Natick, MA) to select the number of patient pairs to average (Np) and truncation limits to eliminate large deltas. Again using Matlab, we simulated SE by adding positive or negative bias at fixed intervals in the arrays of paired patient results for serum assays of albumin, aspartate aminotransferase, amylase, bicarbonate, calcium, creatinine, potassium and magnesium. For each assay the average number of deltas to detection (ANDD) was calculated in Matlab in response to induced SE conditions.

Results: The ANDD for SE equal to the reference change value for easy assay varied between assays and between positive and negative SE. For albumin, a +0.4g/dL shift for was detected with an ANDD of 24.6 intra-patient deltas while a -0.4 g/dL SE was detected with an ANDD of 8.8. The AoD protocol with the lowest ANDD was amylase with a +20 U/L SE detected with an ANDD of 6.2 intra-patient deltas and a -20 U/L SE detected with an ANDD of 6.6. Creatinine had the highest ANDD in our validation set with an ANDD of 44.6 intra-patient deltas for a +0.3 mg/dL shift and an ANDD of 43 for a -0.3 mg/dL shift.

Conclusion: We have demonstrated that the AoD can quickly detect development of SE conditions. The AoD strategy is complimentary to other alternative QC strategies such as MA in that the AoD detects SE in assays such as amylase which are challenging for MA. AoD's limitation is that daily laboratory analyses of patients are required; however this is typically not problematic for most inpatient facilities. This initial study demonstrates the validity of the AoD strategy and we are developing further protocols to assess and optimize AOD's capabilities. It is our belief, given the power of AoD, that in the near future that AoD will be implemented in most clinical laboratory analyzers.

B-022

Evaluation of the Performance of JEOL BioMajesty JCA-BM6010/C Automated Clinical Chemistry Analyzer

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Background: Automated clinical chemistry analyzer has been designed for improved quality and speed, and to meet the various demands of different laboratory environments. The JEOL BioMajesty JCA-BM6010/C (JEOL, Japan) is a recently developed, ultra-compact automated analyzer. In this study, we evaluated the performance of JCA-BM6010/C on 11 analytes.

Methods: Precision, linearity, method comparison, accuracy and sample carryover of 11 analytes; alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransaminase (ALT), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), calcium, uric acid, and total bilirubin were evaluated in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI). We also further evaluated linearity using three open reagents (Sekisui, Japan) in the same JCA-BM6010/C instrument. For the correlation study, we compared the JCA-BM6010/C with Cobas 8000 modular analyzer (Roche diagnostics, Basel, Switzerland), and also compared JEOL exclusive reagents to other open reagents (Sekisui, Denka Seiken and Roche) for AST, ALT, GGT, glucose, and uric acid in the

same JCA-BM6010/C analyzer. To assess accuracy, three analytes with open reagents were evaluated (creatinine for Roche, glucose for Denka Seiken, calcium for Sekisui).

Results: The total coefficients of variation (CV) for imprecision evaluation of all analytes showed good values between 1.0 and 2.7% in the JCA-BM6010/C. Linearity was observed for all analytes over the entire analytical range ($R^2 \geq 0.99$). The JEOL exclusive reagent showed a wider linear range than the Sekisui open reagent in ALT and GGT. The JCA-BM6010/C showed good correlation coefficients ($R^2 > 0.975$) for all evaluated analytes except LDH ($R^2 = 0.945$) compared with the Cobas 8000. In the accuracy evaluation, the recovery rates were 96.6 to 101.5% (JEOL exclusive reagents) vs. 98.7 to 109.3% (open reagents) for three analytes (creatinine, glucose, and calcium). The sample carryover was less than 0.34%.

Conclusions: The JCA-BM6010/C showed excellent performance in terms of precision, linearity, comparison, accuracy, and sample carryover. Additionally, the instrument's performance is comparable with the Roche Cobas 8000. We conclude that the JCA-BM6010/C could be used well for the medical services in the routine laboratories.

B-023

implementation of new total laboratory automation system and analysis of its outcome for laboratory turnaround time: experience of a core clinical laboratory in a large tertiary care hospital

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Background: The continuous pressure to improve laboratory efficiency and reduce turnaround time (TAT) have made the use of laboratory automation pervasive in clinical laboratories and brought about continual evolution and expansion of its capabilities. Recently, our laboratory has implemented a new total laboratory automation (TLA) system and informatics tool (Aptio™ Automation and CentraLink™ Data Management System, Siemens Healthcare Diagnostics), which can automate the laboratory processes from specimen transportation to refrigerated storage and disposal. In this article, we have drawn on our experience on new TLA system in clinical chemistry and immunology at a 1400-bed tertiary care hospital, Korea, in comparison with old one (ADVIA LabCell, Siemens).

Methods: The major changes between Aptio™ and ADVIA LabCell were as follows: use of bulk input module, incorporation of centrifugation module in the TLA line (routine samples only) and implementation of refrigerated storage module (RSM) capable of automated sample storage, retrieval and disposal. To evaluate the performance of Aptio™ Automation, TAT from sample collection to reporting and intra-laboratory TAT (from sample loading to reporting) after Aptio™ implementation (October 2014-January 2015) were compared to those in the ADVIA LabCell period (October 2015-January 2016) using 3 representative chemistry (AST, TSH, and troponin I) and 1 immunology (anti-HBs) items. The benefits of RSM were evaluated using the number and intra-laboratory TAT of reflex tests arising from the results exceeding analytical measurement range, which were processed automatically (Aptio™), or manually (LabCell).

Results: During the study period, the proportion of routine vs. stat orders was 40% vs. 60%, and it meant 40% of manual centrifugation was reduced under Aptio™ system. The mean intra-laboratory TAT for stat and routine samples was 19 and 21 min (LabCell) vs. 21 and 35 min (Aptio™), while TAT from sample collection to reporting was 60 and 67 min (LabCell) vs. 66 and 72 min (Aptio™), respectively. Under both system, 0.2% of samples (4,979/2,463,709 on LabCell; 6,491/3,343,082 on Aptio™) needed reflex tests and mean intra-laboratory TAT of reflex tests was 34 (LabCell) vs. 37 min (Aptio™).

Conclusion: Our experience on the new TLA system showed that Aptio™ Automation did not shorten TAT compared to previous one, while it reduced pre-analytical and post-analytical manual process considerably with minimal delay in intra-laboratory TAT by incorporating centrifugation module and storage module. Although the new features of Aptio™ Automation were appreciated, it seems that strategies to optimize laboratory TAT are needed.

B-024

Development and application of a network glucometer quality control program for a tertiary hospital

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Background: The use of point-of-care (POC) glucometers for hospitalized patients has been increased. However, it has never been subjected to traditional quality control assessment. Here, we developed a novel network quality control program for glucometers using Unity (Bio-Rad Laboratories, Irvine, USA). We evaluated more than 200 glucometers (ACCU-CHEK, Roche Diagnostics) in service in a tertiary hospital.

Methods: Quality control (QC) data on glucometers were collected from September 2014 to June 2015. In Unity, we made the instrument number to be recognized as a lot number in a single laboratory containing more than 200 different lots. The QC data were transferred to a laboratory information system (LIS) using the Roche docking system, and then to Unity, in real time. The data were analyzed daily to detect violations of Westgard rule and monthly to get mean and standard deviation (SD) for each instrument. The acceptance criteria for accuracy and precision were, respectively, the mean±12 mg/dL or 12.5% and a coefficient of variation (CV) ≤7.1%.

Results: About 250 POC glucometers were subjected to QC each month. The mean number of QC runs for each instrument was 55.4. Pooled CVs for low and high control materials were 2.7 ~ 3.8% and 2.1 ~ 2.7%, respectively. During the study period, all the instruments met the accuracy criteria, while 0.0 ~ 0.4% and 0.3 ~ 1.6% of instruments could not meet the precision criteria for low and high QC materials, respectively. When the QC check failed, the instrument was checked and the operative given additional education on how to perform QC measurements. During the study period, seven instruments were changed because of abnormal QC results.

Conclusion: We developed a network QC program for glucometers using LIS and the Unity program. We successfully monitored QC results of POC glucometers. To our knowledge, this is the first attempt to apply QC to glucometers systematically. Our method will be useful in large hospitals with numerous POC glucometers.

B-025

Evaluation of the Analytical Performance of a Thyroid-stimulating Hormone Assay on the Atellica Immunoassay Analyzer$_1$

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Introduction: Measurement of thyroid-stimulating hormone (TSH) concentration with a high degree of sensitivity, accuracy, and precision is important in the diagnosis and management of thyroid and pituitary disorders. The primary objective of this study was to demonstrate the analytical performance of the TSH3-Ultra (TSH3-UL) assay on the Atellica™ Immunoassay (IM) Analyzer[1], an automated, high-throughput immunoassay analyzer under development by Siemens Healthcare Diagnostics.

Methods: The Atellica IM TSH3-ULassay uses the same reagents and calibrators as the ADVIA Centaur® TSH3-UL assay, a third-generation TSH assay. The Atellica IM TSH3-UL assay employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, a FITC-labeled anti-TSH capture monoclonal antibody, and a tracer consisting of another anti-TSH monoclonal antibody and acridinium ester (AE), both conjugated to BSA. The AE is a patented high quantum yield hydrophilic NSP-DMAE-HEG-Glutarate-NHS molecule. Precision of the TSH assay was evaluated for serum and plasma samples spanning the measuring range (0.034 to 132 µIU/mL) according to CLSI protocol EP05-A3. LoB, LoD, and LoQ were determined as described in CLSI protocol EP17-A2. Interference testing followed CLSI protocol EP07-A2.

Results: Detection capability for the Atellica IM TSH3-UL assay was estimated to be 0.001, 0.005, and 0.007 µIU/mL for LoB, LoD, and LoQ (functional sensitivity at 20% total CV), respectively. Observed repeatability ranged from 1.09 to 4.87% CV, and within-lab precision ranged from 1.82 to 5.95% CV over the assay range. The assay showed no significant effect (less than 5% bias) from endogenous interferences, including red blood cell lysate up to 600 mg/dL hemoglobin, triglycerides up to 2000 mg/dL, and conjugated and unconjugated bilirubin up to 60 mg/dL. There was no high-dose hook effect for the Atellica IM TSH3-UL assay in samples up to 9239 µIU/mL TSH. Comparison between the Atellica IM and ADVIA Centaur XP TSH3-UL assays yielded the following Deming regression equation: Atellica IM TSH3-UL = 1.07(ADVIA Centaur XP TSH3-UL) + 0.00 µIU/mL; n = 347 serum samples ranging

from 0.008 to 148.79 µIU/mL; r = 0.994. The on-system stability of the Atellica IM TSH3-UL reagents was determined to be at least 60 days.

Conclusion: The Atellica IM TSH3-UL assay has demonstrated excellent analytical performance capable of measuring TSH with a high degree of sensitivity, accuracy, and precision for use in the diagnosis and management of thyroid and pituitary disorders.

[1.] Under development. Not available for sale.

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B-026

Total laboratory automation in a high-volume clinical laboratory: assessment of economic savings, improvement of processes and productivity increase.

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Background: In the last years, the increasing demand on clinical laboratories required improvements on workflow and cost efficiency, as well as reduction in turnaround time (TAT) and error rates. All these factors propelled to the use of total automation systems (LAS) as a solution for routine and emergency sample management. LAS also allows the laboratory manager to have a clear path of each process in the analytical area and act directly in bottlenecks to improve processes , leading to "Lean" laboratories. In order to measure whether LAS improve sample and process management, we investigated daily test release rates, productivity/staff, tube and waste reduction rates in a laboratory that produces 34 million biochemistry and immunology tests per year.

Methods: A new laboratory configuration was proposed in order to connect 17 Siemens ADVIA CentaurXP®, 8 ADVIA Chemistry 2400 ® and 2 IMMULITE 2000® to the 65 meters Siemens Aptio® LAS equipped with 3 bulk input module, 1 input/output module and 3 rack output modules. This configuration allowed sample loading onto the system without rack-placing procedure with an input up to 3,000 tubes/hour and 35,000 tubes/day. Relevant data, such as, number of tubes, exams/hour, exams/personnel and time to report results, was collected from Laboratory Information System (LIS) and CentraLink Data Management System® before and after LAS installation.

Results: After 3 months of LAS operation, 86% of biochemistry and 80% of hormone-related tests results were reported at the same day of sample collection(versus 67% and 50% in the pre-LAS condition, respectively). An increase of 55% in number of tests performed by technician/day were observed. Cost savings with tubes reached up to US$ 6,500, per month (US$80,000/year) with 97,500/month tube handling decrease and biological waste reduction to more than 1,13 tons/month. Moreover, processes involving loading, sorting, and error handling of samples were dramatically reduced.

Conclusions: Here, we describe a successful LAS implementation, with gains in TAT, number of tests/personnel and tube reduction. The workflow was substantially simplified, turning from a multi-step process to a one-way route through pre-analytical to post-analytical phases. Thus, LAS allowed significant cost reduction and raised the productivity in a high-volume laboratory.

B-029

Analysis of kidney stones by quantitative automated method

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Background: The analysis of kidney stones (KS) are essential for management of patients with nephrolithiasis. Most KS are composed of calcium oxalate, uric acid, calcium phosphate or magnesium-ammonium-phosphate (struvite). KS are usually analyzed by semi-quantitative colorimetric manual method. The aim of this study was to evaluate the analysis of KS by automated quantitative method to identify the following type of KS: calcium oxalate, uric acid, calcium phosphate and struvite. **Methods:** KS were analyzed by two methods: 1. Reference method: manual and semi-quantitative by colorimetric analysis (Merck®), following the manufacturer's instructions and determining the components of the calculation with the highest percentage (oxalate, calcium, uric acid, phosphate, ammonium and magnesium). They were classified into calcium oxalate, uric acid, calcium phosphate and struvite. 2. Method to evaluate: automated and quantitative. KS was crushed and degraded with 100 µL of sulfuric acid. Then 50 µL of the sample degraded was diluted with 450 µL of distilled water and the following biochemical parameter were determined in the autoanalyzer Dimension EXL (Siemens diagnóstic®): calcium, uric acid, phosphorus and magnesium. Statistical analysis was performed using the software MedCalc®. **Results:** We analyzed 58 KS, 35 were calcium oxalate, 17 calcium phosphate, 5 uric acid and 1 struvite, according to the semi-quantitative method reference. The range and median of biochemical parameter determined by automated quantitative method for each type of KS is shown in the following table:

	Calcium oxalate	Calcium phosphate	Uric acid	Struvite
Calcium (mg/dL)	**55.7 (18.6-169.8)**	**63.0 (25.3-155.2)**	2.1 (0.1-4.8)	10.0
Phosphorus (mg/dL)	1.7 (0-9.6)	**18.7 (13.0-52.1)**	0.1 (0-0.1)	**21.1**
Uric acid (mg/dL)	0.2 (0-1.4)	0.2 (0-0.9)	**101.3 (44.6-296.1)**	0
Magnesium (mg/dL)	1.7 (0-5.7)	2.2 (0-2.9)	0.9 (0.4-1.0)	**55.1**

Using the Mann-Whitney test, we found statistically significant differences ($p<0.0001$) with:

a) Calcium levels to identify KS composed of calcium oxalate or calcium phosphate. b) Phosphorus levels to identify KS composed of calcium phosphate or struvite. c) Uric acid levels to identify KS composed of uric acid. **Conclusions:** This automated quantitative method can be used for analysis of KS. Calcium, phosphorus, uric acid and magnesium levels in KS identify the type of KS.

B-030

Automated Sigma Metric Analysis for Monitoring Quality in a Standardized Healthcare System.

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Background:

As healthcare systems continue to merge and expand, additional tools are needed to monitor analytical performance metrics, more efficiently and effectively. This poster demonstrates the utility of sigma metric analyses for evaluating quality across multiple instruments and assays in networked healthcare system. Automated techniques are leveraged in the analysis, demonstrating the future possibilities for quickly and efficiently capturing large amounts of quality control data for use in sigma metric calculations. In addition, this study will demonstrate the use of sigma metrics for evaluating instrument and or assay performance changes over time and which variables play a role in these changes.

Methods: This study collected 400 days of quality control data from 5 networked laboratories, comprising 284,676 quality control data points, across seven Abbott ARCHITECT platforms. In total, 25 assays were evaluated using QC levels near medical decision points. Target means were obtained through Biorad™ Unity peer data reports for bias estimates, and focused on one lot of unassayed Multiqaul control. Before sigma metrics were calculated, an outlier identification method was determined. Two methods were evaluated and compared to the number of values outside of the laboratory defined ranges. 1.) Using an instrument calculated +/- 3.5SD multiplier. 2.) Using instrument calculated mean and quartiles. To test for sigma stability or variability over time, 400 days of data were divided into ~180 day quarters. The impact of reagent lot changes, calibration lot changes, and calibrations were collected for investigating their contribution to sigma variability over time.

Results: The 3.5SD outlier method proved to be the preferred approach by successfully eliminating QC errors attributable to human error. In contrast, the Quartiles method incorrectly eliminated data when quartile widths were made extremely small due to a large number of identical QC results. Sigma metrics revealed nine assays exceeded 6 sigma for all seven analyzers over 4 quarters. Additionally, ten more analytes exhibited median sigma levels exceeding 6. Sigma metrics also revealed performance differences between instruments and over time.

Conclusion: Successful automation of sigma metrics for assessing and detecting quality changes in a networked healthcare system has been demonstrated. This data illustrates how instrument-assay combinations can be quickly obtained and reviewed for differences, prompting further investigations where needed. By collating data into statistically significant quarters, lab managers and directors can monitor performance trends over time. This tool allows a high level overview of networked instrument performance, and helps describe how robust a sigma level will be over time. Through automation tools and summary statistics such as sigma metrics information leading to QC cost reductions and improved performance will continue to play a role in clinical laboratory management.

B-031

Development of a risk model to predict urgent dialysis among advanced chronic kidney disease (CKD) patients

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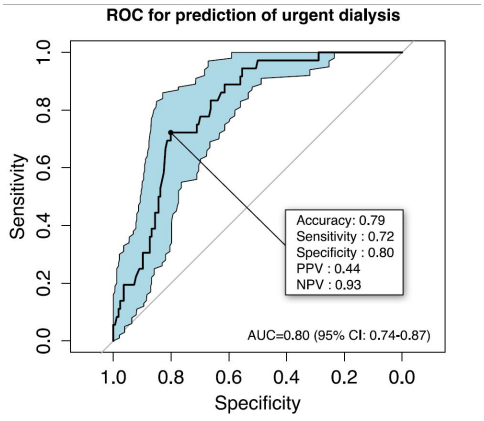
Background: Urgent in hospital dialysis starts are associated with increased costs and high morbidity and mortality. While previous studies have assessed risk factors for urgent dialysis among CKD patients, no study has developed a risk score to predict urgent dialysis. The objective of this was to develop a risk model to predict urgent dialysis among advanced CKD patients.

Methods: The study population included all advanced CKD (eGFR < 30 ml/min/1.73m^2) patients who were referred to multidisciplinary chronic kidney disease at The Ottawa Hospital between January 01, 2010 and December 31st 2014 (n=1010). This was a retrospective cohort study, which included the following data: Patient demographics (age, sex, race), physical examination variables (e.g. height, weight, blood pressure), laboratory test results (e.g. creatinine, eGFR, hemoglobin, urea, albumin); co-morbidities (e.g. coronary artery disease, hypertension, CHF); medications (e.g. anti-hypertensive, statin). A random forest (RF) classification algorithm was developed using these variables to predict patients at risk for urgent dialysis. Data were divided into training (60%), crossvalidation (20%), and test (20%) sets to optimize and test the performance of the model. The algorithm was optimized by adding features to the random forest model to maximize the ROC area-under-the-curve (AUC).

Results: The random forest model identified the following variables as the most important predictors: changes in CO2, calcium, albumin, weight, potassium, and phosphate along with age, urine protein:creatinine ratio, and creatinine. The RF model had an AUC of 0.80 (0.73-0.87), with sensitivity of 72% and specificity of 80% at maximum efficiency for prediction of urgent dialysis.

Conclusion: The model developed herein represents a potential mechanism to identify patients at risk for urgent dialysis. Identification of this population may allow for earlier interventions to improve outcomes in CKD patients progressing urgently to dialysis; implementation of this algorithm is also likely to reduce the associated costs of urgent in-hospital dialysis.



B-032

Optimizing use of business analytics and lab-oriented statistical software to establish robust and pertinent reference intervals

A. B. Muenzenmeyer, E. Z. Reineks. *Cleveland Clinic, Cleveland, OH*

Background: The patient population served by our automated chemistry laboratory has grown outside our local population due to rapid expansion of our health system, as well as the increased nationwide geographical footprint of the Cleveland Clinic's reference laboratory. Population-based reference intervals are a set of values classified by upper and lower reference parameters, which typically represents the central 95% of values from the reference population of normal, healthy, control subjects. Given our growing and likely evolving patient population, our existing reference intervals were re-evaluated for their appropriateness. Various challenges accompany the process of validating or verifying reference ranges. Traditional approaches may have some limitations, including a lack of laboratory resources, inadequate availability of specimens from normal, healthy subjects (especially for partitioned reference intervals, e.g. due to patient demographics), insufficient or inefficient access to LIS or EMR data, or inappropriate starting point reference intervals based on literature or vendor-provided information. Establishing *de novo* reference intervals for common metabolic analytes was preferred (vs. verifying other intervals) because the new ranges would reflect the actual patient population being served. The goal of this study was to establish laboratory-specific *de novo* reference intervals for 12 common metabolic analytes by leveraging multiple software tools and existing patient results. This is an example of how laboratories can efficiently utilize analytics to drive better patient care. **Methods:** This study utilized Altosoft (*Kofax*, Irvine, CA), "code-free" business intelligence software to readily identify suitable existing patient samples where results were stored in our laboratory information system (*Sunquest*, Tucson, AZ). Data was exported into Excel (*Microsoft*, Redmond, WA) and filtered according to pre-defined, medical director-approved qualifications (including visit-related diagnosis codes) which resulted in the datasets used for further analysis. The datasets were evaluated using an EP Evaluator® (*Data Innovations*, South Burlington, VT) statistical module, entitled "Establish Reference Interval (EST)." This Establish RI module uses the nonparametric method in accordance with CLSI: C28-A guidelines to calculate the reference interval (based on central 95% of results from healthy subjects). **Results:** Reference intervals were established using analysis of historical data for 12 common metabolic analytes. The number of patient results used in establishment (N) far exceeded the traditionally recommended minimum of 140 samples, and ranged from 540 to 646 for each analyte. **Conclusions:** Data mining tools and real-time analytics utilization was used for robust establishment of reference intervals that would not be feasible to achieve with our historical methods. Improvements over our previous methodology included: 1) Establishing reference intervals for common analytes utilized large datasets to produce *de novo* ranges that are truly representative of our patient population. 2) After gaining familiarity with the analytic tools, the process proceeded quickly. 3) Readily accessible and exportable data via the business intelligence software allowed us to bypass our previous reliance on IT expertise and availability to conduct our data searches. 4) Although we utilized EP Evaluator® software in this project, the statistical analysis of the healthy population dataset could be performed in most spreadsheet programs with either default functions or via add-in packages.

B-033

Integration of steroid analysis in serum using LC-MS/MS with fully-automated sample preparation

B. J. Feild[1], D. Kawakami[2], T. Minohata[2]. *[1]Shimadzu Scientific Instruments, Columbia, MD, [2]Shimadzu, Kyoto, Japan*

Background: Currently sample preparation for the detection of steroids in serum by liquid chromatography-mass spectrometry (LC-MS/MS) involves complex offline extraction methods such as solid phase extraction or liquid/liquid extraction, all of which require additional sample concentration and reconstitution in an appropriate solvent. These sample preparation methods are time-consuming, often taking 1 hour or more per sample, and are more vulnerable to variability due to errors in manual preparation. Our approach to offering a high sensitivity steroid detection method and timely, automated analysis of multiple samples is to use the automated sample preparation system coupled to the detection capabilities of a high-sensitivity triple stage quadrupole mass spectrometer.

Methods: 10 steroid hormones (cortisol, aldosterone, 11-deoxycortisol, corticosterone, 17-alpha-hydroxyprogesterone (17-OHP), 4-androstene-3,17-dione (androstenedione), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), progesterone and testosterone) in serum were verified using CHS™ MSMS Steroids Kit (PerkinElmer, USA). Serum sample was loaded directly into the automated sample preparation system (CLAM-2000 Shimadzu, Japan). The CLAM-2000 was programmed to perform protein precipitation using acetonitrile followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2000 to the HPLC without human intervention for LC-MS/MS analysis. The treated samples were trapped using a MAYI-ODS column (2mm x 5mm) and then separated by Core-Shell Biphenyl HPLC column (Kinetex Biphenyl, 100mm x 2mm, 2.6µm, Phenomenex) at 40°C with a binary gradient system at a flow rate of 0.3 ml/min in 11 min.

Results: We evaluated this system using calibrator and control serum spiked with 10 steroids in Kit and carried out concurrent analysis over a range of concentrations for each steroid: cortisol (1.51-320 ng/mL), aldosterone (0.03-7.05 ng/mL), 11-deoxycortisol (0.08-18 ng/mL), corticosterone (0.29-62 ng/mL), 17-OHP (0.12-26

ng/mL), androstenedione (0.08-18 ng/mL), DHEA (0.31-65 ng/mL), DHEAS (12.9-2750 ng/mL), progesterone (0.12-26.5 ng/mL) and testosterone (0.03-7.2 ng/mL). The calibration curves that were generated had linear regression values of r^2 >0.997 for each curve. The reproducibility (N=3) at seven concentrations, including LLOQ of each compounds was excellent (CV<10%). We found that the sample preparation time was reduced from 60 minutes to 10 minutes by the automated system.

Conclusion: We completed steroid analysis using the automated sample preparation system coupled to LC-MS/MS. The results shows the capability of the system for large sample set analyses with improved accuracy and precision by eliminating human error associated with manual sample handling.

Wednesday, August 3, 2016

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

Electrolytes/Blood Gas/Metabolites

B-034

A Microtiter Plate Assay for the Quantitative Measurement of Pyruvate in Whole Blood

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Background: Pyruvate is a critical cellular metabolite derived from the oxidation of glucose during glycolysis. Its measurement in blood is useful in the evaluation of patients with inborn errors of metabolism. Methods to measure pyruvate are not available commercially and laboratories that perform pyruvate testing traditionally use spectrophotometric techniques that require the use of individual cuvettes. The objective of this study was to develop a high-throughput analytical method for measuring pyruvate in whole blood.

Methods: Samples were prepared by the addition of 1 mL anticoagulated (heparin or EDTA) whole blood to 2 mL 8% (w/v) cold perchloric acid, incubated on ice for 10 min, then centrifuged to obtain a protein-free supernatant. Samples, Trizma base, and NADH were added to wells of a 96-well microtiter plate and an initial absorbance at 340 nm was measured on a Spectramax 384 Plus plate reader. Lactate dehydrogenase was added to each well and a final absorbance was measured following a 5 min incubation at room temperature. The decrease in absorbance at 340 nm due to the oxidation of NADH was used to determine the concentration of pyruvate. Performance characteristics including precision, linearity, analytical sensitivity, and accuracy were determined as were reference intervals and sample stability. The University of Utah's Institutional Review Board approved this study.

Results: Reactions with samples anticoagulated with EDTA, but not heparin, failed to reach an end-point and were considered to be unsuitable. Precision was determined using samples created by the addition pyruvate to perchloric acid and measuring pyruvate in three replicates for five days. Within-run and total CVs were both 2.4% at 0.157 mM and 5.8 and 5.9% at 0.062 mM, respectively. Linearity was determined by combining samples with high and low pyruvate concentration to prepare six samples that were tested in duplicate. The assay was linear within the measuring range of 0.06 to 0.40 mM ($y=1.007(x)+0.0008$, $R^2=0.999$). Perchloric acid-treated 0.9% saline and a 0.055 mM patient pool were each tested in 12 replicates to determine the limit of blank (LOB) and limit of detection (LOD), respectively. The LOB and LOD were calculated as 0.0158 and 0.0259 mM, respectively. Accuracy was evaluated by a method comparison study using a pool of perchloric acid-treated heparinized whole blood to which volumes of a 7 mM pyruvate standard were added to create 40 pairs of samples (0.063 to 0.369 mM). The comparison method was the previously validated cuvette-based method used in our laboratory. Linear regression yielded a slope of 0.941, intercept of 0.007 and Sy/x of 0.013. The previously established reference interval of 0.03-0.107 mM was verified using freshly collected whole blood samples from 20 healthy, fasting donors. Sample stability was determined using freshly collected heparinized blood samples (0.118 and 0.087 mM). Pyruvate was stable for four hours, two days, and four weeks at room temperature, 4°C, and -20°C, respectively.

Conclusions: The pyruvate assay in a microtiter plate format demonstrates acceptable performance characteristics for quantifying pyruvate in heparinized whole blood. Whole blood anticoagulated with EDTA should not be used for pyruvate testing.

B-035

Performance Characteristics of a Urine Oxalate Reagent System Adapted for Use with Plasma

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: Oxalate is the end-product of the glyoxylate and glycine metabolism and is excreted in the urine. The measurement of oxalate in plasma is used to assess the body pool size of oxalate in patients with primary hyperoxaluria, chronic renal failure, and oxalate toxicity. Oxalate in unacidified plasma can increase due to the conversion of ascorbate to oxalate. As such, plasma must be rapidly frozen or acidified after collection. The purpose of this study was to adapt Trinity Biotech Urine Oxalate Kit for use with plasma.

Methods: Oxalate was oxidized to CO_2 and H_2O_2 by oxalate oxidase. The amount of H_2O_2 produced was determined by a colorimetric reaction that produced an indamine dye. The absorbance at 590 nm was directly proportional to the concentration of oxalate. Residual and freshly collected heparin or EDTA plasma was used for this study. Immediately before testing, samples were thawed, acidified to pH ~2.5 by the addition of 17 uL concentrated HCl to 1.2 mL plasma, and filtered through a 30 kDa centrifugal filter device. Performance characteristics including precision, linearity, analytical sensitivity, and accuracy were determined as were reference intervals and sample stability. The University of Utah's Institutional Review Board approved this study.

Results: Precision was determined using sample aliquots stored at -20°C in three replicates, once each day, for ten days. Within-run and total CVs were 1.3 and 4.4% at 17.3 µM and 9.1 and 20.2% at 5 µM, respectively. Linearity was determined by diluting a high calibrator (50 µM) with 0.01 M HCl to create a set of six samples that were tested in duplicate. The assay was linear within the measuring range of 2 to 50 µM (linear regression $y=1.00(x)+1.10$, $R^2=1.00$). The zero calibrator (0.01 M HCl) and the 3.3 µM calibrator were each tested in 12 replicates to determine the limit of blank (LOB) and limit of detection (LOD), respectively. The LOB was 1.4 µM as calculated from the mean+3 SD. The LOD was 2 µM as calculated as the LOB+3 SD of the LOD. Accuracy was determined by recovery studies performed by adding volumes of the 50 µM calibrator to two plasma samples with oxalate concentrations of 4.4 and 17.3 µM. Calculated recoveries were 104.9% and 102.6%, respectively. The reference interval of <1.8 µM was verified using 20 freshly collected plasma samples. Sample stability was determined using acidified and unacidified plasma, and acidified and deproteinized ultrafiltrates. Oxalate increased by 0.8 to 3.2 µM in unacidified plasma after two hours at room temperature. In both unacidified and acidified plasma, oxalate was stable for up to 7 days at -20°C. Oxalate in acidified and deproteinized ultrafiltrates was stable for up to 21 days at -20°C.

Conclusions: Plasma oxalate can be measured accurately using a reagent platform designed for use with urine although the assay is less precise at low oxalate concentrations. To avoid false increases in oxalate, plasma must be stored frozen or acidified and stored frozen after collection.

B-036

Development of a Reference Material for Low Level Creatinine in Human Serum Using Artificial Serum Matrices

J. E. Camara. *NIST, Gaithersburg, MD*

Background: The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials® (SRMs) intended for use as accuracy controls in the analysis of clinical samples. NIST has also been involved in the standardization efforts of the National Kidney Disease Education Program (NKDEP) of the National Institute of Diabetes and Digestive and Kidney Diseases-National Institutes of Health for many years, which has resulted in the production of several SRMs supporting creatinine measurements in serum and urine. NIST currently sells SRM 967a Creatinine in Frozen Human Serum, which provides two levels of creatinine in serum at adult normal and high levels to support clinical measurements for assessment of kidney disease. However, pediatric ranges for serum creatinine are significantly lower than the adult normal range. Therefore, the current SRM 967a does not support the accurate measurement of creatinine in the range necessary for measuring serum creatinine and screening for kidney disease in the pediatric population, which is a concern that has been voiced in recent years through the NKDEP. **Methods:** As it is not feasible to obtain large volumes of pediatric serum for the production of a new lower level creatinine serum material, NIST has begun an investigation into the use of commercially available artificial serum matrices as serum diluents or bases for a new material with a target value of 4 µg/g (0.4 mg/dL) creatinine. NIST obtained SeraFlx BIOMATRIX and SeraFlx LCMSMS artificial serum from Cerilliant. In addition, a pre-market SigMatrix Ultra Serum Diluent was provided by MilliporeSigma. NIST measured the background creatinine levels and performed spiked recovery studies on each material using an isotope-dilution liquid chromatography-mass spectrometry (ID-LC-MS) Reference Measurement Procedure for creatinine in serum. SRM 914a Creatinine was used as the calibrator and SRM 967a Creatinine in Frozen Human Serum was used as the control for all ID-LC-MS measurements. **Results:** The endogenous levels of creatinine in these artificial matrices were determined to be 0.50 µg/g, 3.30 µg/g, and 0.00 µg/g for SeraFlx LCMSMS, SeraFlx BIOMATRIX, and SigMatrix Ultra, respectively. In addition, none of these materials displayed interfering peaks in the internal standard

LC-MS channel, eliminating this as a possible source of bias. For spiked recovery studies, each material was spiked with multiple levels of creatinine in the clinically-relevant range of 4 µg/g to 35 µg/g and processed in triplicate by ID-LC-MS. The percent recovery results were 94 % to 98 %, 104 % to 105 %, and 100 % to 104 % for SeraFlx LCMSMS, SeraFlx BIOMATRIX, and SigMatrix Ultra, respectively. **Conclusion:** Based on the results of ID-LC-MS analysis, these three artificial serum matrix materials remain viable candidates for use as diluents of normal serum or bases for creatinine spiked materials. However, additional studies are needed to determine if such materials would be fit-for-purpose in routine clinical creatinine assays, such as enzymatic or Jaffe-based methods. NIST is currently organizing a round robin study with assay manufacturers and clinical laboratories to further evaluate candidate reference material mixtures based on these artificial matrices.

B-037

Analysis Of Biochemical Profile In Post-Operative Neuro-Oncology Patients With Hyponatremia

P. CHAVAN, P. PARIKH, V. BHAT, P. SHETTY, A. MOIYADI. *ACTREC-TATA MEMORIAL CENTRE, Navi Mumbai, India*

Introduction: Hyponatremia is not an uncommon electrolyte disorder in post-operative neurology patients. The common causes of hyponatremia in these patients are Syndrome of Inappropriate Secretion of Antidiuretic Hormone (SIADH) and Cerebral Salt Wasting (CSW). The two conditions though present with similar characteristics, can be differentiated on the basis of clinical evidence of a contracted extracellular fluid (ECF) volume in CSW. It is important to differentiate between these two conditions as the treatment for SIADH is fluid restriction and that for CSW is vigorous sodium and volume replacement. Certain biochemical parameters help in distinguishing between SIADH and CSW in addition to clinical findings.

Objective: In this study we analysed cases of persistent hyponatremia (lasting more than three days) with hypotonicity of plasma in post-operative neuro-oncology patients. We compared the changes in select biochemical parameters with those in published literature for their utility to differentiate between SIADH and CSW

Methods: Out of 826 cases operated during a period of three years, 14 were investigated for persistent hyponatremia. Of these, six cases also showed hypotonicity of plasma. They were further analysed for change in following biochemical parameters: Blood Urea Nitrogen (BUN)/ Serum Creatinine Ratio, Serum Uric Acid, Serum Albumin, Serum Potassium, and Hematocrit. The values from the preoperative work-up were compared with those during the period of hyonatremia. An average percent change in the values was calculated for each parameter.

Results: All six cases showed hyponatremia (serum sodium<136 mmol/l), hypotonicity (serum osmolality<275mosm/kg), urine osmolality greater than serum osmolality. All six cases showed decrease in serum albumin (42.63%), serum potassium (10.26%), serum uric acid (56.52%) and hematocrit (26.67%) levels. BUN/ creatinine ratio was decreased in four cases. One case showed no change, while other case showed raised BUN/creatinine ratio with increased BUN levels, however, serum creatinine levels were not raised ruling out kidney disease. All cases were diagnosed as SIADH and responded to fluid restriction.

Conclusion: According to literature serum uric acid, serum potassium and BUN/ creatinine ratio are either decresed or remain unchanged in SIADH. Serum albumin and hematocrit show no change. In our study serum potassium, serum uric acid and BUN/creatinine ratio showed a decrease in the post-operative phase with hyponatremia which is comparable to current literature. However, serum albumin and hematocrit levels too were decreased significantly. Tracking these biochemical parameters in addition to standard tests for differential diagnosis of hyponatremia (osmolality and urine sodium levels) will be helpful in differentiating between SIADH and CSW early before clinical symptoms appear in post-operative neuro-oncology patients. However, a study with larger group of patients is required.

B-038

Evaluation of a New ICT (ISE) Module for the Abbott ARCHITECT® cSystem™

Q. Li, Z. Xu, S. M. Osborn. *Abbott Laboratories, Irving, TX*

Background: Abbott Integrated Chip Technology (ICT) module consists of solid state ion selective electrodes (ISE) to measure sodium, potassium and chloride simultaneously in serum, plasma or urine from 15 µL samples, within 3 minutes, on the ARCHITECT Clinical Chemistry Systems. ICT module enhancements have led to extended calibration intervals, from 8 hours to 24 hours, and module use life, from 2 months to 3 months. More recently, the ICT module was further enhanced for product reliability. The objective of this study was to evaluate the enhanced ICT module performance using Six Sigma Metrics.

Methods: Quality controls, 200 to 300 human serum samples and 20 to 30 urine samples were tested everyday on each ICT module. Over 22,000 samples (66,000 tests) were tested after three months. Then precision was evaluated with 5 replicates and 2 runs per day using commercial controls over 5 days following CLSI Guideline EP5-A2. Linearity was evaluated according to EP6-A. Sigma metrics were evaluated using 9 replicates of NIST SRM 956d, where Sigma= (TEa(%)-Bias(%))/CV(%) per Westgard QC using RiliBäk TEa targets.

Results: Daily quality control results were stable and within ranges. The precision ranged from 0.23% to 0.42% for serum assays and 0.21% to 1.30% for urine assays. All assays met the linearity ranges and acceptable deviations listed in the table below. Sigma values ranged from 8 to 28.

Conclusion: The enhanced ICT Module for Abbott ARCHITECT cSystems consistently demonstrates excellent precision and linearity performance over its warranty claim of 20,000 samples (60,000 tests) or 3 months use. Sigma metrics greater than 6 demonstrate the new ICT Module has a low frequency of error and confirms the new ICT Module is well suited for use in the clinical laboratory.

Assay	**Na^+ (Serum)**			**Na^+ (Urine)**			**K^+ (Serum)**			**K^+ (Urine)**			**Cl^- (Serum)**			**Cl^- (Urine)**		
Precision (%CV), n=50	0.23			0.21			0.42			1.30			0.28			0.35		
Linearity (mmol/L)	100 - 200			20 - 400			1.0 - 10.0			1 - 300			50 - 150			20 - 300		
	≤5%			≤5% / 3 mmol/L			≤5% / 0.25 mmol/L			≤5% / 3 mmol/L			≤5%			≤5% / 3 mmol/L		
Sigma Analysis (NIST SRM 956d)	**Na^+ (Serum)**						**K^+ (Serum)**						**Cl^- (Serum)**					
	Level 1	**Level 2**	**Level 3**				**Level 1**	**Level 2**	**Level 3**				**Level 1**	**Level 2**	**Level 3**			
Certificate Value (mmol/L)	120.0	139.3	158.7				5.752	3.730	1.611				94.53	108.5	122.6			
Architect Mean (n=9)	118.3	137.9	158.7				5.780	3.720	1.684				93.76	107.6	122.2			
Bias (%)	-1.42	-1.01	0.00				0.49	-0.27	4.53				-0.81	-0.83	-0.33			
% CV (5 day precision study)	0.23						0.42						0.28					
TEa (%) (RiliBäk)	5.0%						8.0%						8.0%					
Sigma	**16**	**17**	**22**				**18**	**18**	**8**				**26**	**26**	**28**			

B-039

Sigma Analysis of Automated Chemistries Over Time

J. H. Nichols, J. Colby, J. Wiencek, A. Woodworth. *Vanderbilt University School of Medicine, Nashville, TN*

Introduction: Sigma metrics are a good estimate of clinical laboratory assay performance. Sigmas can be used to estimate risk of reporting an unreliable patient result because they incorporate both analytical performance characteristics and total allowable error for each assay.

Objective: To evaluate the performance of our new automated chemistry system by calculating sigma metrics over time using several different sources for allowable total error (TEa). **Methods:** Performance of general chemistry methods on four Abbott Architect analyzers was estimated using 12 months of Bio-Rad quality control data from the first year of operation (November 2013 to October 2014) and compared to second year of operation (December 2014 - January 2016). Method imprecision was determined by the cumulative coefficient of variation (CV) of quality controls and percent bias was defined by comparison of laboratory mean to peer group means. Sigma was estimated for each analyzer method as [(TEa - Bias%)/CV%] using allowable total error from three sources; the CLIA evaluation limits, biological variation (Ricós C et.al. Scand J Clin Lab Invest 1999;59:491-500) and the 2011 Australasian Association of Clinical Biochemists allowable limits of performance (RCPA Quality Assurance Programs Pty Limited, Adelaide, Australia). Sigma estimates were averaged among our four chemistry analyzers to compare performance between first and second year of operation. Analysis of NIST SRM1950 standards in January 2016 allowed estimation of true bias versus Abbott Architect control peer group bias.

Results: The average sigma metrics were stable over time between our first and second year of performance for both urine and serum analytes. Lower sigmas were generated with the tighter (Ricós and RCPA) allowable error guidelines compared to

CLIA limits especially for serum albumin, ALT, direct and total bilirubin, calcium, HDL, direct LDL, chloride, creatinine, glucose, potassium, lactate, LDH, sodium, phosphorus, total protein and urine creatinine, glucose and magnesium. Bias estimated from NIST SRM1950 standards gave comparable sigmas to peer group estimates except for serum calcium, cholesterol, magnesium, total protein and urea. Several test methods met optimal sigma performance, >6 sigma, across all three sources of TEa; serum AST, CK, triglycerides and urine chloride, potassium, sodium, phosphate, total protein, and uric acid.

Conclusions: This is the first publication comparing sigma metrics for serum and urine chemistry methods over time. The average sigma is stable over time; however sigma analysis presented several limitations. Sigma estimates vary depending on TEa limits used. Tighter TEa limits lead to lower sigmas. Use of the peer group to estimate bias, may not account for true bias of the method. Sigma estimates vary with concentration of control material. This study also noted sigma variability among analyzers. Averaging sigmas across several analyzers does not account for inter-analyzer variability. This variability makes defining a single control strategy based on sigma estimates from one analyzer or a single control material challenging and unlikely to be acceptable for a group of analyzers across the entire reportable range. Laboratories should consider the lowest individual analyzer sigma estimates when setting control strategies for a group of analyzers rather than utilizing an average.

B-040

Anion gap lactate testing.should not be used to indicate the need for lactate testing.

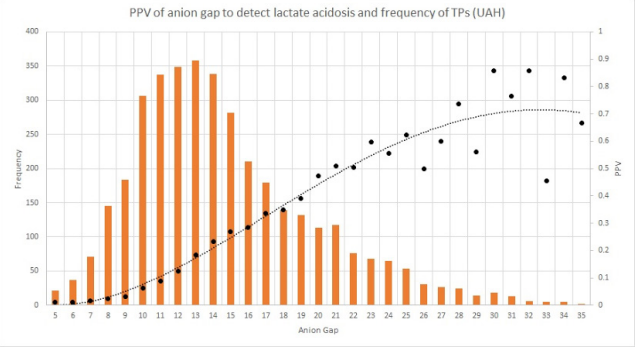
Q. Xu[1], G. S. Cembrowski[2], S. HowlettClyne[2], A. Fuezery[2]. *[1]University of Manitoba, Winnipeg, MB, Canada, [2]Alberta Health Services, Edmonton, AB, Canada*

Introduction: Lactic acidosis represents the pathologic accumulation of lactate and hydrogen ion. High lactates are associated with increased severity of illness; e.g., in septic patients, lactic acidosis is associated with a threefold increase in mortality. It is important to efficiently diagnose lactic acidosis. In emergency departments (ED), serum lactate levels may not be rapidly available, and elevated anion gaps are used to indicate elevated lactates. Most Edmonton metropolitan hospitals have Radiometer blood gas/electrolyte instruments in the ED or close by. As lactate is provided with each electrolyte determination, we could determine the sensitivity of anion gap to detect lactic acidosis.

Methods: Lactic acidosis is defined as a whole blood lactate of 4 mmol or greater. For all of the electrolytes/blood gases ordered in 5 different metropolitan EDs, we determined the positive predictive value of detecting an elevated lactate for each unique anion gap as well as the number of elevated lactates.

Results: Two years of ED lactates and electrolytes from 5 Edmonton metropolitan hospitals were analyzed. The Figure shows for the University Hospital ED a histogram of elevated lactates gathered over 2 years graphed against anion gap (42,311 electrolytes and lactates performed). Also shown is the positive predictive value of the anion gap to detect lactic acidosis. If a physician used an anion gap limit of 13 to trigger the ordering of lactate, approximately 40% of the lactic acidosis would be missed. At a level of 13 mmol/L, the predictive value of an anion gap would be about 30% to indicate lactic acidosis. The graphs for the other 4 Edmonton hospitals are similar.

Conclusions: Anion gap is an inadequate marker of lactic acidosis. We recommend that lactate be done with each set of electrolytes and/or blood gases. In this way lactic acidosis should not be missed.



B-041

Performance Evaluation of an Integrated Multisensor Technology Na, K, and Cl assays on the Atellica CH Analyzer*

J. T. Snyder. *Siemens Healthcare Laboratory Diagnostics, Newark, DE*

Introduction: Measurements of sodium are used in the diagnosis and treatment of aldosteronism, diabetes insipidus, adrenal hypertension, Addison's disease, dehydration, inappropriate antidiuretic hormone secretion, or other diseases involving electrolyte imbalance. Measurements of potassium are used to monitor electrolyte balance in the diagnosis and treatment of disease conditions characterized by low or high blood potassium levels. Chloride measurements are used in the diagnosis and treatment of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis. The Atellica™ CH* Integrated Multisensor Technology (IMT) Na, K, and Cl assays from Siemens Healthcare are intended for the quantitative measurement of sodium, potassium, and chloride (Na, K, Cl) in human serum, plasma, and urine. The objective of this study was to evaluate the performance of the IMT assays on the Atellica CH Analyzer.

Methods: Assay precision was evaluated using Clinical and Laboratory Standards Institute (CLSI), guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3 with Passing-Bablok regression of patient sample results compared with the ADVIA® 1800 Clinical Chemistry System.

Results: For Na, within-lab precision ranged from 0.8 to 1.0% CV. Serum samples ranged from 71.6 to 154 mmol/L and 1.0 to 1.5% CV. Urine samples ranged from 32.2 to 284 mmol/L. For K, within-lab precision ranged from 0.7 to 1.0% CV. Serum samples ranged from 2.70 to 7.31 mmol/L and 0.9 to 1.1% CV. Urine samples ranged from 31.2 to 258 mmol/L. For Cl, within-lab precision ranged from 0.7 to 1.5% CV. Serum samples ranged from 78.1 to 189 mmol/L and 0.8 to 1.7% CV. Urine samples ranged from 42.7 to 280 mmol/L.

In method comparison testing, 106 serum Na samples yielded the following relationship: y = 1.00x - 4.00 mmol/L. 101 urine Na samples yielded the following relationship: y = 1.01x + 0.97 mmol/. 103 serum K samples yielded the following relationship: y = 0.96x + 0.10 mmol/L. 105 urine K samples yielded the following relationship: y = 1.03x - 0.67 mmol/L. 108 serum Cl samples yielded the following relationship: y = 1.00x + 0.00 mmol/L. 102 urine Cl samples yielded the following relationship: y = 0.99x - 0.28 mmol/L.

Conclusion: The IMT assays for the Atellica CH Analyzer enable measurement of sodium, potassium, and chloride (Na, K, Cl) in human serum and urine with excellent precision and accuracy.

*Under development. Not available for sale.

B-042

Blood Collection Device Specific Bias in Hematocrit Measurements

P. V. Pamidi, E. Kovalchick, M. Velankar. *Instrumentation Laboratory, Bedford, MA*

Background: Hematocrit and hemoglobin measurements are routinely measured for assessing risk for anemia in point of care (POC) settings using blood gas analyzers and in central laboratories using either blood gas analyzers or hematology analyzers. Hematocrit measurements in blood gas analyzers are based on electrochemical conductivity measurements and typically use heparin based blood collection devices. Laboratory based hematology analyzers measure hematocrit based on either electrical impedance or light scattering methods using EDTA based blood collection devices. The accuracy of Hematocrit measurements between different analyzers is critical for the commutability of results between POC and laboratory within a hospital system. Since the use of EDTA (hematology) or heparin (POC) based blood collection devices is common, this study aims to understand the impact of EDTA or heparin on hematocrit measurements.

Experimental Data: Blood samples were collected from 6 healthy volunteers in both heparin and EDTA based blood collection tubes. Blood samples from each collection device were run in duplicate on 2 GEM Premier 4000 analyzers for both hematocrit and hemoglobin measurements.

Results: As shown in the Table below, for each of the 6 donors, a systematic negative bias of 4-7% was observed between EDTA and Heparin tubes, with EDTA tubes measuring lower than heparin tubes for hematocrit. However, with hemoglobin measurements, a small random bias of no more than 0.5 mg/dL was observed, indicating that hemoglobin measurements were not affected by the anti-coagulant.

Conclusions: Blood samples collected in EDTA tubes measure 4-7 units low for hematocrit compared to blood collected in heparin tubes. Such bias can cause correlation differences between laboratory hematology and POC analyzers.

Donor#	1	2	3	4	5	6
EDTA - HCT, %	38.5	37.0	38.8	36.3	43.0	44.0
Heparin HCT, %	45.0	42.8	45.0	40.5	49.0	51.0
Delta (EDTA -Heparin)	-6.5	-5.8	-6.3	-4.3	-6.0	-7.0
EDTA tHB, g/dL	14.5	12.6	15.0	13.4	15.9	16.1
Heparin tHB, g/dL	14.5	13.1	15.1	13.7	15.9	16.3
Delta (EDTA -Heparin)	0.0	-0.5	-0.1	-0.3	0.0	-0.2

B-043

Comparison of Electrolyte and Hemoglobin Values between Four Blood Gas Analyzers and Central Laboratory Analyzers

B. Lynn[1], L. Filipova[1], B. B. Suh-Lailam[2]. *[1]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, [2]Ann & Robert H. Lurie Children's Hospital of Chicago/Northwestern University Feinberg School of Medicine, Chicago, IL*

Background: Measurement of blood gases, electrolytes, lactate, hemoglobin and some other analytes are important in the rapid assessment of critically ill patients. These measurements are commonly performed at the point-of-care using blood gas analyzers (BGAs). With the increased availability of BGAs, it is common to find different types of BGAs within an institution. Because different BGAs use different methods, it is important to know how each instrument performs with respect to the reference method for that institution which is the central laboratory analyzer (CLA). This study compares the electrolytes sodium (Na) and potassium (K), and hemoglobin values measured by 4 different BGAs; ABL90 (Radiometer), i-STAT (Abbott Laboratories), GEM4000 and GEM3000 (Instrumentation Laboratory), used at our institution to the CLAs.

Method: A minimum of 40 left over samples collected during cardiovascular surgery (CVS) for blood gas and other analyte evaluations were analyzed by four blood gas analyzers (whole blood-based); electrolyte and hemoglobin values were compared to cobas 6000 (Roche Diagnostics) (plasma-based) and ADVIA 2120i Hematology System (Siemens Healthcare) (whole blood-based) values respectively. Cobas and ADVIA assays were used as the reference methods. In addition 21 excess whole blood samples submitted from different parts of the institution for hematology evaluation were analyzed by i-STAT and ADVIA. Results were compared by Deming regression and Bland-Altman plots.

Results: Correlation between methods for K was good with slopes between 0.97 and 1.06 and intercepts of -0.27 to 0.13 mmol/L for all 4 methods compared with the cobas. Observed bias for K versus cobas for all 4 methods ranged from 0.00 to 0.05 mmol/L. Correlation between methods for Na had slopes between 0.88 and 1.14 and intercepts of -16.4 to15.1 mmol/L for all 4 methods compared with the cobas. Observed bias for Na versus cobas for all 4 methods ranged from 1.1 to 3.3 mmol/L. Correlation between methods for hemoglobin had slopes between 0.94 and 1.16 and intercepts of -2.22 to 0.71 g/dL for all 4 methods compared with the ADVIA. Hemoglobin measurement by GEM3000 showed the biggest bias to the ADVIA at -9.21%, followed by the i-STAT at -4.38%, 2.28% for the ABL90 and 0.54% for the GEM4000. BGAs that use conductivity-based methods for the determination of hematocrit and hemoglobin can be influenced by substantial hemodilution which can happen during CVS. The negative bias observed with the GEM3000 and i-STAT could therefore be attributed to the use of samples from patients undergoing CVS. To test this assumption, 21 non-CVS samples were analyzed by i-STAT and ADVIA. Correlation between the i-STAT and ADVIA hemoglobin was good with a slope of 1.11 and intercept of -0.93 g/dL. The observed bias was 2.88%.

Conclusion: All 4 BGAs showed acceptable performance compared to the cobas for electrolyte measurement. The negative bias observed with i-STAT and GEM3000 hemoglobin results in CVS patients may confound the interpretation of the patient condition including the need for transfusion, if multiple methods are used within the same institution.

B-045

Analytical Performance of Creatinine Methods: A Proficiency Testing Provider Perspective

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Background: The National Kidney Disease Education Program (NKDEP) has developed recommendations to improve the standardization of creatinine measurements. Standard reference materials (SRM 967) and accuracy-based proficiency testing (PT) programs have been invaluable in achieving standardization efforts. The Institute for Quality Management in Healthcare (IQMH) provides ISO 17043:2010 accredited PT programs for creatinine. Data was analyzed for method bias and imprecision to evaluate changes in performance.

Methods: The retrospective review included 79 survey materials that were distributed between May 2005 and May 2015. Spiked human sera were used as PT specimens. Reference results were obtained using an IDMS-traceable measurement procedure. Participant results were assessed against the reference results with pre-determined criteria. Robust statistics based on ISO 13528:2005 were used to calculate peer group means and standard deviations to eliminate the effects of outliers. All participants used commercially-available assays traceable to IDMS.

Results: Creatinine concentrations in PT samples ranged between 0.72-8.94 mg/dL (64-790 µmol/L). Method CV and bias were investigated across 439 peer groups. Median (inter-quartile range) of the peer group CVs and biases are presented in Table 1. Overall, CVs and biases showed a slight negative correlation with reference results (r= -0.297 and -0.203, respectively). Therefore two separate groups were made based on creatinine concentration. Over time peer group biases declined which was more prominent at high concentrations ≥1.1 mg/dL (97 µmol/L). In this group, peer group medians decreased from 6.8% (2005) to 2.4% (2015). Peer group CVs did not change significantly in the group with values ≥1.1 mg/dL, while an increase was observed for values <1.1 mg/dL from 3.6% (2005) to 5.3% (2015).

Conclusion: Biases and CVs demonstrated variation between manufacturers for serum creatinine assays. While imprecision is not yet optimal for all methods, creatinine method bias has improved, most notably for concentrations above 1.1 mg/dL.

Peer group performance for creatinine assays during 2005-2015

	Creatinine <1.1 mg/dL			Creatinine ≥1.1 mg/dL		
Method	N	Median CV (%)	Median Bias (%)	N	Median CV (%)	Median Bias (%)
All Methods	46	5.2 (4.4-6.3)	2.9 (1.2-6.9)	23	3.2 (2.4-4.0)	1.7 (1.2-4.3)
Abbott	37	3.1 (2.2-4.0)	4.7 (2.2-8.5)	30	2.2 (1.7-3.3)	2.8 (1.2-5.4)
Beckman Coulter	46	4.4 (3.7-5.1)	5.8 (3.0-8.6)	30	2.0 (1.9-2.7)	2.0 (0.6-4.6)
Ortho	46	2.5 (1.9-3.2)	4.8 (2.3-9.9)	30	2.0 (1.8-2.3)	2.6 (1.6-5.6)
Roche	32	3.7 (2.6-5.4)	4.7 (2.2-6.2)	22	2.4 (1.8-3.2)	2.3 (0.6-3.5)
Roche (BMC)	42	3.4 (2.8-4.2)	2.5 (1.2-4.9)	28	2.4 (1.8-3.2)	1.6 (0.7-3.8)
Siemens (Bayer)	34	2.6 (1.9-3.8)	4.6 (2.4-8.3)	21	1.7 (1.4-2.3)	2.2 (0.7-3.8)
Siemens (DB)	30	6.2 (4.9-8.3)	7.6 (3.5-10.8)	18	3.5 (1.8-4.8)	3.5 (3.1-4.7)

Wednesday, August 3, 2016

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

Infectious Disease

B-047

Development of alternative diagnostic methods for acute, chronic, and post-treatment phases of *Schistosoma mansoni* infection

V. S. Moraes[1], P. Z. Coelho[1], D. Harn[2], L. V. Siqueira[1], N. B. F. Almeida[1], W. C. Borges[3], R. F. Q. Grenfell[1], L. M. Shollenberger[2]. *[1]Rene Rachou Research Center - Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]University of Georgia, Athens, GA, [3]University of Ouro Preto, Ouro Preto, Brazil*

Background: Schistosomiasis is a serious global public health problem. The standard for diagnosis of infection is the Kato-Katz method, which has low sensitivity and therefore does not work well on patients with low-level infections, representing the majority of cases. Thus, the development of improved diagnostic tests is necessary for disease control. Adding tests such as ELISAs using soluble egg antigens (SEA), increases diagnostic accuracy in low burden endemic and non-endemic areas of Minas Gerais, Brazil. However, crude SEA antigens have low-specificity and cross-react with other helminthes. Therefore, the goal of this work is to identify SEA proteins with high schistosomiasis specificity, as well as the sensitivity to differentiate between active (acute and chronic) and cured (post-treatment phase) infections, in order to develop point-of-care (POC) tests and improve traditional ELISAs. **Methods:** SEA was generated from livers of female Swiss Webster mice 45 days after infection with *Schistosoma mansoni*. Using a protocol approved by the Brazilian Ethical Committee, human serum was obtained in Minas Gerais from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminthes. Fifteen serum samples from each group were pooled and submitted to two-dimensional Western blot (2D-WB) using 60 µg of native SEA. 2D-WB were repeated using sodium metaperiodate (SMP) treated SEA, to denature sugar moieties. The immunoreactive spots were identified by mass spectrometry and analyzed by bioinformatics tools. Promising sequences will be cloned, proteins expressed, and monoclonal antibodies produced. **Results:** A majority of the 23 spots identified by serum from *Schistosoma* infected patients were related to housekeeping proteins (heat shock, energetic metabolism, structural). Among these, 22 spots were identified by serum from patients infected with other helminths, and 9 by negative control samples. One spot was uniquely recognized by sera from *Schistosoma*-infected patients and detection remained after sugar denaturation by SMP treatment, suggesting serum antibodies were binding to peptide epitopes. **Conclusions:** We identified putative new schistosome molecules, which will be produced as recombinant proteins. These proteins will be combined with monoclonal antibodies in the development of highly specific ELISAs and POC immunodiagnostic tests. These tests will be invaluable due to their high specificity and sensitivity as well as their ability to distinguish active and treated cases. Further, the fast, simple POC assay requires minimal equipment and will be an accurate screening tool for epidemiologic surveying in low resource regions.

B-048

Paraoxonase 1 (PON1) and Oxidative Status in Patients with Active Pulmonary Tuberculosis

A. Nagila. *Gandaki Medical College and teaching Hospital, Pokhara, Nepal*

Background: Paraoxonase 1 (PON1), a high density lipoprotein (HDL)-associated esterase/lactonase is a potent antioxidant and its activity is influenced by PON1 polymorphism. Mycobacterial infection induces oxidative stress which might promote tissue injury and inflammation. However, the PON1 activity in Tuberculosis infection remains poorly understood. Therefore, the aim of this study was to investigate the effect of Tuberculosis infection on oxidative status and PON1 activity and to explore the polymorphism of PON1, Q192R, L55M genes in Pulmonary Tuberculosis (PTB) patients.

Methods: A total of 108 (52 newly diagnosed active PTB and 46 healthy control) subjects were recruited from western Regional Tuberculosis Center, Pokhara, Nepal for this study with the mean age of 37.31±1.72 years. Anthropometric variables, Lipid profile, total protein, albumin, uric acid, glycolysed haemoglobin (HbA1c), C reactive protein (CRP), total peroxide, total antioxidant substance, oxidative stress status, PON1 arylesterase and paraoxonase activities were determined in control and PTB subjects. *PON1*, Q192R, L55M polymorphisms were also determined in both healthy controls and PTB patients

Results: Significant difference in BMI, SBP and DBP was observed between PTB and control subjects ($p<0.001$, $p<0.01$ and $p<0.01$, respectively). Total protein, albumin, TC and HDL were significantly lower in PTB subjects in comparision to healthy controls ($p<0.05$, $p<0.001$, $p<0.01$ and $p<0.01$, respectively). Although, HbA1c, TG and LDL levels were also found decreased, were not statistically significant. The level of CRP, uric acid and globulin were significantly increased in PTB patients ($p<0.01$, $p<0.01$, $p<0.001$, respectively). Serum PON1 arylesterase and paraoxonase activity and total antioxidant substance were significantly lower in patients than control ($p<0.05$, $p<0.01$, and $p<0.05$, respectively) while total peroxide level and oxidative stress level were significantly higher ($p<0.01$, $p<0.001$, respectively). In PTB patients oxidative stress level was significantly correlated with PON1 paraoxonase activity ($r= -0.390$, $p< 0.001$). A statistically significant difference in PON1 L55M polymorphism was found between the PTB patients and the control group ($p=0.05$), however, there was no statistically significant difference in PON1 Q192R polymorphism between control and patients group ($p>0.05$). **Conclusion:** Patients with active pulmonary tuberculosis are exposed to potent oxidative stress and have decreased PON1 activity. These predisposing factors might play a role in inflammation and the pathogenesis of atherosclerosis in PTB.

B-049

An Evaluation of Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay at two European Trial Sites.

C. Chandler[1], P. Contestable[2], L. Holm Harritshoej[3], T. Kjerstadius[4]. *[1]Ortho Clinical Diagnostics, Marlow, Buckinghamshire, United Kingdom, [2]Ortho Clinical Diagnostics, Rochester, NY, [3]Rigshospitalet, Copenhagen, Denmark, [4]Centralsjukhuset Karlstad, Karlstad, Sweden*

Objective: To assess the clinical performance (sensitivity and specificity) of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with MicroWell capability, in routine use at two external testing laboratories in Europe. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. The p24 antigen detection is accomplished using monoclonal antibodies (MAbs) against HIV p24. Biotinylated antigen or MAb are pre-bound to microwells coated with streptavidin. Sample is added to the coated wells in the first stage of the reaction and HIV analyte from the sample is captured by the biotinylated proteins. After washing, HRP conjugated envelope proteins and anti-p24 MAbs are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent.

Specificity and sensitivity testing was performed at two external hospital laboratories, one in Denmark and one in Sweden. The Danish hospital laboratory used one assay lot on both a VITROS 5600 Integrated System or a VITROS ECi/ECiQ Immunodiagnostic System. The Swedish laboratory used a different assay lot, run on a VITROS 3600 Immunodiagnostic System. Assay specificity was assessed using 5077 blood donor samples and 607 samples from hospitalised patients. Assay sensitivity was evaluated by running 20 commercially available seroconversion panels and 34 fresh (less than 24 hours old) known HIV positive patient samples. All sample results were compared against results from a commercially available fourth generation HIV Combo assay.

Results: The specificity of the VITROS HIV Combo Assay for the donor population was calculated as 99.84% (5069/5077) 95% exact CI (99.69-99.93 %). The specificity of the VITROS HIV Combo Assay for the clinical population was calculated as 100% (607/607) 95% exact CI (99.39 -100 %).

When used to test 20 commercially available seroconversion panels, the HIV Combo assay was first positive at the same panel member as a commercially available 4th generation assay for 17 of the 20 seroconversion panels and was positive one panel member earlier, for the remaining three panels.

All 34 of the fresh HIV positive samples tested were positive in the VITROS HIV Combo Assay*

Conclusion: The VITROS HIV Combo Assay* provides comparable sensitivity and specificity performance when compared with a commercially available 4th generation assay.

*In Development

B-051

Performance of a Prototype *T. cruzi* Assay* on the VITROS® ECi/ECiQ and 3600 Immunodiagnostics Systems for the Detection of Chagas Disease

S. J. Danielson, T. Grupp, J. Ramerman. *Ortho Clinical Diagnostics, Rochester, NY*

Background / Objective:

Chagas Disease is caused by infection with the blood-borne protozoan parasite *Trypanosoma cruzi (T. cruzi)*. The acute infection can resolve into a chronic, asymptomatic stage during which the infection can still be transmitted. This disease is endemic in many parts of Latin America. We have assessed the performance of a prototype *T. cruzi* Assay* on VITROS Immunodiagnostic Systems.

Method:

Antibody detection in the VITROS® *T. cruzi* Assay* is achieved using antigen coated microwells which capture *T. cruzi* antibodies present in the sample. After addition of the HRP-labeled anti-human IgG and the VITROS® Signal Reagent, the bound HRP conjugate is measured by a chemiluminescent reaction. The signal is compared to a cutoff signal generated using a positive calibrator. All specificity and sensitivity testing was performed using one lot of reagent on a VITROS ECiQ Immunodiagnostic System with the exception of the dilutional, seroconversion, and performance panels which were tested on a VITROS ECiQ and a VITROS 3600 Immunodiagnostic System to compare performance across systems. Assay specificity was assessed using 1361 serum and plasma blood donor samples and 142 samples from individuals with unrelated medical conditions. Assay sensitivity was evaluated by testing 105 serological presumed positive samples from a diverse geographic endemic population, and 2 commercial seroconversion and performance panels. Results from 5 serially diluted positive patient samples were compared to results from the ORTHO® *T. cruzi* ELISA Test System on the ORTHO VERSEIA® Integrated Processor. An endogenous interferent panel was also evaluated. Total within lab precision was evaluated over 20 days in accordance with CLSI EP05-A2 using 1 VITROS 3600 and 1 VITROS ECiQ Immunodiagnostic System.

Results:

The assay specificity was 99.93% (1360/1361, 95%CI: 99.5% - 99.99%) for donor patient samples. Of the patient samples with unrelated medical conditions, 119 of the 122 non-Leishmania samples (97%) were nonreactive and 3 (2%) were repeatedly reactive. 14 of the 20 Leishmania infectious samples (70%) were found to be repeatedly reactive. The assay sensitivity was 100% (105/105; 95% CI: 96.55%-100.0%) with the presumably positive patient panel. The seroconversion and performance panels were found to be reactive on both VITROS Systems at the same bleeds as all of the other commercially available assays. The VITROS *T. cruzi* Assay* generated similar dilutional performance as the Ortho *T. cruzi* ELISA Test System on both VITROS Systems. The assay showed no interference to hemolysis (<500 mg/dL), conjugated and unconjugated bilirubin (<20 mg/dL), total protein (<100 g/L), and triglycerides (<500 mg/dL). Within-lab precision of the assay ranged from 7.74 to 10.61 %CV above the cutoff (>1.0 S/C) and 10.06 to 12.7 %CV below the cutoff on both VITROS Systems.

Conclusion:

The VITROS *T. cruzi* Assay* has been demonstrated to be sensitive and specific and has acceptable precision. Results are comparable to the ORTHO *T. cruzi* ELISA Test System, which received FDA approval in 2006.

* Under Development

B-052

Can Medicare and Medicaid Claims Data be used for Influenza Activity Surveillance?

l. fan[1], r. astles[1], h. burkom[2]. *[1]CDC, ATLANTA, GA, [2]Johns Hopkins University, Baltimore, MD*

Objective: To investigate the utility of claims data from the Centers for Medicare and Medicaid Services (CMS) as early indicators of increased influenza incidence. We evaluated diagnosis-based counts of selected claims data as influenza activity indicators.

Methods: We compared CMS claims data, including Medicare outpatient (OTP), physician office visits (Carrier) and Medicaid (MAX) data, with the National Respiratory and Enteric Virus Surveillance System (NREVSS) data from CDC as the "gold standard" for flu activity. Our statistical comparison used 2007-2012 claims data from 10 states, representing each HHS region. We calculated correlations of weekly time series of counts of CMS test orders and diagnosis counts with gold standard time series. Regarding the uncertainty of diagnosing influenza and seasonal expectations, we tried three separate case definitions to classify influenza-related CMS diagnoses: CD1=Specific code for influenza, CD2=CD1 or code for any influenza-like illness, CD3=CD2 or unspecified viral infection.

Results: Table 1 shows significant ($p<0.01$ throughout) correlation coefficients (CC) between CMS and gold standard time series, with bold font for values ≥ 0.7. All states' OTP flu test count CCs exceeded 0.75. Diagnosis count CCs for CMS were less consistent, with OTP tracking the gold standard better than Carrier or MAX data. The OTP CCs exceeded 0.7 in nine of ten states for CD1 and in seven states for less specific CD2.

Conclusions: Correlations with gold standard influenza data support the surveillance utility of CMS flu test data when physicians are ordering tests. Diagnosis code count correlations based on influenza-like illness and unspecified infection codes indicate utility even when physicians do not expect or test for influenza. Understanding of differences in insurance coverage and reimbursement practices is needed to clarify state-specific utility. The approach for creating/validating case definitions is applicable to investigations of electronic health record data for surveillance of multiple disease outcomes.

Table 1. Correlation coefficients (**bold font** for values ≥ 0.7) between weekly time series of CMS claims data and gold standard reference surveillance data (NREVSS)

State	CMS/gold standard corr. coeffs. based on weekly test count volumes			CMS/gold standard correlation coefficients based on diagnosis codes for three CMS data types, three case definitions								
				Carrier			OTP			MAX		
	Carrier	OTP	MAX	CD1	CD2	CD3	CD1	CD2	CD3	CD1	CD2	CD3
CA	0.64	**0.86**	**0.84**	0.51	0.51	0.48	**0.72**	**0.77**	0.69	**0.83**	**0.81**	**0.82**
CO	**0.77**	**0.76**	**0.76**	0.56	0.44	0.41	**0.74**	0.63	0.67	0.60	0.58	0.61
FL	**0.73**	**0.81**	**0.73**	0.6	0.57	0.53	**0.80**	**0.74**	**0.78**	**0.77**	**0.74**	**0.71**
IA	**0.85**	**0.84**	**0.75**	0.68	0.50	0.43	**0.78**	**0.71**	**0.73**	0.52	0.50	0.51
IL	**0.77**	**0.80**	0.48	0.26	0.14	0.14	**0.82**	0.67	**0.73**	0.59	0.50	0.56
MA	**0.75**	**0.78**	**0.74**	0.48	0.36	0.35	0.65	0.55	0.66	0.51	0.45	0.58
NY	**0.81**	**0.85**	**0.82**	0.47	0.39	0.34	**0.79**	**0.74**	**0.82**	**0.81**	**0.74**	**0.81**
PA	**0.85**	**0.86**	**0.88**	0.64	0.52	0.46	**0.84**	**0.83**	**0.82**	**0.81**	**0.81**	**0.82**
TX	**0.77**	**0.84**	**0.76**	**0.7**	0.59	0.56	**0.84**	**0.83**	**0.79**	**0.84**	**0.82**	**0.82**
WA	**0.84**	**0.85**	**0.79**	**0.73**	0.63	0.62	**0.8**	**0.77**	**0.83**	**0.78**	**0.75**	**0.77**
Overall	**0.76**	**0.77**	**0.74**	0.54	0.50	0.42	**0.71**	**0.71**	0.64	**0.79**	**0.76**	0.63

B-053

Simultaneous detection of zika, chikungunya and dengue viruses in EDTA-plasma samples by RT-qPCR: If their vector is versatile their detection assays also should be

G. Barra, T. Santa Rita, P. Mesquita, R. Jácomo, L. Abdalla, S. Costa. *Laboratório Sabin, Brasília, Brazil*

Background:

Currently, multiple arbovirus are circulating in Brazil: Zika, chikungunya and dengue. They have similar clinical pictures, which can lead to misdiagnosis based on clinical grounds. RNA detection tests such as the RT-qPCR can reliably and specifically distinguish the three viruses and the specific diagnosis can be important in anticipating, preventing, and managing complications. Thus, the aim of the present study was to validate a RT-qPCR assay for simultaneous detection of these 3 viruses.

Methods:

This validation enrolled 90 EDTA-plasma samples from the arbovirus laboratory routine, 20 positive for zika (RT-qPCR), 6 positive for chikungunya (RT-qPCR), 18 positive for dengue (NS-1) and 46 negative for all 3 viruses. Nucleic acids were extracted from 1mL of sample by using an automated DNA extractor. An in-vitro transcribed random RNA sequence, which is not found in the nature, was spiked into plasmas during the nucleic acids extraction to function as a process control. Primers/probes for chikungunya were specifically designed for this study. Primers/probes for zika and dengue were obtained from literature. Zika, chikungunya and dengue viruses were assessed simultaneously by RT-qPCR, but in independent reaction wells. The control RNA was co-amplified in all instances. The viral loads of specific samples were quantified against a serial dilution of synthetic ssDNA and the limits of detection of each assay were determined by probit regression analysis (serial dilutions of each viral material from ~500 to ~0.5 copies/mL). To investigate the precision of the assays, three samples at ~72, ~7.2 and ~0.72 copies/mL of each viruses were evaluated by using the CLSI EP12-A2 method during 5 days in quadruplicate by two operators. The assays accuracies were evaluated by the agreement of the proposed RT-PCR with NS-1 assay for dengue and a second set of primers/probes for chikungunya and zika.

Results:

The limits of detection were 26 copies/mL (95%CI 14-89 copies/mL) for zika, 23.5 copies/mL (95%CI 13-81 copies/mL) for chikungunya and 25.6 copies/mL (95%CI 14-85 copies/mL) for dengue. The ~72, ~7.2 and ~0.72 copies/mL samples yielded 18/20 (90%), 2/20 (10%) and 1/20 (5%) positive results for zika, 18/20 (90%), 4/20 (20%) and 0/20 (0%) positive results for chikungunya and 20/20 (100%), 16/20

(80%) and 0/20 (0%) positive results for dengue, respectively. The total, positive and negative agreements between compared methods were 95.5% (95%CI 89-98%), 90% (95%CI 70-97%) and 97.5% (95%CI 90-99%) for zika, 100% (95%CI 95-100%), 100% (95%CI 61-100%) and 100% (95%CI 95.6-100%) for chikungunya and 95.6% (95%CI 89-98%), 100% (95%CI 81.5-100%) and 94.5% (95%CI 86.7-97.8%) for dengue, respectively. No cross-reaction was observed.

Conclusion:

The proposed RT-PCR method for simultaneous detection of zika, chikungunya and dengue viruses is highly sensitive, all assays showed limit of detection below 50 copies/mL. Moreover, cut-off regions were characterized and acceptable precisions were observed for positive (~72 copies/mL and above) and negative (~0.72 copies/mL and below) results. Finally, the agreements with the comparative methods were very good, above 90% of concordance in all instances. The main drawback of the study was that only 6 chikungunya samples were available.

B-054

Prevalence and Genotype Distribution of Cervical Human Papillomavirus in Korean Women

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Background:Persistent infection of human papillomavirus(HPV) can cause the cervical intraepithelial lesions and carcinoma. The prevalence and genotype distribution of HPV infection have been known to be different among the geographical regions. For the development of strategies for prevention of cervical cancer, it is important to clarify the prevalence of HPV infection and genotype distribution in their own population. This study was performed to evaluate the prevalence and genotypic distribution of HPV infection according to the age and cervical cytological findings in Korean women.

Methods:A total of 18,815 health examinees who were selected for this study, who took both tests of cervical cytology and HPV genotyping using multiplex PCR for screening of cervical cancer in 16 health promotion centers of 13 cities in Korea. The twenty-eight HPV genotypes were divided into two categories; high-risk HPV (carcinogen such as HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 69, and probable carcinogen such as HPV-68, 26, 53, 66, 70, 73, and 82), and low-risk HPV (HPV-6,11, 40, 42, 43, 44, 54, and 61).

Results:The overall HPV prevalence was 27.8%, with 22.2% of high risk HPV (HR-HPV), and 11.4% of low-risk HPV (LR-HPV). Among HR-HPV genotypes, HPV-53 was the most frequent (3.8%), followed by HPV-52(3.2%), HPV-70(3.2%), HPV-68(3.0%), HPV-58(2.7%), and HPV-16(2.0%). The prevalence of overall HPV, and HR-HPV infection increased along with the severity of cervical cytological findings (*P* for trend <0.001). The proportion of HR-HPV in relation with the age and cervical cytological findings, showed U-shape curve, with being the highest at age below 30 year-old, declining gradually down to the bottom at the age between 50-59, and then increasing afterwards in ASCUS/LSIL (*P*=0.001).

Conclusion:The prevalence and distribution of HR-HPV were different according to the age and cervical cytological findings, which provides a genotypic support for more effective screening of cervical cancer and appropriate vaccination.

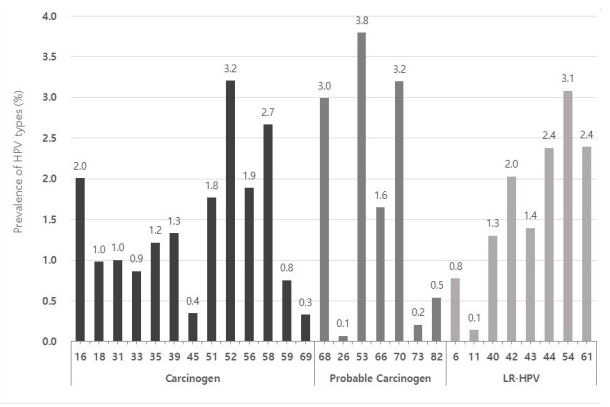

Fig. 1. Prevalence of HPV types in 18,815 Korean women.

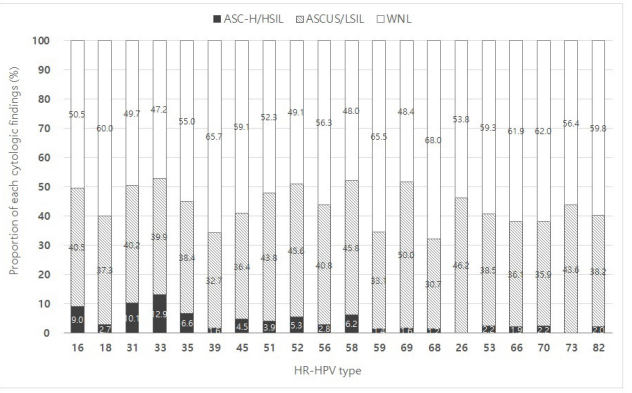

Fig. 2. Proportions of cervical cytology findings in each HR-HPV type.

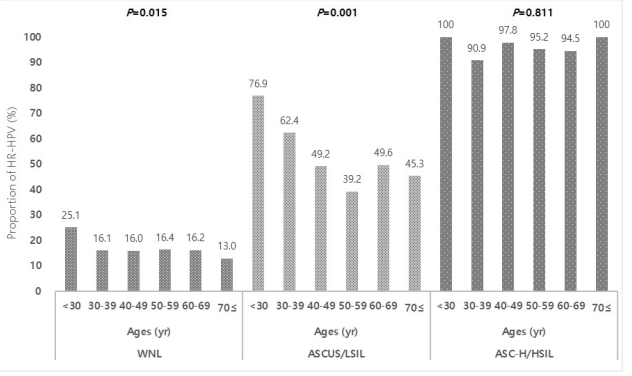

Fig. 3. Proportions of HR-HPV according to age groups and cervical cytology findings.
P for trend derived from Mantel-Haenszel test.

B-055

HIV-1 and HCV sequence-based genotyping methods validated against TRUGENE commercial kits.

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Background: The HCV and HIV-1 genotype determination have clinical importance because the response to antiviral treatment varies with the genotype. Furthermore, the classification of HIV-1 subtype and HCV genotypes provides a scientific opportunity to study the worldwide spread of these viruses. Sequence-based genotyping is the method that is most widely used to genotype. Some laboratories use in-house sequencing, but a number of commercial assays are also extensive used. **Methods:** Two genotyping assays were validated: one for HIV-1 and another for HCV genotyping in human plasma samples. For both assays, the viral RNA was extracted using the Abbott molecular m2000 sample preparation instrument. The HIV-1 genotyping assay

included two regions of the HIV-1 virus RNA: *env* gp41 immunodominant and *pol* IN. The sequences were amplified by RT-PCR using specific primers for each one. The *env* gp41 immunodominant region was amplified using the forward primer, JH35F, and the reverse primer, JH38R. The sequences from the *pol* IN region were obtained using primers poli5_OF and poli8_OR, followed by a nested PCR using primers poli7_IF and poli6_IR. Samples with low viral loads (< 500 copies/ml) were analyzed using an alternative protocol designed to amplify the *env* gp41 immunodominant region. Reverse transcription and primary PCR were performed using primers GP40F1 and GP41R1. Primers GP46F2 and GP47R2 were then used for the nested PCR. The HCV genotyping assay was carried out using sequences obtained from the NS5b region. The sequences were amplified by RT-PCR using the specific primers Pr1 and Pr2. Alternatively a hemi-nested PCR was carried out using primers Pr3, Pr4 and Pr5. The obtained sequences were blasted against available HIV and HCV sequence databases. **Results:** Results were validated against previously obtained results from the TRUGENE HIV-1 genotyping kit and TRUGENE HCV 5'NC genotyping kit. All 20 samples (100%) for the HIV-1 subtypes showed concordant results in both methods. However, at sub-subtype level, one sample was not coincident showing an F sub-subtype with TRUGENE method and an F2 sub-subtype in this study. The HCV genotypes determined by the two methods were concordant in 100% (21/21) of the samples but the results differed at subtype level (15/21). On the other hand, the NS5b method was able to classify all samples at subtype level whereas the TRUGENE HCV 5'NC genotyping kit did not in four cases (20%). **Conclusion:** It is concluded that these methods are reliable and convenient for HIV-1 and HCV genotyping. Moreover, the HIV-1 genotyping assay permitted subtype identification in samples with a low viral load and the HCV genotyping assay showed more resolution at the subtype level in comparison with TRUGENE methods.

B-056

Clinical application of a rapid POC immunoassay for Serum Amyloid A (SAA)

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Background:

Serum amyloid A (SAA), an apolipoprotein of high density lipoprotein (HDL) particles, is the precursor of AA fibrils in reactive amyloidosis. It is a sensitive acute-phase protein, which has been shown to have diagnostic utility in infections, unstable angina and in pediatric diseases. To permit early detection of these conditions, a rapid POC assay for the determination of SAA in plasma or whole blood samples was developed. The objective of this study is to demonstrate the clinical utility of the SAA determination using S-SPOT (Upper Biotech, Shanghai, PRC) which provides results in three minutes.

Methods:

Clinical samples were analyzed in parallel by both S-SPOT and a commercially available latex-enhanced rate nephelometry immunoassay for SAA, using samples from both healthy adults and patients. The S-SPOT method was calibrated using the WHO standard (92/680). Whole blood samples were measured on S-SPOT. Plasma samples derived from these specimens were re-assayed on S-SPOT to determine the impact of using whole blood as a sample material on S-SPOT. Samples from children with bacterial infection (n = 386) and with a viral infection (n = 219) were also assayed.

Results:

a. Healthy Subjects

The median of serum SAA from 240 normal healthy adults was 5.7mg/L, and the 95th percentile was 0-9.6mg/L.

b. Serum samples from patients

SAA values for patients ranged from 5 to over 1,700mg/L on S-SPOT. The study showed very good correlation between the S-SPOT and a commercially-available SAA test. A linear regression analysis showed y (S-SPOT) =1.134x+1.836; r^2=0.961, n=118.

c. Whole blood vs Plasma

The results obtained using whole blood correlated well with those obtained on the corresponding plasma samples. Linear regression: y (Plasma) =1.096x-2.704; r^2 = 0.976, n = 41.

d. SAA and CRP

SAA values for the pediatric patients were significantly increased compared to the values found for the classic and highly sensitive acute-phase reactant, C-reactive protein (CRP). The SAA values in patients with viral infections (Influenza virus, respiratory syncytial virus, adenovirus, etc.) were significantly higher than those in the control group ($P<0.05$) while the CRP in these patients showed no significant difference ($P>0.05$).

In viral infections, using CRP in conjunction with SAA improves diagnostic efficiency slightly but is significantly better than CRP alone. (AUC: SAA alone 0.83, SAA plus CRP 0.85, CRP alone 0.54).

Conclusion:

In most circumstances the serum concentration of SAA correlates well with that of CRP, but SAA reaches higher concentrations and may respond more rapidly. SAA results obtained by S-SPOT showed excellent discrimination between healthy adults and patients with viral and bacterial infections. Whole blood can be used on S-SPOT, saving the centrifugation step. S-SPOT is a reliable, rapid quantitative test which takes less than 3 minutes and is especially useful in pediatric care and emergency situations.

B-057

Comparison of serologic Lyme testing with the Immunetics C6 ELISA, EUROIMMUN EUROLINE-WB and Viramed Biotech ViraStripe assays

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Objective:

The objective of this study was to compare the performance of the EUROIMMUN Westernblot Anti-Borrelia Burgdorferi US EUROLINE-WB IgG assay with an integrated recombinant VlsE (variable major protein-like sequence, expressed) chip to that of the Immunetics® C6 B. burgdorferi ELISA™ Kit. A secondary goal was to determine if utilization of the VlsE antigen chip could replace the use of the current Immunetics C6 ELISA. Evaluation of the EUROIMMUN Westernblot Anti-Borrelia Burgdorferi US EUROLINE-WB IgG and IgM assays compared with the Viramed® Biotech AG - Borrelia B31 IgG and IgM ViraStripe® assays was also performed.

Methodology:

The Immunetics C6 ELISA (Immunetics, Inc., Boston, MA) utilizes a synthetic C6 peptide, derived from the VlsE protein of B. burgdorferi, bound to the wells of a microwell plate. Antibodies in the patient serum are captured by the immobilized antigen and then detected by the addition of a horseradish peroxidase-conjugated goat anti-human IgG/IgM conjugate. Incubation with a chromogenic peroxidase substrate results in color development, which is then read via a spectrophotometer.

The EUROIMMUN EUROLINE-WB IgG and IgM assays (EUROIMMUN AG, Luebeck, Germany) contain test strips with antigen extracts of B. burgdorferi. Test strips also contain a membrane chip coated with B31 antigens and recombinant VlsE antigen (IgG). Antibodies present in the patient serum bind to the antigens on the strip and are detected using an enzyme-labeled anti-human IgG/IgM which in the presence of substrate results in color development.

The Viramed Biotech IgG and IgM ViraStripe assays (Viramed Biotech AG, Planegg/Steinkerchen, Germany) contain specific B31 antigens that are bound to a solid phase nitrocellulose support membrane. Antibodies present in the patient serum bind to the antigens on the strip. Enzyme labeled anti-human IgG/IgM is added to detect bound antibody. Substrate is added which undergoes a color change.

Results:

Two-hundred and forty-nine serum specimens were available for testing by all methods. Comparison between the Immunetics C6 ELISA and the EUROIMMUN EUROLINE-WB IgG intregrated VlsE chip assay demonstrated an overall agreement of 91.7% with 96.8% of the positive specimens and 90.0% of the negative specimens demonstrating agreement between the methods. The Immunetics ELISA called more specimens positive while the EUROIMMUN assay generated a greater number of borderline results. These same specimens were also analyzed by the Viramed Biotech IgG and IgM ViraStripe assays and the EUROIMMUN EUROLINE-WB IgG and IgM assays. An overall agreement of 85.1% was observed between the IgG WB assays while the IgM WB assays gave an overall agreement of 76.3%. Both WB assays demonstrated good negative agreement at 95.2% and 98.8% for IgG and IgM, respectively with lower rates of positive agreement at 54.8% and 36.0%, for IgG and IgM, respectively.

Conclusion:

Findings from this study demonstrate that testing performed utilizing a recombinant VlsE chip is generally comparable to that provided by current screening assays. The assays employed for WB analysis demonstrate good agreement when ruling out Lyme disease, however confirmation of disease via positive results was less definitive.

B-059

Incidence study of sexually transmitted infections in asymptomatic patients and association with cervical intraepithelial neoplasia.

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Cervical carcinoma is the third most common cancer among women worldwide and most cases in developing countries. The carcinogenic human papillomavirus (HPV), a highly prevalent sexually transmitted infection (STI), is a necessary cause for the development of cervical intraepithelial neoplasia (CIN) and cervical carcinoma. However, among women infected with high-risk HPV, only some will develop CIN/ cervical carcinoma, suggesting that carcinogenic process following HPV infection and CIN is likely influenced by biological cofactors, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Mycoplasma hominis,* and *Mycoplasma genitalium* infection that are often asymptomatic infections. Epidemiological studies about the impact of these infections on HPV acquisition and development to CIN or cervical carcinoma have yielded equivocal results due to the difficulty in separating biological from behavioral effects. The aim of this study was to investigate the incidence of HPV, *C. trachomatis*, *N. gonorrhoeae*, *U. urealyticum*, *M. hominis,* and *M. genitalium* in sexually active women and to evaluate the association these infections with CIN. A total of 126 patients was subjected to collection of cervical cytology. The samples (vaginal swabs) were examined by Real Time PCR and TaqMan detection system with specific primers and probes for each infectious agent. For the statistical analysis, the chi-square test was used considering the 0.05 significance level, and a multivariate analysis was performed by logistic regression. The results obtained showed that incidence of STIs was HPV 46.8%, *C. trachomatis* 27.8%, *M. genitalium* 28.6%, *M. hominis* 0.8%, *U. urealyticum* 4.8%, *N. gonorrhoeae* 4.8%. HPV (p= 0.024), *C. trachomatis* (p= 0.009), *M. genitalium* (p= 0.040) infection. Coinfection with HPV and *C. trachomatis* (p= 0.023), and HPV infection associated with the presence of at least one STI (p= 0.011) were associated with CIN. After multivariate analysis, a positive association was found between HPV and CIN (p= 0.040, OR= 2.48, CI: 1.04-5.92) and between *C. trachomatis* and CIN (p= 0.028, OR= 2.69, CI: 1.11-6.53). Therefore, in our study HPV, *Chlamydia*, *M. genitalium* infection and coinfection with HPV and *Chlamydia* and HPV infection associated with the presence of at least one STI constitute significant risk factors for the occurrence of CIN. Although these findings did not significantly differ by HPV genotype, the high frequency of these infections in asymptomatic women confirms that the inclusion of a diagnostic screening for detection of HPV in patients with STIs seems to be relevant in clinical and laboratory routine.

B-060

Comprehensive Clinical Evaluation of BacT Alert 3D Resin Bottles for Blood Cultures

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Background: We prospectively evaluated the performance of blood culture resin media: FA Plus and FN Plus of BacT Alert 3D System (bioMerieux) in a tertiary university-affiliated hospital in 2014. Resin may enhance the growth of microorganisms by adsorbing antibiotics in the blood sample. Positivity and time to detection (TTD) is the key indicator to assess the performance of blood culture system. TTD is defined by the time interval from the entry to the automatic blood culture machine to the positive signal of microorganism.

Methods: We obtained 2,994 blood culture sets. Ten ml of venous blood were collected for each puncture and distributed into each bottle evenly. Blood culture media were immediately entered in the BacT Alert 3D System. We categorized the bottles into two groups based on the prior antibiotic treatment. Terminal subculture was performed for the signal-negative bottles after 5 days of incubation to evaluate the false negative in the blood culture machine. Positivity and TTD of microorganisms were analyzed for the clinically significant microorganisms.

Results: Of 2,994 sets received, 371 sets (12.4%) yielded 385 clinically significant pathogens. There was no statistically significant difference between prior antibiotic users (13.1%, 65/498) and antibiotics naïve patients (12.3%, 306/2,496) (P>0.05) in the positivity. Growth was observed in 0.35% (9/2,623 sets) in the terminal subculture. *Staphyloccus aureus*, gram-positive cocci, and all microorganisms were detected significantly faster in FA Plus (13.81 h, 14.09 h, and 11.12 h, respectively) than in FN Plus (14.82 h, 15.60 h, and 11.88 h, respectively). Otherwise, there was no significant difference in TTD for Gram-negative rod and yeasts. The distribution of the TTD was noteworthy. Over 88% of positive signals were encountered in the first 24 h. More than 97% were detected in both FA and FN bottles within 48 h. Within 72 h, 99.7% were detected in FA Plus and 99.0% in FN Plus.

Conclusions: Detection of microorganisms was equally complementary between FA Plus and FN Plus. Gram-positive cocci including *S. aureus* grew earlier in FA Plus. The positive rate was not affected by prior antibiotic therapy in BacT Alert 3D resin media. False-negative in the machine seems negligible. Since 99.0% of TTD were detected within within 72 hours, laboratories with limited resources could apply 3 day incubation protocol instead of current 5 day incubation.

B-061

Evaluation of Performance of Enzyme Linked Fluorescent Assay, Multiplex PCR and Real time PCR for Detection of Clostridium difficile

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Background: *Clostridium difficile* causes antibiotic-associated diarrhea and pseudo-membranous colitis. *C. difficile* generates toxin A (enterotoxin) and toxin B (cytotoxin) which are the major factors of *C. difficile* associated diarrhea (CDAD). These toxins or toxin genes are the targets for the laboratory diagnosis of CDAD. We evaluated the performance of variable detection methods of *C. difficile*.

Methods: We obtained 354 clinical fecal specimens which were requested for *C. difficile* toxin tests in 2014. The enzyme linked fluorescent assay (ELFA), VIDAS C. difficile Toxin A & B (VIDAS CDAB; bioMerieux, France) was used for the toxin tests. Toxin B gene was detected using multiplex PCR, Seeplex Diarrhea ACE Detection panel B1 (Seegene, Korea) and both toxin A and toxin B genes were detected by Advansure CD real time PCR (LG Life science, Korea). Positive rate and concordance rate of each tests were compared.

Results: Among 354 stool specimens, 38 (11.4%) were positive by VIDAS assay, 60 (16.9%) by PCR, and 82 (23.2%) by real time PCR. In comparison of real time PCR with VIDAS, positive concordance rate was 100%, negative concordance rate was 89.1% (P<.001), and discordance rate was 9.7%. Between PCR and VIDAS results, positive concordance rate was 84.2%, negative concordance rate was 93.4% (P<.001), and discordance rate was 7.6%. When real time PCR was compared with PCR, positive concordance rate was 93.3%, negative concordance rate was 91.2% (P<.001), and discordance rate was 8.5%.

Conclusion: Comparative evaluation of three methods showed that real time PCR was a relatively appropriate test to detect *C. difficile* since it is fast and convenient with the highest detection rate. Real time PCR and VIDAS showed the highest concordance rate.

B-062

Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens

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Background: Prompt diagnosis of central nervous system (CNS) disease is critical to guide intervention and appropriate therapy. Development of novel laboratory approaches to rapidly classify acute-onset CNS disease is in great demand. Serious microbial pathogens, especially viruses, are quickly expanding beyond their historic geographic range and may not even be considered in the clinician's differential diagnosis. Unlike bacterial cultures, current viral testing targets a limited number of viruses. Additionally, despite diversity in etiology, signs and symptoms of both infectious and non-infectious CNS disorders can be remarkably similar, which can confuse the clinical picture and delay treatment. Bacterial, viral, fungal and parasitic CNS pathogens are sensed by pattern recognition receptors of the immune system, stimulating immediate release of measurable levels of chemokines and cytokines

into the CSF. Our objective is to use pathogen-specific chemokine/cytokine profiles to classify CNS disease as infectious versus non-infectious and further discriminate between viral and non-viral infections. **Methods:** Levels (pg/ml) of chemokines and cytokines were determined in the CSF of 45 patients with documented infectious meningitis or meningoencephalitis (mean age 19.2 years) and in the CSF of 25 patients who were negative for CNS infection (mean age 27.4 years). MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panels (Millipore) were used to measure CSF chemokines and cytokines levels (pg/ml). Innate immune analytes quantified included IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L. Samples were analyzed in duplicate by a FlexMAP 3D (Luminex). Standard curves were generated for each cytokine and median fluorescent intensities were transformed into concentrations by 5-point, non-linear regression. For univariate analysis, comparisons between groups were made using the Mann-Whitney test. We utilized receiver operating characteristic (ROC) curve analysis to calculate areas under the ROC curve (AUC) for each analyte to access the utility of chemokine/cytokine levels as discriminating tests. The ROC generated sensitivity and specificity values were then used to determine clinically optimal cutoff values for the informative analytes. **Results:** Univariate analysis utilizing Mann-Whitney tests demonstrated that median values (pg/ml) of IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L were all significantly higher in CSF from patients with infectious brain disorders than in CSF from patients with non-infectious disorders (p-value < 0.05). MDC (CCL22) demonstrated statistical significance, when comparing viral infections versus non-viral infections (with the non-viral infection group having higher analyte levels). IP10 (CXCL10) can reliably distinguish between an infectious versus non-infectious CNS process (AUC 0.9778) with an optimal cut-off value of 2023 pg/ml (sensitivity, specificity; 93.0%, 92.0%). In the infectious group, MDC (CCL22) can reliably differentiate between viral and non-viral CNS infection (AUC 0.9545) with an optimal cut-off value of 194 pg/ml (sensitivity, specificity; 91.67%, 87.88%). **Conclusion**: CSF levels (pg/ml) of IP-10 (CXCL10) can reliably distinguish infectious versus non-infectious CNS disorders, and in the infectious group, MDC (CCL22) can reliably distinguish between viral and non-viral CNS infections. These results suggest that CSF chemokine/cytokine quantification can serve as a useful laboratory tool for the rapid triage of CNS diseases to help guide prompt therapy and further testing.

B-063

Next Generation Sequencing-based HIV-1 Drug Resistance Monitoring System

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Background: The most common cause for therapeutic failure in people infected with Human Immunodeficiency Virus (HIV) is the resistance of HIV to antiretroviral drugs. Objective of this study was to compare two sequencing-based HIV-1 drug resistance monitoring systems: an CLIP-based system (TruGene HIV-1 Genotyping Kit) and a novel Next Generation Sequencing (NGS)-based test (*Sentosa* SQ HIV-1 Genotyping Assay).

Methods: We used a newly developed automated NGS-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction and NGS library preparation (*Sentosa* SX101); 2) Ion Torrent instruments for template preparation and deep sequencing; 3) kits for RNA extraction, HIV NGS library preparation, template preparation and deep sequencing, and 4) data analysis and reporting software. Reporting includes 86 Drug Resistance Mutations (DRMs) across the Reverse Transcriptase (RT), Protease (PR) and Integrase genes. 111 prospective EDTA plasma clinical samples from patents infected with HIV-1 were tested for this study.

Results: All 111 HIV-1 positive samples were tested on both systems. 97.3% (108/111) samples were subtyped as CRF01_AE. In total, 647 DRMs were detected (435 in the RT gene, 199 in the PR gene and 13 in the Integrase gene). The *Sentosa* SQ HIV Genotyping Assay detected 100% (199/199) of all DRMs in the PR gene and more that 98% DRMs (427/435) in the RT gene. The Integrase gene was not included into the comparison study because it is not covered by the TruGene test. In total, 130 DRMs were detected by the *Sentosa* SQ HIV Genotyping Assay, that were not found by TruGene and 8 DRMs were missed by the *Sentosa* HIV Genotyping Assay (but detected by TruGene). Mutation detection rate for the HIV PR gene was 100% (95%CI: 98.11-100%) for the *Sentosa* SQ HIV Genotyping Assay and 90.45% (95%CI: 85.57-93.80%) for the TruGene system. In the RT gene 98.16% (95%CI: 96.41-99.07%) of DRMs were recorded by the *Sentosa* SQ HIV Genotyping Assay and 74.48% (95%CI: 70.18-78.35%) by TruGene. Overall DRM detection rates aggregated were 98.74% (95%CI: 97.53-99.36%) for the *Sentosa* SQ HIV Genotyping Assay and 79.5% (95%CI: 79.02-79.62%) for the TruGene HIV-1 Genotyping Kit. All HIV strains were carrying 1 or multiple DRMs in 61, 16 and 9 AA positions of the RT, PR and Integrase genes respectively. The most prevalent DRMs in the RT gene were: M184V was present in 48,7% (54/111) of the samples, K103N in 29.7% (33/11), Y181C in 27,9% (31/111), G190A and D67N (both 18.9% (21/111)). In the PR gene: M36I 91.9% (102/111), K20R 21.6% (24/111) and L10I 20.7% (23/111).

Conclusion: Timely detection and reporting of DRMs is critical for drug regiment and can minimize the development of resistance to antiviral drugs. In this perspective the NGS-based workflow appears as a promising new tool for detecting clinically relevant variants in HIV-1. Given its high sensitivity (up to 5% mutation frequency) compared to Sanger sequencing-based systems and the comparatively short turnaround time of 2.5 days the workflow provides comprehensive, clinically relevant information for optimal selection of HIV treatment regimens.

B-064

HCV Genotyping and Resistance-Associated Variants Detection Using Next-Generation Sequencing

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Background: Both conventional interferon based regimens as well as the increasingly used direct acting antivirals (DAAs) provide current treatment options for HCV. Accurate genotyping remains a prerequisite for IFN based treatment. However, with regard to DAAs one of the most important considerations is the potential development of Resistance-Associated Variants (RAVs) that may negatively affect sustained virological response. Timely detection and reporting of RAVs and HCV genotypes (GTs) is critical for drug regiment and can minimize the development of resistance to antiviral drugs. In this study we investigated the frequency of RAVs across HCV GT1, which is highly prevalent across geographic regions.

Methods: 346 prospective and retrospective EDTA-plasma and clinical serum samples from patents with chronic HCV infection across all 6 HCV GTs were tested in this study. We used a line probe-based test (VERSANT HCV Genotype 2.0 LiPA) in conjunction with the AutoBlot 3000H platform (SIEMENS) and a novel automated Next Generation Sequencing (NGS)-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for RNA extraction and NGS library preparation (*Sentosa* SX101); 2) Ion Torrent technology for deep sequencing; 3) kits for nucleic acid extraction and NGS library preparation (*Sentosa* SQ HCV Genotyping Assay) and deep sequencing, respectively, and 4) data analysis and reporting software. The data reports on GTs 1a and 1b include 136 known RAVs in the NS3, NS5A and NS5B genes. Sanger sequencing was used as a reference method for all discordant and indeterminate samples.

Results: All 346 samples were tested on both platforms. For 47/346 (13.6%) samples GT results by VERSANT were "indeterminate". In 19/299 (6.4%) of the samples, discordant results between the two methods were obtained. The ability to correctly determine HCV genotypes was 93.7% (95%CI: 90.3-95.9%) for VERSANT and 100% (95%CI: 98.7-100%) for *Sentosa* HCV Assay. Sanger sequencing confirmed that all 19 discordant samples were incorrectly classified by line probing. GT distribution among the 47 samples indeterminate by VERSANT was: 5 GT1a, 1 GT2, 19 GT3, 1 GT4, 20 GT6 and 1 mixed infection (GT2 and GT3). Clinical sensitivity aggregated was 86.4% (95%CI: 82.4-89.6%) for VERSANT and 100% (95%CI: 98.9-100%) for *Sentosa* HCV. 56 GT1a and 54 GT1b samples were used for further analysis of RAVs distribution among the GT1 population. 52.7%(58/110) of HCV strains were carrying 1 or multiple RAVs in 23 positions across all target genes. An unequal distribution of 4 mutations across the GT1 subtypes was observed. Frequency of the Q80K mutation (NS3) was 25%(14/56) in GT1a and 1.9%(1/56) in GT1b. While mutations Q54H and Y93H (NS5A) were prevalent in GT1b: 42.6%(23/56) and 18.5%(10/56) respectively. No Q54H mutation was present the GT1a population studies; Y93H in this group reached 1.8%(1/56). Mutation V499A in the NS5B gene was present in the GT1b population at 25.9%(14/54) and absent in the GT1a population.

Conclusion: Simultaneous determination of HCV genotypes and detection of RAVs in single NGS runs provides comprehensive, clinically relevant information for optimal selection of HCV treatment regimens.

B-065

A qPCR assay that simultaneously detects *Mycoplasma genitalium* and mutations associated with macrolide resistance has the potential to improve patient management

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Background: In the 2015 CDC STD treatment guidelines, Mycoplasma genitalium (Mg) was highlighted as an emerging issue based on increasing evidence linking it with nongonococcal urethritis (NGU) in men and cervitis in women. However, to-date diagnosis has been difficult as it is a slow growing organism and can take up to 6 months to culture. It has only been with the advent of NAAT testing that routine detection has been made possible. Further complications have arisen by emerging resistance to the standard treatment for Mg which is a single dose of 1g azithromycin, a macrolide antibiotic. Widespread use of azithromycin, has been associated with the emergence of macrolide resistance and ineffective cure rates. A new assay, PlexPCR™ *M. genitalium* ResistancePlus™ kit, has been developed to simultaneously identify Mg and 5 mutations in the 23S rRNA gene (positions 2058 and 2059 (*E. coli* numbering)) associated with macrolide resistance. The assay has previously been demonstrated to have good clinical performance on 400 retrospective samples (sensitivity and specificity for Mg and 23S rRNA mutation detection was 99.1% and 98.5%, and 97.4% and 100%, respectively). This study evaluates incorporating the assay into a diagnostic algorithm to direct faster and more appropriate clinical management and reduce the spread of antibiotic resistant Mg by testing for resistance upfront.

Methods: In this prospective study, 1087 consecutive urogenital samples from symptomatic and asymptomatic patients were evaluated with the PlexPCR *M. genitalium* ResistancePlus kit. This is a real-time PCR kit that employs novel PlexPrimer and PlexZyme technology. PlexPrimers selectively amplify mutants over wild-type and PlexZymes allow for efficient multiplexed detection and signalling. This unique combination allows "stacking" of the 5 mutation assays for a single readout. This kit was run in parrellel to an in-house test for Mg detection and sequencing of Mg positives to determine 23S rRNA mutation status.

Results: The prevalence of Mg was 6.0% (65/1087) and in the Mg positive samples 23S rRNA mutation prevalence was 63.1% (41/65). The PlexPCR *M. genitalium* ResistancePlus assay showed very high clinical performance compared to the reference methods with sensitivity and specificity for Mg detection of 98.5% (95%CI: 91.7-100%) and 100.0% (95%CI: 99.6-100%), and 23S rRNA mutation detection of 92.7% (95%CI: 80.1-98.5%) and 95.7% (95%CI: 78.1-99.9%) respectively. The PlexPCR *M. genitalium* ResistancePlus assay showed a limit of detection (LOD) of 10 - 15 copies for each mutant when LOD study was performed on synthetic template and no cross-reactivity of related organisms was seen.

Conclusion: The PlexPCR *M. genitalium* ResistancePlus kit demonstrated excellent clinical performance for the simultaneous detection of Mg and assessment of macrolide resistance. Performance of this assay as a rapid screening assay can offer better clinical management of macrolide resistant Mg infection.

B-066

Performance Evaluation of a Prototype CMVIgG Assay on the ADVIA Centaur® System

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Objective: Siemens Healthcare (Tarrytown, NY) is currently developing a cytomegalovirus IgG (CMVIgG) assay to detect the presence of lgG antibodies to cytomegalovirus (CMV). Anti-CMV IgG antibodies act as a specific marker to aid in the diagnosis of CMV infection. Changes in the seroconversion status of CMV lgG are an indicator of either a reinfection or reactivation of CMV. The Siemens ADVIA Centaur® CMVIgG assay* is a chemiluminescent magnetic microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay System. The objective of this study was to evaluate the positive and negative agreement, precision, and cross-reactivity of a prototype, automated ADVIA Centaur CMVIgG assay.

Methods: The fully automated ADVIA Centaur CMVIgG assay is being developed as an indirect sandwich assay for the detection of CMV IgG antibodies in human serum and plasma. The assay was evaluated for positive and negative agreement (via method comparison), cross-reactivity, repeatability, and within-lab precision. The positive and negative agreements of the assay were evaluated using a total of 2315 patient samples across two reagents lots. Cross-reactivity was evaluated using samples negative for CMV IgG but known to have at least one of the following disease states: Epstein-Barr virus (EBV), herpes simplex virus (HSV), toxoplasma, Rubella, Chlamydia, Measles, Varicella Zoster Virus (VZV), Rheumatoid Factor (RF), Hepatitis C virus (HCV), and Hepatitis A virus (HAV). The results were assesses based on index values as reactive (≥1.00) and nonreactive (<1.00). The serological status of all samples was initially determined by the bioMerieux VIDAS® CMV IgG assay. Discordant samples were tested on the Siemens IMMULITE® 2000 and Roche cobas® e 411 CMV IgG assays, when available. Per CLSI EP5-A3, precision was evaluated by testing four samples with index values concentrations spanning the assay range in two runs per day for 20 days on the ADVIA Centaur system for a total of 80 replicates.

Results: Evaluation of the patient samples using the ADVIA Centaur CMVIgG assay indicated that the positive agreement ranged from 98.7% to 99.4% and the negative agreement ranged from 98.5% to 99.5%, when compared to the VIDAS CMV IgG assay. Additionally, the ADVIA Centaur CMVIgG assay displayed a total agreement of 99.3% (133/134) to the resolved clinical status of all CMV IgG-negative cross-reactive samples evaluated. The assay demonstrated good precision, with an average repeatability and within-run %CV of <5.0% and <9%, respectively, for samples yielding Index values between 0.50 and 30.00.

Conclusion: The results of this study demonstrate good performance of the prototype ADVIA Centaur CMVIgG assay.

*For investigational use only. Not available for sale. The performance characteristics of this product have not been established.

B-067

Heparin Binding Protein; a potential new biomarker for diagnosis of Acute Bacterial Meningitis

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Background: Acute bacterial meningitis is a life-threatening neurological emergency. It is one of top 10 causes of infection-related death worldwide. Untreated, its mortality approaches 100%. Even with current antibiotics and advanced intensive care, the mortality rate of the disease is approx. 10% & 30-50% of its survivors have permanent neurological sequelaeThe clinical distinction between viral & acute bacterial meningitis is difficult in the acute phase of illness because the symptoms often are similar. Cerebrospinal fluid (CSF) examination is essential to establish the diagnosis & to identify the etiological agent. Bacterial culture is the gold-standard technique for confirmation. However, approx 50% of suspected cases are not culture-confirmed.It is also not uncommon that antibiotics are instituted before lumbar puncture, reducing the chance of a microbiological diagnosis. A rapid noninvasive biomarker for diagnosis & differentiation of ABM from is much needed. Heparin-binding protein (HBP) forms a part of the innate defenses of human neutrophils. HBP is rapidly mobilized from migrating neutrophils. **Methods:** 90 Egyptian individuals divided into three groups; 30 patients diagnosed with ABM**,** 30 diagnosed with patients with aseptic meningitis & 30 subjects with normal CSF examination findings (controls). Diagnosis was based on history, clinical criteria & CSF examination; macroscopic examination, microscopy (CSF cells and Gram stain), chemical examination (CSF Protein, glucose and lactate), latex agglutination & culture and sensitivities. HBP was measured using ELISA technique in both serum & CSF. **Results:** CSF HBP levels in the controls averaged 0.82 ± 0.3 ng/mL. In viral meningitis, mean CSF HBP levels 3.3 ± 1.7 ng/mL. In bacterial meningitis, mean CSF HBP levels was 174.8 ± 46.7 ng/mL. The mean serum HBP levels in the controls was 0.84± 0.3 ng/mL. In viral meningitis, mean serum HBP levels was 3.7 ± 1.9 ng/mL. In bacterial meningitis, mean serum HBP levels was 192.2 ± 56.6 ng/mL. CSF and serum HBP levels were significantly higher in patients with ABM than in patients with viral meningitis and controls. A cut-off of CSF HBP level of 56.7 ng/ml and serum HBP level of 45.3 ng/ml showed 100% sensitivity, specificity & positive & negative predictive values of 100% and overall accuracy of 100%. Area under the ROC curve for HBP was 1.0. Even in ABM patients who received antibiotics 48 - 72 hours prior to lumbar puncture, HBP levels in CSF & serum remained elevated. A significant positive correlation between CSF and Serum HBP levels and other CSF findings in all three groups. **Conclusion:** HBP levels in CSF & serum serve equally as strong potential diagnostic markers for bacterial meningitis and differentiation between bacterial and viral meningitis. HBP might be able to assist in early identification of bacterial meningitis even in empirically partially treated bacterial meningitis cases.

B-068

The Prevalence of Six Common Sexually Transmitted Pathogens by Multiplex Real-Time PCR in Cervico-Vaginal Specimens Collected from Sexually Active Women in Korea

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Background: Sexually transmitted infections (STIs) present a major public health concern worldwide. Reduction of complication rates and disease spread requires the use of rapid and reliable laboratory diagnostic tool that can identify causative pathogen. Multiplex real-time PCR assay is a highly sensitive and effective diagnostic tool for detection of urogenital pathogens, especially for culture-difficult organisms. The objective of this study was to assess the prevalence of six common sexually transmitted pathogens and the co-infection rate by multiple pathogens in female genital tract using a multiplex real-time PCR assay. **Methods:** The authors retrospectively analyzed the STIs multiplex real-time PCR results from 3,460 women (mean age: 36.7±10.1 years). STIs tests were requested to Seoul Medical Science Institute from 43 medical institutions nationwide in Korea between January 2015 and September 2015. Multiplex real-time PCR assays were performed with cervico-vaginal swabs by use of *AccuPower®* STI8A-Plex Real-Time PCR Kit (BIONEER, Korea). Detection of the six types of STI pathogens including *Micoplasma hominis* (MH), *Ureaplasma urelyticum* (UU), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Trichomonas vaginalis* (TV), and *Neisseria gonorrhoeae* (NG) was performed simultaneously in a single multiplex real-time-PCR reaction using a combination of primers for each pathogen. **Results:** Of the 3,460 subjects, 1,266 (36.6%) tested positive for at least 1 pathogen, with 836 (24.2%) positive for 1 pathogen, 337 (9.7%) for 2 pathogens, 87 (2.5%) for 3 pathogens, and 7 (0.2%) for 4 pathogens (n =1,799 pathogens total). Among the 836 subjects infected by single pathogen, the prevalence of each microorganisms were as follows; 349 tested positive for MH (41.7%), 337 for UU (40.3%), 68 for CT (8.1%), 46 for MG (5.5%), 25 for TV (3.0%), and 11 for NG (1.3%). Among the subjects infected by 2 pathogens, co-infections with MH and UU were most frequently observed (185/337, 54.9%). Other combinations of infection by 2 pathogens were as follows; MH and CT, 44/337 (13.1%): MH and MG, 24/337 (7.1%): MH and TV, 23/337 (6.8%): UU and CT, 23/337 (6.8%): CT and MG, 9/337 (2.7%): UU and MG, 7/337 (2.1%): UU and TV, 6/337 (1.8%): MH and NG, 5/337 (1.5%): UU and NG, 4/337 (1.2%): CT and NG, 3/337 (0.9%): CT and TV, 2/337 (0.6%): MG and TV, 2/337 (0.6%). Among the subjects infected by 3 pathogens, con-infections with MH, UU, and CT (20/87, 23.0%) were the most frequent. **Conclusion:** STIs are prevalent in sexually active women in Korea, and infections by MH and UU are especially frequent. As the proportion of co-infection by multiple pathogens was noted up to 34.0% among the STIs-positive subjects, guidelines establishment regarding screening of STIs by multiplex PCR approach in high risk group is needed. Correct diagnosis of STIs by multiplex real-time PCR apprach will guide the appropriate interventions targeted against specific pathogens. Effective treatment of STIs in sexually active women has important implications for the reduction of side effects and risk to fetal development, and for the prevention of onward transmission.

B-069

The utility of QuantiFERON-Gold in Tube assay to diagnosis of active tuberculosis: Using new equation

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Objective

Interferon-γ release assay (IGRA) measure a cellular immune response to *Mycobacterium tuberculosis (Mtb)* specific antigens. QuantiFERON-Gold in Tube assay (QFT-GIT) is a kind of interferon-γ release assay (IGRA) for the detection of *Mycobacterium tuberculosis (Mtb)*. QFT-GIT is known to be highly specific and sensitive but cannot distinguish active from latent tuberculosis (TB) infections. The purpose of this study was to evaluate a new equation using interferon-γ (IFN-γ) levels for diagnosis active TB in positive QFT-GIT population.

Method

During the period from January 2013 to May 2015, 295 cases with positive QFT-GIT and having final diagnosis of active TB or non-TB by work-up including liquid and solid TB cultures, TB polymerase chain reaction (TB-PCR), or acid fast staining were retrospectively studied. We compared individual IFN-γ levels and new equation levels between TB and non-TB. The IFN-γ levels were recalculated by standard curve and used without treatment in case with higher than 10 IU/mL. The characteristics of IFN-γ responses were analyzed by statistics including Mann Whitney U-test, Pearson's Chi-square test and ROC curve analysis.

Results

The number of active TB and non-TB was 144 (age 51.5±19.0, M:F=86:58) and 151 (age 63.5±14.2, M:F=98:53), respectively. The INF-γ levels of NIL and Mitogen tube were inversely correlated with age but not in TBAg tube. The active TB showed higher IFN-γ levels than non-TB in Nil tube (0.24±0.24 vs. 0.11±0.16, $P<0.001$) and in TBAg tube (4.84±3.45 vs. 3.19±2.93, $P<0.001$). But there was no difference in IFN-γ levels of Mitogen tube between TB and non-TB. The cut-off of INF-γ in Nil and TBAg were >0.08 (AUC 0.72, sensitivity 72%, specificity 64%) and >2.83 (AUC 0.65, sensitivity 63%, specificity 64%). Newly derived equation maximizing the discrimination power was $(Nil \times 10)^2 (TBAg - Nil)$. The AUC of new equation was 0.75 (cut-off >4.42, sensitivity 59%, specificity 80%). The combination of new equation and Nil for active TB diagnosis showed sensitivity 57% and specificity 80%.

Conclusion

The QFT-GIT assay is in vitro test with high sensitivity and low positive predictive value. To help to find real active TB infection among positive QFT-GIT cases, this study suggests a new equation. The diagnostic value of new equation was better than individual IFN-γ levels of QFT-GIT. TB suspects who present with stronger IFN-γ responses in Nil tube and TBAg tube and high calculated value through the new equation should be carefully evaluated to exclude the possibilities of active TB infection in TB-endemic area. To accurate differential diagnosis of active TB using IGRAs more clinical researches involving the IGRAs of new formats are needed. New format of IGRAs should have additional cytokines or diagnostic formula for discrimination of active TB infection.

B-071

Prevalence of HIV-1 genetic/recombinant subtypes and drug resistance mutations in Brazil, updated 2015

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Background: Genetic variability is a major feature of the human immunodeficiency virus type 1 (HIV-1). This viral diversity has impact on the diagnosis, monitoring, therapy and development vaccines. Subtyping is a powerful molecular tool for the choice of an effective therapy and monitoring the evolution of the HIV-1 epidemic. In Brazil, the majorities of published data about the prevalence of HIV-1 are from south and southeast remain scarce in other regions. **Objective:** To estimate the prevalence of HIV-1 subtypes and resistance profiles in order to contribute to the implementation of public health *policies in Brazil.* **Method**: Plasma samples from 184 diagnosed HIV-infected patients of different geographical regions of Brazil with viral loads above 1,000 copies/mL were used. Viral cDNA fragments corresponding to the reverse transcriptase and protease regions were amplified and sequenced. The subtype and mutations profile were assessed using Stanford HIV Drug Resistance Database. **Results:** The mean age of the patients was 37.17 (range 4-74 years), and most samples were from men (80.43%). Among the 184 patients, 144 (78.26%) presented mutation in the analyzed region (21.74%), of which 96 (52.17%) to NRTIs, 104 (56.52%) toNNRTIs and 82 (44.57%) to protease inhibitors. According to analyses, a variety of subtypes and recombinant forms were detected (Figure 1A), the most frequently been the subtype B. The viral recombinant forms was identified at 17 patients, and seven different types of recombinant were recognized(Figure 1B). The distribution of HIV-1 subtypes and recombinants within each region in Brazil is revealed in Figure 1C. **Conclusion:** The maintenance of HIV-1 genotyping programs is important in the management of patients for first line and rescue therapy, and the attempted monitoring of the HIV-1 subtype prevalence in Brazil. Furthermore, these surveys produce data that should be an important resource for all HIV scientists and public health officials.

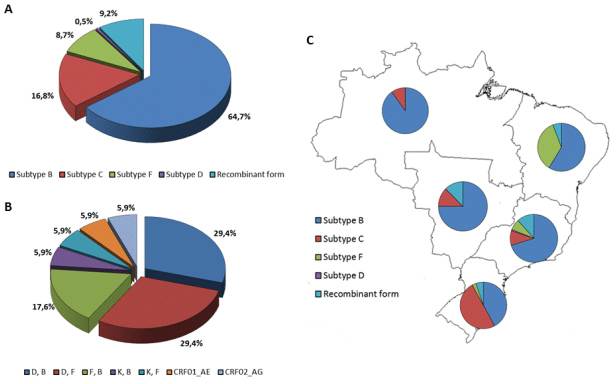

Figure 1: (A) Frequency of HIV-1 subtypes and recombinant forms identified at present study. (B) Frequency of different types of recombinant form. (C) Recent geographical distribution of HIV-1 subtypes and recombinants in Brazil.

B-072

Influenza Rapid Testing Course and New Influenza Pandemic Preparedness Course Offered for Clinicians - Utilization of Courses and E-Resources

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Objective: To enhance the knowledge of clinicians when using rapid influenza diagnostic tests (RIDTs) by offering a free online influenza rapid testing course, "Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS)", with continuing education credits (CEs). The course focuses upon RIDT utilization. Further objectives were to gather feedback from course participants about SIRAS and about other educational needs, and based on that feedback, to develop and offer a new online influenza pandemic preparedness course with CEs. Data on course utilization and on utilization of associated e-resources are presented. **Relevance:** RIDTs are often used for making treatment decisions for patients suspected of having influenza. Concerns persist about the variable predictive value of RIDTs, within the context of the prevalence of circulating influenza viruses in the population being tested. This is of particular concern in outpatient and ambulatory settings where RIDTs are often the only readily-available influenza tests. **Methods**: The SIRAS course was originally offered in Oct. 2012, and restructured in 2013 from a single to four 30- minute modules (http://www.jointcommission.org/siras.aspx). The new influenza pandemic preparedness course, "Influenza Preparedness and Response in Ambulatory Settings" (http://www.jointcommission.org/topics/influenza_pandemic_preparedness.aspx) was developed and launched in Apr. 2015 and restructured in Oct. 2015 into two shorter segments: 1) pandemic planning, the response phase and 2) laboratory testing/diagnosis and patient management/treatment. Both courses were developed with a technical panel of influenza experts. Specimen collection videos https://www.youtube.com/playlist?list=PLNQfL_CJ36fK08KEPjxu1ZKJn7GuFtn-N. , and other e-resources and helpful links (such as CDC FluView) are offered with the courses. Courses are updated annually and relaunched in Oct. of the respective year. Multiple social media and Joint Commission communication channels are used for marketing. Course utilization data and e-resource utilization data are tracked. The SIRAS course is currently being translated into Spanish. **Validation**: There were 5,864 unique visitors to the SIRAS webpage and 1,615 enrollments in SIRAS between Oct. 2014 and Sept. 2015. There were 1,722 visitors to the pandemic course webpage and 608 course enrollments since its original launch in Apr. 2015 through Sept. 2015. Since updating and relaunch of both courses in Oct. 2015, there have been 458 enrollments in SIRAS and 301 enrollments in the pandemic course, representing upward trends. The SIRAS certificate issuance rate has increased to 82% (Oct. - Dec. 2015) compared with 34% during the first year of offering and 48% last year. There were 2,345 downloads of the Infographic describing the courses; 10,162 views of the RIDT post on The Joint Commission's "AmBuzz" blog, and 132,667 views of the specimen collection videos, cumulatively from the initial offering through Nov. 2015. The overall satisfaction rates were 99% for the SIRAS course and 94% for the pandemic course. **Conclusions**: Ambulatory care providers have welcomed the ongoing opportunity for continued education in influenza testing and preparedness. The annual updating of all course modules before the onset of influenza season specifically attracts high usage of the e-resources and increased course enrollments. YouTube specimen collection videos, and other course e-resources are increasingly popular.

B-073

Distribution and Phenotypic Resistance Profile from Bloodstream Infections Isolates from Metropolitan Public Hospitals of Sao Paulo City, Brazil: Data from a Laboratory of Clinical Microbiology

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Background: Nosocomial Bloodstream Infections (BSI) are directly related with high morbidity and mortality rates, hospital lengths of stay in critical ill patients mostly and associated health care costs. Brazilian studies concerning Bloodstream Infections are important tools that can bring specific issues related to antimicrobial resistance. The aim of this study was evaluate the frequency of microorganism isolation and the main phenotypic resistance profiles from BSI in medical centers located throughout in the city of Sao Paulo, Brazil. **Methods:** Data on bacterial and fungal isolates from BSI patients admitted to the 10 public health system medical centers were selected from July 2014 to July 2015. Identification and susceptibility tests were performed using Vitek MS and Vitek 2 (bioMerieux) systems, respectively. Antimicrobial susceptibility profile were evaluated according CLSI, 2014/2015 criteria. Coagulase Negative Staphylococcal (CNS) BSI was only considered in the presence of at least two sets of positive blood cultures with the same organism. **Results:** A total of 8632 microorganisms were evaluated. The frequency of organism group types were Gram-positive (n=4841; 56.1%), Gram-negative (n=3359; 38.9%), yeasts (n=400; 4.6%) (1.5% *Candida albicans*; 2.9% *C.* non *albicans* and 0.2% others yeasts) and 0.4% fastidious organisms (*Moraxella* spp.; *Neisseria* spp. and *Haemophilus* spp.). The most frequent organisms were CNS (26.7%); *Staphylococcus aureus* (17.1%); *Klebsiella pneumoniae* (10.7%); *Acinetobacter baumannii* (7.2%); *Escherichia coli* (6.4%); *Enterococcus* spp. (5.9%); *Pseudomonas aeruginosa* (3.4%); *Candida* non *albicans* (2.9%); *Streptococcus viridans* group (2.9%) and others Gram-negative non-fermenter (2.8%). Antimicrobial resistance was observed mainly in 88.7% CNS (2050/2311) and 52% *S. aureus* (769/1479) to methicillin; and 39.2% (200/510) of *Enterococccus* spp. to vancomycin. Carbapenems resistance was detected in 78.1% (485/621) of *Acinetobacter* spp isolates, 43% (398/926) *K. pneumoniae*; 31.3% (92/294) *P. aeruginosa*; 4.7% (10/211) *Enterobacter* spp. and 2.3% (25/1066) of others Enterobacteriaceae. **Conclusion:** In contrast with other Brazilian studies, *P. aeruginosa* was not the main organism recovered from BSI, although the resistance rate to carbapenem was one of the most higher when compared with others Gram-negative. Rates of antimicrobials resistance reported in CNS, *Enterococcus* spp. and *K. pneumoniae* are reinforce the high resistance rates to antimicrobials in Sao Paulo city that is considered one of the highest observed in Brazil. A continued study is necessary to monitor these national trends.

B-074

Beckman Coulter DxN VERIS Molecular Diagnostic System Sample-To-Sample Crossover Contamination Study

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OBJECTIVE: Sample carry-over and cross-contamination present a high risk for diagnostic medical devices and therefore are monitored to determine their impact. Based on internal and external voice of customer surveys, minimal/no sample carryover and cross-contamination were recognized as critical system characteristics that had to be addressed in the development of a 21st century molecular diagnostic random access instrument, Beckman Coulter's DxN VERIS Molecular Diagnostic System*. However, there are no guidelines on the statistical methodology for data analysis, especially where the absence of carryover is necessary. Furthermore, there is a lack of criteria for analytical specificity that rigorously challenges the system being tested. The DxN VERIS was designed to have a false positive rate due to cross contamination of less than 1 in 500 tests with 95% confidence with an overall design goal of zero. The objective of this study was to assess the sample-to-sample contamination rate using a real-time PCR assay, characterize potential sources of contamination, and assess the effective resolution of carry-over and cross-contamination artifacts.

METHODS: Contamination characterization was performed by swabbing areas of the instrument before and after running a series of high concentration level positive samples to determine potential sources of contamination. Swab assessments identified areas where contamination of liquid handling was occurring on the instrument. Several modifications to the liquid handling parameters were incorporated into the test processing system to eliminate sample-to-sample contamination. The analytical specificity, as a measurement of cross-over contamination, was assessed using the optimized liquid handling parameters with the VERIS Hepatitis B (HBV) assay. In order to fully evaluate the ability to prevent instrument induced cross-contamination,

high positive HBV plasmid samples at a concentration of 1×10^9 IU/mL, which represents a concentration level exceeding 95% of the diseased population for any of the assays developed on DxN VERIS, were tested interspersed with true negative samples composed of sterile tris ethylenediaminetetraacetic acid (TE) buffer.

RESULTS: A total of 2,330 true negative samples were processed alternating with 2,330 high positive samples, providing the statistical power to evaluate the false positive rate using a lower bound two-sided 95% confidence interval. Because PCR can detect single molecules of nucleic acid, a 1 picoliter (1.0×10^{-12} liter) droplet size at a concentration of 1×10^9 IU/mL, would cause a false positive result. Based on these data, the DxN VERIS did not exhibit detectable carryover from the high positive samples to the negative samples and was verified to have a false positive rate due to cross contamination of 0% with a lower bound two-sided 95% confidence interval of 99.9%, providing evidence for the absence of sample-to-sample carryover.

CONCLUSIONS: The Beckman Coulter DxN VERIS Molecular Diagnostic System did not exhibit detectable carryover from high positive samples to negative samples. This was true when the concentration of target in the samples was significantly higher than target concentrations found in clinical samples.

*Not for sale or distribution in the U.S.; not available in all markets.

B-075

Performance Evaluation of Prototype Herpes-I IgG and Herpes-II IgG Assays* on the ADVIA Centaur XP Immunoassay System

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Background: Two members of the herpes virus family, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), are pervasive, and both infect epithelial cells. While HSV-1 produces most cold sores, HSV-2 produces genital herpes. The ADVIA Centaur® Herpes-I IgG and Herpes-II IgG assays* from Siemens Healthcare are indirect sandwich chemiluminescent two-step immunoassays that employ the recombinant HSV-1 and HSV-2 glycoprotein G (gG1 and gG2) respectively. They are designed for the detection of HSV-1 and HSV-2 antibodies in order to allow a prompt recognition of herpes simplex infection and early initiation of therapy. The aim of this study was to evaluate the performance of the assays on the ADVIA Centaur XP Immunoassay System.

Methods: Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the Siemens Herpes-I IgG and Herpes-II IgG assays were assessed by testing on 970 serum and 670 plasma samples against LIAISON (DiaSorin) and HerpeSelect 1 and 2 Immunoblot IgG (Focus) assays, respectively. Samples came from Banc de Sang i Teixits de Catalunya (Spain) (300 sera), from different commercial sources (75 samples), from sexually active adults provided by ProMedDx (Norton, U.S.) and Cerba (Spain) (367 sera), and from pregnant women, also acquired from ProMedDx (Norton, U.S.) (228 sera). Other relevant performance characteristics such as 20-day precision (CLSI EP5-A3), reagent opened onboard stability (CLSI 25-A), interferences (CLSI EP7-A2), and cross-reactivity were also determined.

Results: For the Herpes-I IgG assay, PPA% was 98.6% and 98.0% and NPA% was 95.3% and 95.3% against LIAISON and Immunoblot, respectively. For the Herpes-II IgG assay, PPA% was 97.3% and 96.5% and NPA% was 95.1% and 96.9% against LIAISON and Immunoblot, respectively. Within-run and total precision %CV was found to be between 1.7 and 5.1% and 3.1 and 6.6% for the Herpes-I IgG assay and between 1.2 and 2.0% and 2.8 and 4.1% for the Herpes-II IgG assay. Reagent opened onboard stability was verified up to 8 weeks for both assays. Additionally, for both assays, no interferences (hemoglobin, bilirubin, triglycerides, and cholesterol) or cross-reactivity were observed with 24 different disease conditions.

Conclusions: The results of this study show that the prototype ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays are rapid, precise, highly specific immunoassays capable of determining HSV-1 IgG and HSV-2 IgG antibodies qualitatively in comparison to other assays. The robustness of the ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays, together with the features of the ADVIA Centaur analyzers (random access, easy to use, and full automation), demonstrate their potential use in a laboratory setting.

*Under development. Not available for sale.

B-076

Quantitative Detection of CMV, HBV, HCV, and HIV-1 on the Beckman Coulter DxN VERIS System

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Background: The Beckman Coulter DxN VERIS System* is a fully-automated, random-access, sample-to-answer system for the quantitative/qualitative analysis of molecular targets. DxN VERIS incorporates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using the real-time polymerase chain reaction (RT-PCR). The initial VERIS assay menu includes quantitative detection of Cytomegalovirus (CMV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency virus Type 1 (HIV-1). The objective of this study was to test and report performance of each assay in limit of detection, clinical specificity, and method comparison.

Methods: Study methods were based on Clinical and Laboratory Standards Institute (CLSI) guidelines for "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures" (EP17-A2E) and "Measurement Procedure Comparison and Bias Estimation Using Patient Samples" (EP9-A2). For clinical specificity, assessed with by the Wilson Score method, at least 287 samples were collected for each assay.

Results: Probit analysis calculated the DxN VERIS CMV Assay LoD as 15 IU/mL, supporting the product claim of 30 IU/mL; the DxN VERIS HBV Assay LoD as 3.8 IU/mL, supporting the product claim of 10 IU/mL; the DxN VERIS HCV Assay LoD as 4.3 IU/mL, supporting the product claim of 12 IU/mL; the 1 mL sample volume DxN VERIS HIV-1 Assay LoD as 30 IU/mL, supporting the product claim of 60 IU/mL; and the 0.175 mL sample volume DxN VERIS HIV-1 Assay LoD as 167 IU/mL, supporting the product claim of 400 IU/mL.

The average biases between VERIS assays and Roche COBAS assays were 0.24 log IU/mL for CMV, -0.32 log IU/mL for HBV, 0.20 log IU/mL for HCV, and 0.34 log IU/mL for HIV-1.

The Wilson Score method yielded 100% specificity with a lower bound confidence interval of 98.7% for CMV, 100% specificity with a lower bound confidence interval of 99.5% for HBV, 100% specificity with a lower bound confidence interval of 99.0% for HCV, and 99.8% specificity with a lower bound confidence interval of 99.1% for HIV-1 at the 1 mL sample volume assay.

Conclusions: Based on these data, the Beckman Coulter DxN VERIS CMV, HBV, HCV, and HIV-1 Assays are automated molecular tests with the sensitivity and accuracy of viral load monitoring required for effective patient management.

* The DxN VERIS System and DxN VERIS CMV, HBV, HCV, and HIV-1 Assays are not available for sale or use in the U.S.

B-077

Enabling Random Access Molecular Testing Using Multiple PCR Cells

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OBJECTIVE: Beckman Coulter's DxN VERIS Molecular Diagnostic System* is a fully automated sample-to-answer system for quantitative and qualitative analysis of molecular targets. The system integrates and automates all major processes, from sample introduction and nucleic acid extraction and purification, to real-time PCR amplification and detection. In order to meet the throughput goals of 150 tests per 8 hour shift and the random-access testing goal via a one-piece flow process, 20 PCR cells are required. The objective of this study is to assess the level of uniformity across the 20 PCR cells.

METHODS: A DxN VERIS system was used for testing. The 20 on-board PCR cells each accommodate a single 40 µL sample volume vessel. Sample heating is achieved by applying a voltage across heating tape thermally communicating with the reaction vessel. Sample cooling is achieved by flowing ambient temperature air across the reaction vessel. Laser-induced fluorescence is made using a dual laser excitation / CCD detection system.

To compare the sample temperature experienced across all 20 PCR cells, thermistors were placed in reaction vessels filled with 40 µL water. Thermocycling temperature profiles were collected near the extremes and in the middle of the full temperature range, namely 45°C, 70°C and 95°C, with dwell times of 25 seconds per temperature over 20 cycles.

To compare the reproducibility across PCR cells, a pooled sample containing PCR-enabling reagents and two different genomic DNA targets was distributed among 20 PCR vessels, capped and loaded onto all 20 PCR cells. The samples were thermocycled using the same protocol and the data analyzed to obtain cycle threshold (Ct) values.

RESULTS: The temperature variability across all 20 cells during the 25 second dwell times was found to be less than 0.3°C in all cases. The largest variation, at 0.3°C, was measured at 95°C, while the smallest variation, at <0.1°C, was measured at 45°C. Amplification and real time detection of the two genomic targets resulted in Ct values of (32.46±0.15) and (28.40±0.16) cycles.

CONCLUSIONS: In order to meet throughput and single sample, random-access testing goals, the DxN VERIS Molecular Diagnostics System must contain PCR cells which all yield the same result for a given thermocycling protocol, irrespective of which cell is employed. This work demonstrated that the maximum PCR cell-to-cell temperature variation was less than 0.35°C, ensuring samples undergo comparable thermal experiences during the amplification and detection process. Actual real time PCR testing, using pooled samples, show that the variance among the 20 cells tested was 0.16 cycles, indicating a high level of reproducibility.

*Not for sale or distribution in the U.S.; not available in all markets.

B-078

Workflow Characteristics of the Beckman Coulter DxN VERIS Molecular Diagnostic System Compared Against Four Automated Laboratory Instruments

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OBJECTIVE: Automated molecular diagnostic instruments present standardized processing schemes and workload advantages to the clinical laboratory. Assessments of workflow and maintenance characteristics provide quantitative and objective metrics that accurately measure automated system efficiency and associated labor costs. The objective of these series of studies was to determine the workflow characteristics of the Beckman Coulter DxN VERIS Molecular Diagnostic System* evaluated against four existing batch and semi-automated platforms.

METHODS: Multi-site comparative workflow studies were conducted on the DxN VERIS and four other platforms by Nexus Global Solutions, Inc., an independent third party healthcare and diagnostic consulting firm. The targeted parameters of the study included system complexity (process and consumables), system operating and performance metrics, hands-on time requirements, and total process times for the HIV-1, HCV, HBV, and Cytomegalovirus (CMV) viral load assay workflows. Testing methodology and work practices were captured and documented through direct observations and practices, time study of operators and analytical systems, and targeted interviews with operators. Operational metrics were attained by means of process steps, value streams, operational complexities, testing timelines, and process times.

RESULTS: DxN VERIS had 10 process steps compared to 29 steps for Roche Cobas® Ampliprep/TaqMan®-48 (CAPTM-48) and Siemens kPCR (kPCR), 30 steps for Hamilton MagNA STARlet/Roche MagNA Pure-96/Roche LightCycler-480 (MS+MP+LC), and 26 steps for Abbott m2000 (M2000). DxN VERIS had five consumables required for sample processing. In contrast, kPCR and m2000 had >20 consumables, while all others had >10 consumables. DxN VERIS showed the shortest time to first result (TTFR) at 1.25 h for CMV and HBV and 1.75 h for HIV-1 and HCV, with subsequent results occurring every 2.5 min. DxN VERIS also showed a time to last result (TTLR) at 4.15 h for CMV and HBV (48 tests) and 4.65 h for HIV-1 and HCV (48 tests). The other platforms processed samples in batch mode, therefore having identical TTFR and TTLR. CAPTM-48 yielded a process time of 7.58 h for 48 HIV-1 samples and 7.2 h for 48 HCV samples. kPCR were 6.93 h for 96 HIV-1 Patient Samples. Process time for MS+MP+LC had 3.32 h for 48 HBV and/or CMV samples. m2000 had a process time of 6.42 h for 48 HIV-1/HCV samples, 5.43 h for 24 HBV samples, and 5.87 h for 24 CMV samples. DxN VERIS had a hands-on time requirement of 23 min for 144 patient samples. The hands-on time requirement processing 48 samples for CAPTM-48 was 60 min, MS+MP+LC was 84 min, and m2000 was 63 min. kPCR had a hands-on time requirement of 58 min for 96 samples.

CONCLUSIONS: Considerable time requirements and process complexity differences were observed between instruments. While in these particular studies MS+MP+LC showed the fastest TTLR of 3.32 h for the HBV and CMV assays, compared to the DxN VERIS system at 4.15 h, DxN VERIS was faster for the HCV and HIV-1 assays, had the shortest TTFR, the fewest process steps, the largest test capacity, and the lowest hands-on time requirements.

*Not for sale or distribution in the U.S.; not available in all markets.

B-079

Lumipulse *G* TP-N Assay for Quantitation of Anti-TP antibodies in Human Serum and Plasma

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Background: Treponema pallidum (TP) is a pathogenic bacterium and the causative agent of syphilis. Assays for anti-TP have been adopted in clinical diagnostic testing, and are considered to be useful in the diagnosis of patients where infection with TP is suspected, or in preventing infection via blood transfusion (Larsen, 1995; Singh, 1999; LaFond, 2006 and Meyer, 1996). Lumipulse G TP-N can be used as an initial screening test or in conjunction with a nontreponemal laboratory test and clinical findings to aid in the diagnosis of syphilis infection. WARNING: Lumipulse G TP-N is not intended for blood and tissue donor screening. United States federal law restricts this device to sale by or on the order of a physician.

Methods: Lumipulse G TP-N is an immunoassay for the qualitative detection of anti-TP antibodies in serum or plasma based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE G System. In the assay, serum or plasma was incubated with recombinant TP antigen-linked magnetic particles to capture the analyte, anti-TP antibodies. The particles were then washed and rinsed to remove unbound materials. Alkaline phosphatase-labeled recombinant TP antigens were added to and incubated with the anti-TP antibodies-bound particles. The particles were then washed and rinsed again to remove unbound materials. Substrate 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt (AMPPD) solution was then added to and mixed with the particles. Luminescence signals were thus generated by the cleavage reaction of dephosphorylated AMPPD and converted into the amount of anti-TP antibodies in the serum or plasma.

Results: The Lumipulse G TP-N demonstrated a 20-days total imprecision ≤ 5.3% (n = 80), site to site ≤ 5.4% (n = 120) and Lot to lot ≤ 6.7% (n =120) using 5 serum, one Na Citrate plasma panel and one commercially available control. One thousand seven hundred forty-five (1745) samples were used to compare the Lumipulse G TP-N with the Final Comparator Result using a Treponemal IgG/IgM test (Centaur ADVIA), Nontreponemal test (RPR), and 2nd Treponemal test (Serodia TP-PA). The Positive Predictive Agreement (PPA) was 96.8% and the Negative Predictive Agreement (NPA) was 98.0%.The PPA results of Lumipulse G TP-N for a total of 724 apparently healthy patients (apparently healthy adults, children and pregnant women) were 99.7%. Two samples tested positive (1 apparently healthy adult and 1 pregnant woman) for Lumipulse G TP-N. The apparently healthy adult was confirmed positive by Serodia TP-PA. The pregnant woman was negative per Serodia TP-PA. The Lumipulse G TP-N demonstrated an average interference of ≤ 10% (for each compound) in a study with human serum specimens supplemented with potentially interfering compounds individually at indicated concentrations: 1000 ng/mL HAMA, 1000 IU/mL rheumatoid factor, 40 mg/dL conjugated and free bilirubin, 3000 mg/dL triglycerides, 500 mg/dL hemoglobin, g/dL protein biotin 500 ng/mL, gamma globulin 60 mg/mL, cholesterol 400 mg/mL, and ascorbic acid 3 mg/dL.

Conclusion: Lumipulse G TP-N assay under development demonstrated to be a precise, sensitive, specific and robust assay for the quantitative determination of anti-TP antibodies in human serum and plasma.

B-082

The Diagnostic Accuracy of Various HIV Assays for Discrimination Between HIV-1 and HIV-2 Infections

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Background: Discrimination between HIV-1 and HIV-2 infections is crucial for selection of appropriate treatment options. For this reason, Centers for Disease Control and Prevention (CDC) has recently added HIV1/2 discrimination step in the recommended laboratory HIV testing algorithm. However, lack of experience and limited number of available assays still make it difficult to distinguish between two viruses. In addition, it is often very difficult to interpret the assay results due to strong antibody cross-reactivity. So, we evaluated diagnostic accuracies of several assays which are helpful in diagnosing and discriminating HIV1/2 infections.

Methods Between 2013 and 2015, total 57 plasma samples from HIV infected patients were collected from Togo and provided by Human Serum Bank. To distinguish between HIV-1 and HIV-2 infection, three rapid tests, five Western blot assays, and one line immunoassay were performed. We also performed real-time reverse transferase polymerase chain reaction (RT-PCR) for the confirmation of HIV-1 or HIV-2 infection.

Results: Of 57 samples we confirmed 25 samples as HIV-1 infection and 31 samples as HIV-2 infection, and one sample as dual infection. There were best diagnostic accuracies in SD Bioline HIV-1/2 3.0 for detection of HIV-1 (87.7%) and INNO-LIA HIV-I/II Score for detection of HIV-2 (96.4%). With dilution of samples to reduce the cross-reactivity between HIV-1 and HIV-2 antibodies, there was impressive improvement in diagnostic accuracy of SD Bioline HIV-1/2 3.0 (98.2% for HIV-1 and 100% for HIV-2). Meanwhile, the Western blots except MP HIV Blot 2.2 were little helpful for the discrimination of HIV types due to cross-reactivity and high rate of indeterminate results.

Conclusions: SD Bioline HIV-1/2 3.0 and INNO-LIA HIV-I/II Score showed best performance in accurate diagnosis of HIV-1 and HIV-2 infections. However, severe cross-reactivity was shown in Western blot assays except one MP HIV Blot 2.2, thus this resulted in difficulty of differentiation between two viruses. Therefore line immunoassay and rapid test would be the best option for discrimination between HIV1/2 infections.

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B-084

Development of a highly sensitive Procalcitonin Assay for the LUMIPULSE® G1200 and G600II analyzers.

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Background: Procalcitonin (PCT), a precursor of calcitonin, is synthesized by C-cells in the thyroid under healthy conditions. However, systemic inflammatory responses triggered by severe bacterial infections or sepsis induce systemic synthesis of PCT, and then it results in elevated PCT level in blood. Therefore, the determination of PCT levels in blood can be used to formulate differential diagnosis or to indicate severity of severe bacterial infections and sepsis. We have developed new chemiluminescent enzyme immunoassay (CLEIA) for PCT using fully-automated LUMIPULSE G1200 and G600II systems. Here we report the fundamental performance of the PCT assay.

Methods: B•R•A•H•M•S PCT assay for LUMIPULSE G1200 and G600II systems is a two-step sandwich assay based on CLEIA technology. The resulting reaction signals are derived within 30 minutes/test, and are proportional to the amount of PCT in the sample allowing quantitative determination of serum or plasma PCT.

Results: The detection limit was 0.0047 ng/mL, and the limit of quantitation was 0.0079 ng/mL. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and the imprecision was ≤ 4% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 32-fold dilution was 90-102% for manual dilution and 99-104% for 10-fold automated dilution within the calibration range of 0.02–100 ng/mL. The correlation coefficient and the regression slope and Pearson's correlation coefficient of Lumipulse *G* B•R•A•H•M•S PCT and B•R•A•H•M•S PCT sensitive KRYPTOR, based on Time-Resolved Amplified Cryptate Emission (TRACE) Technology (Thermo Fisher Scientific), were 1.01 and 0.96, respectively (N=85). On the correlation between LUMIPULSE G1200 and G600II systems, the slope and correlation coefficient were 1.00 and 1.00, respectively. No interference was observed with unconjugated (20.0 mg/dL) or conjugated bilirubin (21.1 mg/dL), hemoglobin (294 mg/dL), triglyceride (2,000 mg/dL), and protein (4-12 g/dL).

Conclusion: The performance of PCT assay for LUMIPULSE G1200 and G600II was satisfactory. The measurement value was compatible for the other B•R•A•H•M•S PCT assay. On the other hand, the accuracy and reproducibility at especially low range is one of the best in current PCT assay. The performance of Lumipulse *G* B•R•A•H•M•S PCT is considered as useful for the routine analysis of PCT.

B-085

Evaluation of the Architect Syphilis assay after reformulation

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Background: Testing for Syphilis specific antibodies is recommended for pregnant women to prevent congenital infections and members of at risk populations such as MSM or HIV infected patients to prevent further spread of the disease, and is a mandatory test for blood donations in many countries. As such, any Syphilis test has to be highly specific to reduce the number of false reactive results to a minimum. In response to this need the ARCHITECT Syphilis TP assay was reformulated with the aim of improved precision and specificity.

Methods: Evaluation of the new version of ARCHITECT Syphilis TP assay included testing of 5180 random blood donor specimens (serum and plasma) for determination of specificity and 409 samples pre-characterized as reactive for Syphilis antibodies (by various test methods) to determine sensitivity in comparison to the previous assay version. Samples with reactive results were further tested by 3 immunoblots,INNO-LIA™ Syphilis Score, Mikrogen *recom*Line Treponema IgG and *recom*Line Treponema IgM for confirmation. A sample was defined as positive if reactive in at least two of 4 assays, including the previous ARCHITECT Syphilis assay version. Imprecision was determined according to protocol EP5-A2 using the assay specific negative and positive controls and 4 plasma panels.

Results: Resolved specificity of the new assay version was 99.94 % (5171/5174). One sample was confirmed positive and 5 samples were unable to categorize and excluded from calculation of specificity. All samples pre-characterized as containing Syphilis antibodies were found reactive (409/409). Within-laboratory imprecision was determined to be 3.6 % CV for the Positive Control and ranging from 2.0 - 2.9 % for the 4 plasma panels.

Conclusion: The new version of the ARCHITECT Syphilis TP assay showed improved specificity compared to the previous version without compromising sensitivity. Lot to lot variation was reduced demonstrated by an imprecision of less than 4 %.

B-086

Zika virus in Brazil: a prevalence study

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Background: Zika virus (ZIKV) is an RNA virus of family *Flaviviridae* and genus *Flavivirus* transmitted by mosquitoes of genus *Aedes*, including specie *Aedesaegypti*. It was initially isolated in 1947 in the Zika forest in Uganda, and nonspecific clinical features of Zika fever (FZ) can be confused with most other arboviruses particularly dengue and chikungunya virus infection. The first ever case of ZIKV disease in Brazil was reported in May 2015 and since then the virus has rapidly spread within Brazil and across 22 other countries. Currently, 534 out of 72,062 Zika suspected cases reported in Brazil were confirmed. The outbreaks suggests that FZ is an emerging disease and its might be associated with the increasing numbers of congenital microcephaly cases reported in the country. Therefore, there is an urgent need to detect and study the prevalence of Zika virus in Brazil and regions in order to contribute to the implementation of public health policies in Brazil. **Objective**: To report the prevalence of Zika virus infection in Brazil and federative units during period of December 2015 to February 2016.**Methods**: The detection of ZIKV was performed by RT-qPCR with specific primers and TaqMan fluorescent probe technology. The inclusion criterion was the detection of viral RNA in patients. **Results**: A total of 585 patients were analyzed, among these 137 (23.4%) from Midwest region, 145 (24.8%)

from Northeast region, 42 (7.2%) from North region, 230 (39.3%) from Southeast region, 31 (5.3%) from South region. Only one state (Acre) has not been studied. It was observed that the median age of patients was 35 years, andmost patients were female (73.8%).Among the samples evaluated 12.14% (71/585) were ZIKV positive. The Midwest region presented 19.7% (27/137) of positive cases, North region 19.5% (8/42), Southeast region 12.2% (28/230), Northeast region 4.8% (7/145) and South region 3.2% (1/31). The data showed that state of Bahia had 43% of positive results of Northeast region; Minas Gerais state had 75% of Southeast region, Goias state had 52% of Midwest region and Tocantins had 50% of North region. **Conclusion**: The results obtained by ZIKV specific molecular testshowed that Midwest region has the highest prevalence of zika virus infection followed by North region and Southeast region. However, the published data indicate that the Northeast is the most affected. Thisprevalence study of ZIKV infectionin different regions of Brazil may lead to a clearer indicationofvirus potential to spread and establish in the country. Further investigations are needed to improve our understanding the disease and its clinical effects such as possible connections between the virus and neurologic complications.

B-087

Cross-Sectional Study of Patients Tested for 5 Sexually Transmitted Infections Using Molecular Methods

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Background: According to the World Health Organization more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. There are more than 30 different species of bacteria, viruses, and parasites that are sexually transmitted, and several of these agents can be transmitted during pregnancy and/or shortly after birth. STIs often involve more than one pathogen, difficulting the diagnosis and increasing the treatment cost, besides increase susceptibility to Human Immono deficiency Virus (HIV) infection. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*are major causes of pelvic inflammatory disease and infertility in women. *Mycoplasma genitalium*is detected in urogenital tract and can cause urethritis in men and cervicitis, endometritis, and tubal factor infertility in woman. *Mycoplasma hominis* is associated with non-gonococcal urethritis, pelvic inflammatory disease, spontaneous abortions and infertility. It is often present concurrently with *Ureaplasma* species.*Ureaplasma urealyticum* is related with urogenital tract infections, abortions and infertility. **Objective:** To investigate the prevalence of *Mycoplasma hominis, Mycoplasma genitalium,Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Chlamydia trachomatis* among patients tested for STIs. **Method:**This is a cross-sectional study of data from patients who were tested for STIs in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) in the period of 2010 to 2012. The inclusion criteria was the detection of DNA ofone or more microorganisms by using polymerase chain reaction. **Results:**From a total of 2,332 samples analyzed, 1,393 (59.7%) were positive for one or more microorganisms, and 939 (40.2%) were negative. 1,334 (57.2%) patientswere male and 998 (42.7%) female. *Mycoplasma hominis* was the most prevalent microorganism among both sexes, 509 (78.5%) men and 486 (71.2%) women. Overall, men had a higher positivity rate than woman, with 711 (51.0%) versus 682 (48.9%) for STIs. Among the positive samples, 1,160 (83.2%) were detected a single microorganism, and 233 (16.7%) were detected multiple microorganisms. 995 (71.4%) samples were positive for *Mycoplasma hominis*, 119 (8.5%) for *Chlamydia trachomatis*, 31 (2.2%) for *Ureaplasma urealyticum*, 13 (0.9%) for *Neisseria gonorrhoeae,* and 2 (0.1%) for *Mycoplasma genitalium*. 220 (15.7%) patients were coinfected by two microorganisms which *Mycoplasma hominis/Chlamydia trachomatis* were the most frequent, and 13 (0.9%) were coinfected with three microorganisms which *Mycoplasma hominis/Chlamydia trachomatis/Ureaplasma urealyticum*were the most frequent. **Conclusions:** The most prevalent STI was *Mycoplasma hominis,* followed by *Chlamydia trachomatis, Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Mycoplasma genitalium*. The most prevalent coinfection was *Mycoplasma hominis/Chlamydia trachomatis*, which is consistent with the literature, and also *Mycoplasma hominis/Chlamydia trachomatis/Ureaplasma urealyticum*. It was not found data in the literature describing coinfection with three or more microorganisms. Further studies are necessary to investigate the prevalence of infections with multiple STIs, once this information can help in prevention, behavior modification counseling, partner notification, and early treatment continue to be the mainstays in preventing the spread of STIs.

B-088

Performance of the HIV Ag/Ab Combo and Syphilis assays on Abbott's next-generation immunochemistry analyzer

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Background: HIV Ag/Ab Combo assays are able to detect HIV p24 antigen and anti-HIV antibodies simultaneously, therefore reducing the risk of transfusion transmitted HIV compared to assays detecting HIV antibodies exclusively. Syphilis assays are intended to be used as an aid in the diagnosis of Syphilis infection and as a screening test to prevent transmission of *Treponema pallidum* to recipients of blood, blood components, cells, tissue and organs. The aim of the current study is to evaluate the key performance characteristics like sensitivity and specificity of the HIV Ag/Ab Combo and Syphilis assays on a next-generation immunochemistry analyzer in comparison to the on-market comparator analyzer.

Methods: The HIV Ag/Ab Combo and the Syphilis assays were evaluated internally in regards to specificity and sensitivity. For HIV Ag/Ab Combo and Syphilis results were generated on the next-generation immunochemistry analyzer (test to be evaluated= TBE) and compared to results generated on the on-market comparator analyzer (test of record =TOR).

Sensitivity

HIV Ag/Ab Combo

Antigen Sensitivity: The analytical HIV-1 p24 antigen sensitivity was determined by evaluation of the WHO International Standard HIV-1 P24 Antigen NIBSC (code: 90/636) and the BIO-RAD HIV-1 Antigen Standard (LN 72217).

Antibody Sensitivity: Clinical sensitivity was evaluated on selected HIV-1 (n = 430 including 43 HIV-1 gO specimens) and HIV-2 (n = 115), positive specimens. Seroconversion sensitivity was assessed on sequential specimens from 37 seroconverting donors.

Syphilis

Sensitivity of the Syphilis assay was evaluated on the WHO 05/122 and WHO 05/132 standards and on 415 confirmed positive diagnostic specimens.

Specificity

Specificity of the HIV Ag/Ab Combo assay was investigated on 5340 blood donor specimen and on 211 diagnostic specimens. Specificity of the Syphilis assay was investigated on 5119 blood donor specimen and on 531 diagnostic specimens.

Results: Antigen sensitivity determined per linear regression ranged from 20.41 - 20.81 pg/mL for the TBE and was at 21.01 pg/mL for the TOR using the BioRad HIV Antigen Standard. The same assays exhibited antigen sensitivities of 0.73- 0.75IU/mL and 0.77 IU/mL using the WHO Standard. Antibody sensitivity was at 100% on both platforms. The first reactive time point for the TBE HIV Ag/Ab Combo assay occurred earlier in 2 panel sets and at the same time in the 35 remaining panel sets compared to the TOR. The HIV Ag/Ab Combo assay TBE exhibited a specificity of 99.93% (5336/5340) compared to 99.91% (5364/5369) on the TOR. The diagnostic specificity was at 99.53% (210/211) for both platforms.

The Syphilis TBE showed a clinical sensitivity of 99.52% (413/415). The two missed specimens were also negative on TOR. Donor specificity was at 99.94% (5116/5119), clinical specificity at 100.00% (531/531) on the TBE and 99.76% on the TOR. Analytical sensitivity was 0.01 IU/mL as measured against both WHO standards: 05/122 and 05/132.

Conclusion: The HIV Ag/Ab Combo and Syphilis assays showed equivalent or better performance in terms of sensitivity and specificity on the next-generation immunochemistry analyzer compared to the on-market comparator analyzer.

B-089

Concordance for HIV Combo assay between Chemiluminescence and Eletrochemiluminescence platforms

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Objective: The HIV diagnostic is ever a challenge to the laboratories, differences between methodologies, specificity and sensibility of the tests and the false reactive samples can be verified in the routine. This study aims to compare concordance of HIV combo assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics) in a large laboratory in Cascavel, Brazil.

Materials and Methods: 2602 samples were selected from the laboratory routine, with concentrations within assay linearity range for each assay. Samples were tested

in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies.

Results and Discussion: The results obtained in this study are showed in the Table 1.

Total Samples	*2602*	
Concordant Results	**2531**	**97.27%**
Nonreactive Samples Agreement	2303	88.51%
Reactive Samples Agreement	228	8.76%
Discordant Results	**71**	**2.73%**
Reactive Siemens x Nonreactive Roche	41	1.58%
Nonreactive Siemens x Reactive Roche	28	1.08%
Reactive Siemens x Inconclusive Roche	1	0.04%
Nonreactive Siemens x Inconclusive Roche	1	0.04%

Table 1: Results of comparative.

Conclusion: The results showed a good correlation (97.27%) between the cHIV assays analyzed, considering only the first result for each assay (initially reactive). The differences (2.73%) in the results of cHIV between platforms Siemens and Roche can be clarified by duplicate repetition, confirmatory assay WB and third assay with similar methodology.

B-090

Concordance for CMV assay between chemiluminescence and eletrochemiluminescence platforms

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Objective: Laboratories face a challenge regarding the CMV assays, mainly for the IgM tests. One of the principal issues to be solved is the IgM residual positive results. Some methodologies are more sensible and variations between positive and negative results for the same patient aren't rare. This study aims to compare concordance of CMV assays between the platforms Cobas E-170 (Roche) and ADVIA CentaurXP (Siemens Diagnostics) in a large laboratory in Cascavel, Brazil.

Materials and Methods: 641 and 578 samples were selected from the routine to be processed in the CMV IgM and IgG respectively. Samples were tested in ADVIA Centaur and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both. The discordant results (initially reactive for one of the tests) were processed in an Avidity IgG assay in an attempt to determine the result of better specificity.

Results and Discussion: The results are showed in the Table 1:

Total Samples	*CMV IgM*	*641*	*CMV IgG*	*578*
Concordant Results	**402**	**62.71%**	**571**	**98.79%**
Nonreactive Samples Agreement	339	52.89%	57	9.86%
Reactive Samples Agreement	50	7.80%	514	88.93%
Inconclusive Samples Agreement	13	2.03%	-	-
Discordant Results	**239**	**37.29%**	**7**	**1.21%**
Nonreactive Siemens x Reactive Roche	71	11.08%	7	1.21%
Nonreactive Siemens x Inconclusive Roche	57	8.89%	-	-
Reactive Siemens x Inconclusive Roche	13	2.03%	-	-
Reactive Siemens x Negative Roche	56	8.74%	-	-
Inconclusive Siemens x Negative Roche	32	4.99%	-	-
Inconclusive Siemens x Reactive Roche	10	1.56%	-	-

Table 1: Total Samples Results Classification

IgM discordant results were evaluated analyzing the Abbott IgM results as well as Avidity IgG results. When a patient have IgM variable between the methodologies and a high avidity, the IgM found were considered as a residual. Thus, of the 239 (37.29%) discordant results 21 (3.28%) are considered result resultant of residual for Roche test, 57 (8.90%) for the Siemens test and 9 (1.40%) for both.

Conclusion: The results showed a lower correlation for CMV IgM (62.71%) comparing with the CMV IgG assay (98.79%). Part of the differences in the results of CMV IgM is due the IgM residual results. The percentage of IgM residual could be observed in both assays analyzed. Those differences between platforms Siemens and Roche can be clarified in routine using a third assay and also the Avidity IgG Assay.

B-091

Concordance for HVA Total and IgM assay between chemiluminescence and eletrochemiluminescence platforms

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Objective: The assays for HAV Total and HAV IgM are used to define the serological status for Hepatitis A. Some assays have different specificities and sensitivities and the laboratories deal with some variant results. The present study aims to compare concordance of HAV Total and HAV IgM assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics), in a large laboratory in Cascavel, Brazil.

Materials and Methods: 279 and 408 samples were selected from the laboratory routine to be tested in HAV Total and HAV IgM respectively. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. Reactive samples were used to the precision test. The correlation was performed based in the first result without a duplicate repetition in both methodologies. High and low reactive and samples were used to the precision test in 2 different sample pools repeated 20 times.

Results and Discussion: The results concerning the present study are showed in the Table 1.

Total Samples	**HAV Total**	**n= 279**	**HAV IgM**	**N=408**
Concordant Results	268	96.06%	405	99.26%
Nonreactive Samples Agreement	73	26.16%	341	83.58%
Reactive Samples Agreement	195	68.89%	64	15.69%

Table 1: Comparative results.

The HAV Total assay showed 4.30 % for within run precision and 6.57% for between runs precision. The HAV IgM assay within run precision was 6.13% and 4.08% for the between runs precision.

Conclusion: The results showed a good correlation (96.06% and 99.26%) between the HAV Total and HAV IgM assays analyzed, considering only the first result for each assay (initially reactive). The differences in the results of HAV Total (3.94%) and HAV IgM (0.74%) between platforms Siemens and Roche can be clarified by duplicate repetition, third assay with similar methodology and patient historic.

B-092

Concordance for HBV markers assay between chemiluminescence and eletrochemiluminescence platforms

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Objective: The laboratory labors with different performance to the screenings assays, mainly with the HBV markers. There are some false reactive results due different specificities for the assays. The laboratories are facing a challenge to diagnostic HBV with good performance assays. The present study aims to compare concordance of HBV assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics), in a large laboratory in Cascavel, Brazil.

Methods: 6526 samples were selected from the laboratory routine to be tested in the HBV markers. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies. Reactive samples were used to the precision test in a pool sample repeated 20 times.

Results: The results from this study are demonstrated in the Tables 1 and 2:

Assay (Total Samples)		HBsAg (2473)	Anti-HBs (2258)	HBc Total (517)	HBc IgM (432	Anti-Hbe (441)	HBeAg (405)
Concordant Samples% (n)		97.9 (2420)	96.1 (2170)	97.5 (504)	98.1 (424)	94.3 (416)	99.0 (401)
Discordant Samples% (n)	NR Siemens x R Roche	0.9 (21)	1.3 (30)	2.5 (13)	_	0.5 (2)	_
	R Siemens x NR Roche	0.6 (15)	2.6 (58)	_	0.9 (4)	3.6 (16)	1.0 (4)
	R Siemens x INC Roche	0.3 (9)	_	_	_	_	_
	NR Siemens x INC Roche	0.3 (8)	_	_	_	_	_
	INC Siemens x NR Roche	_	_	_	0.2(1)	1.1 (5)	_
	INC Siemens xR Roche	_	_	_	_	0.5 (2)	_
Precision Results	Within RunPrecision	1.9%	2.0%	3.8%	4.8%	1.9%	3.4%
	Between RunPrecision	7.4%	3.1%	5.4%	6.5%	1.8%	8.7%

Table 1: Comparative and precision results.

Conclusion: The results showed a good correlation between the HBV markers assays analyzed, considering only the first result for each assay (initially reactive): HBsAg 97,86%, anti-HBs 96,10%, anti-HBc Total 97,49%, anti-HBc IgM 98,15%, anti-HBe 94,33% and HBeAg 99,01%. The differences in the results of HBV between platforms Siemens and Roche can be clarified by duplicate repetition and also third assay with similar methodology.

B-093

Evaluation of modified FibroTest Formula in Patients with Genotype 4 Hepatitis C.

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Background: Liver fibrosis is usually diagnosed with core needle liver biopsy which is considered to be an invasive procedure and may be harmful to the patient. Non-invasive methods and formulas have been suggested to replace these invasive procedure. For example, "FibroTest" formula has been validated for the initial diagnosis of fibrosis and for the monitoring of patients. The "Fibrotest" formula is based on the calculations of blood biochemical parameters. Many of these formulas where only validated in developed countries with HCV genotype 1 patients. Conversely, limited studies have been conducted in the developing countries including Saudi Arabia, where genotype 4 predominates. The main objective of this study is to validate and evaluate some the non-invasive modified "Fibrotest" formula in hepatitis C genotype 4 patients with or without liver fibrosis. **Methodos:** A cross sectional study that utilized adult genotype 4 HCV patients who attend the hepatology clinic. Two groups were initially evaluated for fibrosis using transient elastography "FibroScan" , "Fibrotest" and liver biopsy. Metavir histological scoring system were used for staging of fibrosis in liver biopsies. The "Fibrotest" formula which includes 6 serum markers, Alpha-2-macroglobulin, Haptoglobin, ApolipoproteinA1 (Apo A1), Gamma-glutamyl transpeptidase (GGT), Total bilirubin and Alanine transaminase (ALT). Along with patients' age and gender. The modified fibrotest formula was used by replacing the Apo A1 with high density lipoprotein (HDL). All of these markers were measured in serum by Architect analyzer from Abbott. The first group included 26 patients diagnosed with hepatitis C genotype 4 with mild fibrosis (F0, F1) on fibrotest. The second group included 54 hepatitis C patients moderate and severe liver fibrosis (F2, F3, F4) on fibrotest. Fibrotest and modified fibrotes were calculated using markers and compared with the biopsy and fibroscan in both groups. **Results:** The samples consisted of 46 Females (57%) and 34 males (43%) with a mean age of 53 years (std 12). When both fibrotest and the modified fibrotest were compared, agreement was found to be 80% in stage F0, 72% in F1, 67% in F2, 50% in F3 and 96% in F4. When fibrotest was compared with biopsy, agreement was found in 58% of F0, 78% of F1, 18% of F2 and 35% of F4. Similar results were found with the modified fibrotest by 67% in F0, 78% in F1, 15% in F2, and 32% in F4. Finally, comparison of fibrotest and fibroscan were found to be with agreement in 50% of F0, 100% of F1, 10% of F2, 60% of F3 and 54% of F4. Modified fibrotest and fibroscan was agreed in 50% of F0, 100% of F1, 14% of F2, 50% of F3 and 47% of F4 **Conclusion:** Both fibrotest and the modified fibrotest were comparable to each other and moderately to fibroscan. However, the use of noninvasive fibrosis formulas were shown to be less superior to liver biopsy in our study especially at late stage of liver fibrosis.

B-094

Concordance for Rubella IgM and IgG assay between chemiluminescence and eletrochemiluminescence platforms

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Objective: Laboratories regularly find out difficulties in the Rubella assays, mainly for the IgM tests. IgM residual results in IgG positive patients and reactions with unspecific antibodies are some issues to be solved. Some methodologies are more sensible and variations between positive and negative results for the same patient aren't rare. This study aims to compare concordance of Rubella IgM and IgG assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics) in a large laboratory in Cascavel, Brazil.

Materials and Methods: 497 and 471 samples were selected from the laboratory routine to be processed in the Rubella IgM and IgG respectively. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies.

Results and Discussion: The results obtained in this study are showed in the Table 1:

Total Samples	Rubella IgM	497	Rubella IgG	471
Concordant Results	402	80.56%	411	87.26%
Nonreactive Samples Agreement	373	74.75%	32	6.79%
Reactive Samples Agreement	26	5.21%	378	80.25%
Inconclusive Samples Agreement	3	0.60%	1	0.21%
Discordant Results	95	19.04%	60	12.74%
Nonreactive Siemens x Reactive Roche	60	12.02%	3	0.64%
Nonreactive Siemens x Inconclusive Roche	24	4.81%	3	0.64%
Reactive Siemens x Inconclusive Roche	9	1.80%	19	4.03%
Reactive Siemens x Nonrective Roche	-	-	18	3.82%
Inconclusive Siemens x Reactive Roche	2	0.40%	3	0.64%
Inconclusive Siemens x Nonreactive Roche	-	-	14	2.97%

Table 1: Total Samples Results Classification

Conclusion: The results showed similar correlations for Rubella IgM (89.94%) and IgG assay (80.56%). The differences in the results of Rubella IgM are due the IgM residual results, when both IgM and IgG are reactive for the assays. Those differences between platforms Siemens and Roche can be clarified in routine using a third assay and also the Avidity IgG Assay.

B-096

Evaluation of Novel Respiratory Assays on the Fully Automated Panther Fusion System

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Background: Respiratory viral infections remain a leading cause of infectious diseases worldwide. Since most respiratory viruses present with similar symptoms and many patients are co-infected, clinicians rely on molecular diagnostics to rapidly and accurately identify one or more viral agents to ensure appropriate patient management. Multiple novel respiratory assays are being developed to run on the fully automated Panther Fusion system (in development) with random access capability, minimum sample handling, and short load-to-first result. The Panther Fusion Flu A/B/RSV, Para 1/2/3/4, and AdV/hMPV/RV assays can all be run from a single nucleic acid isolate. These qualitative multiplex RT-PCR assays detect multiple targets utilizing individualized assay specific reaction pellets. Performance of the Panther Fusion Flu A/B/RSV assay which detects and differentiates Flu A, Flu B and RSV, and the Panther Fusion Paraflu assay, which detects and differentiates human Parainfluenza (HPIV) types 1, 2, 3 and 4 were evaluated to demonstrate rapid, reproducible, and reliable detection of respiratory targets.

Methods: Reproducibility was tested at 0.5 log above LoD using virus spiked in nasopharyngeal clinical matrix and tested for both assays. Clinical performance of each viral target was compared to various on-market assays.

Results: Percent agreement of the reproducibility study for all intended targets for both assays was 100%. Concordance of clinical performance to on-market assays was high with positive and negative agreements of 96.5-100% and 96.0-100%, respectively, for all intended targets for both assays. Time-to-first result was less than 2.5 hours.

Conclusion: These results demonstrate the Panther Fusion Respiratory Assays, Panther Fusion Flu A/B/RSV, and Paraflu assays are suitable candidates for repeatable and accurate identification of Influenza A, Influenza B, RSV, and HPIV subtypes 1-4.

B-097

Pleural fluid lactate for diagnosis and management of parapneumonic pleural effusion

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Background: Parapneumonic pleural effusions (PPE) are exudates associated to pneumonia, lung abscess or bronchiectasis, they are called empyemas when containing pus. Approximately 40% of patients hospitalized with pneumonia have pleural effusions. Morbidity and mortality are higher in patients with pneumonia and pleural effusion than in those without pleural effusion. In the management of PPE, the relevant challenge is to discriminate between uncomplicated (UPPE) and complicated PPE (CPPE) for identify which PPE require chest tube drainage (CPPE and empyemas). Pleural fluid lactate levels may be useful for diagnosis and management of PPE; however, there are very few published studies on the quantification of lactate in pleural fluid. New studies assessing the diagnostic value of pleural fluid lactate are necessary. The aim of this study was to measure the accuracy of pleural fluid lactate concentration for diagnosis of PPE and to discriminate between UPPE and CPPE. **Methods:** We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. After to centrifugation of sample in sterile tube at 4000 rpm for 5 minutes, lactate was measured in the supernatant of pleural fluid using automated analyzers Dimension EXL (Siemens Diagnostics®). The reference interval of plasma lactate with this method is < 2.1 mmol/L. Patients were classified into two groups according to the etiology of pleural effusion: PPE and NOT PPE. The PPE patients were considered: a) Patients with pleural effusion and pneumonia, abscess, or bronchiectasis diagnosed by radiography, computed tomography or magnetic resonance imaging; or b) Patients with Gram stain or positive culture of pleural fluid. Statistical analysis was determined using receiver operating characteristic (ROC) techniques, analysing the area under the ROC curve (AUC) by the software MEDCALC®. **Results:** We studied 173 patients with ages between 1 to 96 years (median = 64 years), 83 women and 90 men. Thirty patients were PPE (10 UPPE and 20 CPPE) and 143 were NOT PPE (37 transudates, 88 malignant, 5 tuberculosos and 13 other etiologies). The AUC value was 0.831 ($p<0.0001$) and the optimal cut-off value was 5.6 mmol/L exhibiting 70% sensitivity and 90.9% specificity for diagnosis of PPE. Also, pleural fluid lactate could be use to discriminate between UPPE and CPPE, the AUC value was 0.740 ($p=0.0089$) and the optimal cutoff value was 10.2 mmol/L, exhibited 45% sensitivity and 90% specificity. **Conclusions:** Pleural fluid lactate has a high accuracy for diagnosis and management of PPE. Pleural fluid lactate > 5.6 mmol/L may be included among the biochemical parameters used to define PPE, and pleural fluid lactate > 10.2 mmol/L to identify which PPE require chest tube drainage.

B-098

Total bile acid levels in hepatitis C RNA positive patients with liver fibrosis

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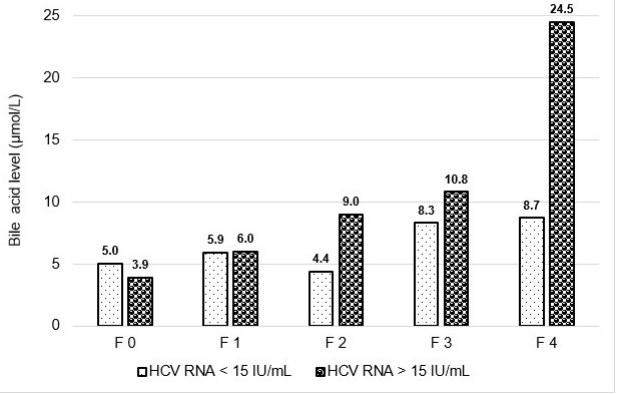
Background: Bile acids (BA) are liver-specific products of cholesterol metabolism, and their measurement in blood is used as test of hepatobiliary dysfunction. Furthermore, BA have been associated with increased viral replication in hepatitis C virus (HCV) infected cells. BA levels were also shown to positively correlate with increasing stage of liver fibrosis in HCV patients. Our objective was to investigate the relationship between BA levels, HCV viral load, and liver fibrosis stage in order to assess the utility of total BA as an HCV monitoring test.

Methods: 172 patient samples (110 men, 62 women) tested for HCV viral load by real-time PCR were frozen at -20° C prior to BA testing. The total BA levels were quantified using Diazyme reagent on Beckman DxC instrument. All patients had fibrosis stage determined by FibroSure.

Results: Among the 172 patient samples, 84 (49%) had HCV RNA > 15 IU/mL, while 88 (51%) had HCV RNA < 15 IU/mL. Patients with fibrosis stages 1-4 in HCV RNA > 15 IU/mL group had higher mean total BA levels compared to HCV RNA < 15 IU/mL group, and they showed increasing BA levels consistent with fibrosis stage (Figure 1). However, the difference in BA levels between HCV RNA < 15 IU/mL and > 15 IU/mL groups was statistically significant only for patients with stage 4 fibrosis, with total BA means of 8.7 µmol/L (95% CI of 8.0 - 13.2) and 24.5 µmol/L (95% CI of 18.5 - 31.2), $p < 0.001$, respectively.

Conclusions: An increasing trend in BA levels relative to fibrosis stage in patients with HCV RNA > 15 IU/mL was observed. The statistically significant difference in BA levels among the two patient groups was confined to stage 4 fibrosis. Total BA test may be useful as a monitoring tool, particularly for patients with stage 4 HCV fibrosis.

Figure 1. Mean Plasma Bile Acid Levels and HCV Viral Load



B-099

Calprotectin evaluation as an inflammation marker in different stages of Kawasaki disease

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Kawasaki disease (KD) is the most common systemic vasculitis syndrome that primarily affects infants and young children. KD is in most cases a self-limited illness resolving within few weeks after fever onset. It preferentially affects small and medium-sized arteries, particularly the coronary arteries, and in 15 to 25% of patients, it will result in coronary aneurysms or dilatation. The natural history of coronary artery lesions in KD furnish evidences for a continuous active pathological process after the decline of acute symptoms. According to histological studies, even apparently normal coronary artery with no history of dilated lesions could show some degree of intimal thickening several years after the disease onset. Consequently, effective markers of inflammation and autoimmunity are needed for an effective follow-up of KD children both during the acute and subacute phase as during the following years. Among several inflammation markers available, Calprotectin (CP), a calcium- and zinc-binding protein of the S100/calgranulin family has been successfully used as a marker in several inflammatory and autoimmune diseases. The objective of the present study is to evaluate the use of Calprotectin (CP) as a serological marker in the assessment of inflammation in patients during the different stages of KD. Thirteen patients (2.5 years ± 1.2) were selected from January 2013 to December 2014, among patients referred to the Brasilia Children's Hospital Jose Alencar (Federal District, Brazil) for KD diagnostic confirmation and follow-up. Serum samples were collected following standard H3-A6 of Clinical and Laboratory Standards Institute (CLSI) and stored at -80°C until analysis. Calprotectin was assayed by ELISA method using a commercial kit according to the manufacturer's instructions. For analysis, the samples were stratified into three groups according to the stage of KD (acute, subacute and chronic) and calprotectin levels were detected at three different times: during the first 10 days of disease (acute phase, n = 3), between 30 and 60 days (subacute phase, n = 4) and ≥150 days after diagnosis (chronic phase, n = 6). The acute phase sample results (up to 10 days of diagnosis) disclosed levels of CP of 231.2 ± 23.3 ng/mL. Levels of 68.43 ± 16.6 ng/mL were detected during the subacute phase, and levels of 113.6 ± 55.6 ng/mL during the chronic phase. Our preliminary data

indicate that high levels of CP can still be detected during the chronic phase of KD suggesting that an inflammatory process continues active. Further study on the presence sustained inflammatory process during KD evolution and on the use of CP as an inflammatory marker are needed and should be performed.

B-100

Robust and reliable Screening of HPV Subtypes by Mass Spectrometry

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Background: High-risk Human Papilloma Virus (HPV) tests were recently approved by the U.S. Food and Drug Administration (FDA) as a primary screening tool for cervical cancer risk in women aged 25-65 years without a simultaneous Pap smear. It can be predicted that pathologists and molecular biologists will be faced with a significantly increased demand for molecular HPV testing in the near future. Consequently, test systems will be required for the specific and sensitive detection of high- and low-risk HPV subtypes in a time and cost effective manner.

The mass-spectrometry-based MassARRAY System® (Agena Bioscience, Inc.) has the potential to meet all these requirements as it comprises of a comprehensive set of tools to run multiplexed panels in clinical studies that achieve an unsurpassed level of specificity, sensitivity and sample throughput, at a low per sample cost.

Methods: We developed an assay for the MassARRAY to screen for the presence or absence of 19 specific HPV types plus a positive control housekeeping assay. The HPV panel consists of a single multiplex-reaction allowing minimal sample input. We compared the MassARRAY data to the results of hybridization assays (COBAS and Chipron). In total 45 formalin fixed paraffin embedded (FFPE) tissue samples and 10 Pap smear liquid samples were analyzed. Moreover we assessed three different DNA isolation methods.

Results: All high risk HPV subtypes detected by the COBAS or the Chipron system could be also detected by the MassARRAY HPV panel. Moreover, the MassARRAY panel outperformed the number of HPV subtypes detected when a high yield DNA extraction process was utilized.

Conclusion: We conclude that the MassARRAY HPV panel represents a highly specific, sensitive and reliable method for the detection of HPV subtypes in FFPE samples and Pap smears in a high throughput setting.

The MassARRAY® System is For Research Use Only. Not for use in diagnostic procedures

B-101

Clinical Utility of Interferon Lambda 3 and BAFF polymorphisms as predictors of treatment response in patients with HCV

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The goal of treatment in patients with chronic hepatitis C virus infection is to eradicate hepatitis C virus (HCV) RNA, which is predicted by the attainment of a sustained virologic response (SVR). A SVR is associated with a 99 percent chance of being HCV RNA negative during long-term follow-up. Treatment with pegylated interferon 2a or 2b combined with Ribavirin (RBV) has been considered the standard of care (SOC) for HCV treatment. Approximately 50% of patients with HCV exhibit Mixed Cryoglobulineamia which is a bengin but prelymphomatous condition. **Aim** of this work was to evaluate the clinical utility of interferon Lambda-3 polymorphism rs12979860 and its association with -871 C/T BAFF polymorphisms as predictors of viral clearance and treatment response as well as MC occurrence among HCV treated patients. **Patients**: Analytical cohort study conducted on 100 HCV patients. They received pegylated interferon and ribavirin as treatment of choice. All the patients have signed an informed consent. **Methods**: HCV quantitation by COBAS AmpliPrep ,TaqMan PCR,Genotyping of HCV using the Abbott Real-time HCV Genotype II . Levels of BAFF mRNA were determined by real-time quantitative PCR. **Results:** Males were more non-responder than females. The level of viremia showed that a total of 68 patients were responders; 23.5% with very low level of viremia (< 200,000 U/ml) with the rest of responders had viremia level < 600,000 to 800,000 U/ml . All responders achieved RVR after 4 weeks and maintained e-RVR after 12 weeks and predict more than 90% SVR. IL-28B polymorphysim rs12979860 showed 68 patients who were responders; 22 (32.4%) of them had IL-28B genotype CC, 37 patients of them (54.4%) had CT type, and 9 patients (13.2%) had TT genotype. The other 32 patients who were non responders showed increasing in CT genotype in 81.2% and 8.8% for TT genotypes. There was statistically significant difference between IL-28B polymorphism C allele in responders group for treatment (MCP=0.002) (P≤0.05).Regarding the BAFF -871 C/Tgenotype the TT genotype was significantly increased in HCV patients whether with MC or without compared with the control group (P=0.036) . Among responders with MC 52 patients were positive for TT with 16 patients with CT genotype which was proved to be statistically significant with P <0.001 when compared with patients without MC . 6 Patients showed CT polymorphism, while those without MC; Baff -871 TTgenotype for CC was positive among 58 patients, 4 patients with CT polymorphism which proved to be statistically significant with P <0.001. **Conclusion**: in the present study, we demonstrated that CC genotypes of rs12979860 significantly determined SVR in patients with HCV mainly genotype 4.Carrying at least one copy of the C allele increased sensitivity to PEG-IFN/Ribavirin therapy, which was 3 times that of rs12979860-negative hosts. The prevalence of the BAFF -871TT genotype was significantly associated with the formation of Mixed Cryoglobulineamia and could be used as a marker for follow up.

Key Words : HCV , PCR , BAFF (B Cell Activating Factor)

B-102

Serum fibrosis marker panels FIB-4 and APRI are statistically equivalent to AST alone at predicting liver fibrosis in a cohort of 1733 hepatitis C virus infected patients

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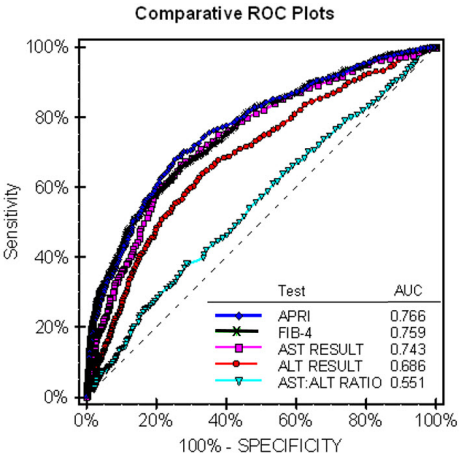
Background: With the recent availability of direct-acting antiviral drugs for hepatitis C virus (HCV) capable of inducing sustained virologic responses, efficient tools are needed to rapidly identify higher stage HCV-infected patients to prioritize them for early treatment. Fibroscan (transient elastography) is less invasive than liver biopsy and compares well to biopsy, however, Fibroscan availability is limited. Compared to biopsy, several studies demonstrated favorable performance of non-invasive multi-analyte serum fibrosis marker panels FIB-4 (index derived from alanine amino-transferase [ALT], aspartate aminotransferase [AST], platelets, and patient age) and APRI (AST-to-platelet ratio index). Serologic tests capable of identifying cohorts with higher stages of fibrosis at the time of HCV diagnosis would greatly aid in prioritizing patients for treatment. Our objective was to validate FIB-4 and APRI for staging fibrosis in our HCV-infected population.

Methods: Fibroscan results from 1733 HCV-infected patients were mapped to an F0-F4 equivalent scale. AST, ALT, and platelet results obtained 5 months before or 1 month after the date of the Fibroscan were used to calculate FIB-4 and APRI scores.

Results: The areas under the receiver operating characteristic curve (AUC) for distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) were not significantly different between FIB-4 (0.76; 95% CI 0.73-0.78), APRI (0.77; 95% CI 0.74-0.79), and AST (0.74; 95% CI 0.72-0.77). The AUCs for ALT (0.69; 95% CI 0.66-0.71) and AST:ALT ratio (0.55; 95% CI 0.52-0.58) were significantly worse. (See Figure 1.)

Conclusions: We concluded that AST alone was as effective as FIB-4 and APRI at distinguishing severe from mild-to-moderate fibrosis. Using a cutoff of ≥56 IU/L (normal AST reference range is 10-40 IU/L), AST is 59% sensitive and 79% specific for identifying severe fibrosis and could be used to prioritize patients for early treatment. In contrast to published studies, we concluded that no test was sufficiently sensitive to rule out severe fibrosis.



B-103

Increase Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) Producing Enterobacteria in Urinary Tract Infection in Long-Term Care facilities.

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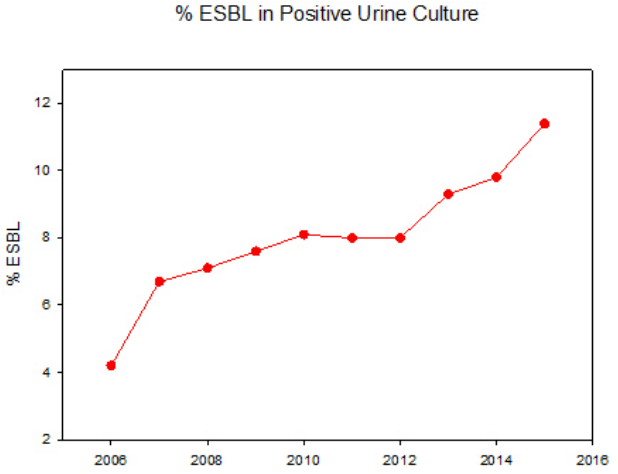
Background: Urinary tract infection (UTI) is one of the most common infections in the geriatric population; it is considered the second cause of community-acquired infection and nosocomial infection. Most of the UTI in long-term care facilities are caused by gram negative bacteria; however, Extended-Spectrum Beta-Lactamase producing enterobacteria are a big concern in these facilities where they can spread rapidly where most of the residents are elderly, frail and are on multiple medications. In addition, identifying these organisms in urine culture is becoming a challenge to physicians by limiting their therapeutic options since they are resistant not only to extended-spectrum cephalosporins but other antibiotics such as aminoglycosides and sulfonamides.

Design: We analyzed data from 604,890 specimens collected for urine culture from 2006-2015 from residents in Long-Term Care facilities. Cultures were done utilizing MicroScan Walkaway 96 conventional panels. We calculated the prevalence of positive cultures and the prevalence of ESBL among the population tested. No growth and <10,000 colonies were considered negative, cultures with > 50,000 colonies were considered positive.

Results: More than 50 % of cultures were positive across the years tested, and >70% of the positive cases were due to gram negative bacteria. ESBL producing bacteria accounted for 15.2% of the gram negative bacteria and 11.4% of all positive culture in 2015; the number reflects more than double the results from 2006 (6.0% and 4.2% respectively).



Conclusion: Our results showed a progressive increase in ESBL producing enterobacteria in elderly patients and it supported the notion that Long-Term Care facilities could be a reservoir for these microbes. An infection control and antibiotic stewardship should be implemented to identify the risk factors for ESBL and optimize the use of antibiotics to improve patient outcome, limit the spread of these bacteria in the geriatric population, and reduce the cost of patient care.

B-104

cerebro spinal fluid adenosine deaminase levels in tuberculous and pyogenic meningitis

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Background:

Tuberculosis is still leading cause of mortality and morbidity in India particularly in rural and tribal population. The annual death rate is more than 400,000 a year. Although there were several programmes like NTPC (National tuberculosis control programme) and RNTCP (Revised national tuberculosis control programme still the the mortality is high. Mostly due to lack of early diagnostic and discontinuity of treatment. Tuberculos meningitis is one of the emergencies in these population especially in children which requires immediate hospitalization and treatment. Though there are diagnostic procedures like sputum AFB,CSF analysis and still needs better methods for diagnostic and prognostic use. The present study is determination of Adenosine deaminase enzyme which is important in purine degradation in tuberculosis meningitis patients and pyogenic meningitis patients before and after treatment.

Methods:

60 clinically symptomatic cases of meningitis admitted between 16 and 50 years of age in medical wards of King George Hospital, Visakhapatnam, India between January 1999 and November 1999 were enrolled the present study. Out of these 60 cases there was 23 cases with tuberculous meningitis, 9 cases were diagnosed as pyogenic meningitis and remaining with other causes which were not included in study. There were 12 normal adults were also in this whose diagnosis was not meningitis. Lumbar puncture was done on the day1, day 8 and day 30 for tuberculosis patients, day1 and day 8 for pyogenic meningitis patients and was only once to the controls. CSF was collected and examined for levels of Adenosine Deaminase by authentic method. Anti tuberculous treatment was started for tuberculous meningitis patients and anti bacterial treatment was started for pyogenic meningitis patients.

Results: The levels of adenosine deaminase significantly high in both pyogenic and tuberculosis patients when compared with controls p-value < 0.05 (3.86+/-0.15). The levels of adenosine deaminase was significantly decreased on day 8 after treatment p-Value < 0.05(13.04+/-1.74 on day 1 and 9.4 +/- 1.2 in day 8.). In tuberculous meningitis it was the levels of adenosine deaminase levels were significantly elevated on day 8 after treatment(p-value < 0.05) and significantly reduced on day 30 of the treatment (p-value < 0.05) (15.08+/- 3.15 on day 1 ,17.99+/-4.81 on day8 and 9.4+/-1.9 on day 30) .

Conclusion:

The present study the therapeutic response of pyogenic meningitis was immediate according to the levels of adenosine deaminase and is delayed in case of tuberculosis. Hence this ADA activity is useful tool in bacterial meningitis as well as in tuberculous meningitis.

B-105

Use of AccuPlex Recombinant Sindbis Virus Technology to Produce a Noninfectious, Whole Process Zika Control

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Background: An outbreak of the mosquito-borne Zika virus occurred in Brazil in spring of 2015. Since that time, the virus has gained global attention due to its rapid spread through at least 21 countries of the Americas and its possible link to neurological birth defects. In response to this outbreak, diagnostic laboratories and test developers need to design, manufacture and validate molecular diagnostic assays and this requires stable, reproducibly manufactured positive reference materials. SeraCare has developed the AccuPlex™ Zika Positive Reference Material using recombinant Sindbis virus technology. This well established recombinant viral technology has many advantages as a NAT quality control material: it mimics clinical samples because it undergoes the entire extraction procedure; it is non-infectious and ensures biological safety for lab personnel; and it has extended stability at 2-8 °C and does not require freezer storage.

Methods: The Zika virus is a positive sense RNA virus whose genome is approximately 10.7 Kb. The Zika genome was divided into four (4) segments and each segment was used to generate an AccuPlex™ recombinant virus using Sindbis vector system. Construct #1 contains Capsid (C), precursor Membrane (M), envelop (E), and non-structural protein 1 (NS1) genes. Construct #2 contains NS2A, NS2B, and NS3 genes. Construct #3 contains NS4A and NS4B, and Construct #4 contains NS5 gene. Each construct is designed to contain ~150 bp of sequence which overlaps with the other constructs to assure that a mixture of the recombinant viruses is compatible with all Real Time PCR targets (even those that may span the junction between genes). Each construct was used for in vitro RNA transcription, and RNA was introduced into BHK-21 cells where recombinant Sindbis virus particles were assembled. The viruses were heat inactivated and purified. The strategy of dividing the Zika genome into four different recombinant viruses such that each recombinant virus is not functional assures the safety of the reference material and the heat inactivation serves as an additional safety precaution.

Results: Initial titering of the purified viral supernatant used digital PCR analysis and either primers/probes specific for the Sindbis vector or primers/probes to the Zika envelop region as in Lanciotti et al.[1]. The recombinant viruses were mixed and diluted

into defibrinated human plasma at a concentration of 1.0E+05 copies/mL. Ongoing accelerated and real time stability studies indicate that AccuPlex™ recombinant viruses are stable at room temperature for at least 17 months and at 4 ºC for at least two years. Accuracy and precision data on PCR based assays will be presented.

Conclusions: SeraCare has developed a stable, well-characterized whole process control for Zika virus. This reference material will enable laboratories to validate tests and train technicians to ensure preparedness. The Accuplex™ Zika product demonstrates the utility of recombinant virus technology to produce non-infectious controls for dangerous viruses that are difficult to source or propagate.

1. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis Volume 14, Number 8. 2008 Aug.

B-106

EVALUATION ANTIFUNGAL ACTIVITY OF DRUGS ANTIFUNGAL AND CRUDE EXTRACTS FROM BRAZILIAN CERRADO PLANTS AGAINST CLINICAL ISOLATES CANDIDA SPECIES

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Most cases of systemic candidiasis were assigned to the *Candida albicans* species. However, non-*albicans Candida* have been identified as human pathogens in infections representing a change in prevalence with a percentage at times related to *Candida albicans*. This change in the etiology of systemic candidiasis is related to new diagnostic methods, such as identification of species using molecular techniques and may reflect on the high rate of resistance to antifungal *Candida* non- *albicans*. This study´s main proposed objective is to characterize clinical isolated cases of *Candida* spp. to epidemiological and therapeutic aspects resisting to antifungal agents during treatment. Plant extracts of Brazilian Cerrado were used to evaluate. Microbiological identification on non-molecular and molecular methods, and disk diffusion test were used as a protocol reference. The microbiological identification methods characterized 150 isolated clinical in patients suspected of systemic infection in the Federal District, Brazil, between January/2011 and December/2012. *Candida albicans* is the most commonly isolated species (50.6%), followed by *Candida parapsilosis* (21.3%), *Candida tropicalis* (16.6%), *Candida glabrata* (8.0%), *Candida krusei* (0.7%), *Candida guilliermondii* (0.7%), *Candida intermedia* (0.7%) and *Kadamaea ohmeri* (0.7%), which were isolated in bronchoalveolar lavage samples, blood and mucus, predominantly in male patients aged between 21 and 60 years. The disk diffusion test was used to determine the antifungal activity of conventional agents and plant extracts. In conventional antifungal agents, most isolated clinical cases of *Candida* were sensitive to amphotericin B (97.9 %); 91.9 % were susceptible to voriconazole; 91.3 % to fluconazole; 73% itraconazole and 2.7 % 5- fluorocytosine. The resistance to azoles has been demonstrated by isolated cases of C. glabrata and 5-fluorocitocina for all *Candida* species. When evaluating the antifungal activity of the extracts, the six species of plants, *Eugenia dysenterica*, *Pouteria ramiflora*, *Pouteria* torta, *Erythroxylum subrotundum*, *Erythroxylum daphnites* and *Bauhinia rufa* showed inhibitory effect on various *Candida* species, and *Candida glabrata* the most inhibited species. Our results suggest new therapeutic targets with potential antifungal activity in extracts evaluated primarily on *Candida glabrata*.

B-107

Molecular Epidemiology of the Clonal Relationship among *Klebsiella pneumoniae* Carbapenemase Strains recovered from Six Brazilian Hospitals using DiversiLab System

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Background: The emergence and intra-hospital dissemination of multidrug-resistant Gram-negative microorganisms and carbapenemases producers have been described in Brazil, as well as in many parts of the world. The inter-hospital evaluation of the relationship among *Klebsiella pneumoniae carbapenemase* (KPC) recovered from different hospitals could be helpful to understand the dynamic of the resistance dissemination in a specific region. The aim of this study was to evaluate the relationship among KPC samples isolated from several Brazilian public hospitals represented by the North, South and East regions of the São Paulo city. **Methods:** Between November and December 2014 was selected a group of *K. pneumoniae* strains resistant to cephalosporins and carbapenems. The samples were isolated from six different public hospitals of the São Paulo city. The hospitals were identified by the letters HA to HF. Eight samples per hospital and only one per patient was included in this study. The bacteria identification was performed using mass spectrometry (Vitek-MS, bioMerieux) and the minimal inhibitory concentration (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux). The presence of cabapenemases (KPC, VIM, IMP, NDM and OXA-48) and $bla_{\text{CTX-M}}$ gene were determined by PCR using specific primers for each gene. The genetic relatedness of the strains was characterized by automated repetitive extragenic palindromic polymerase chain reaction (Rep-PCR) based DiversiLab system (bioMerieux) and the analysis of the profiles was performed using Pearson's Correlation in the DiversiLab software (version 3.4). Isolates with similarities of < 95% were considered different, and isolates with similarities of >97% were considered indistinguishable. Isolates with similarities of >95% and <97% were categorized manually using the pattern overlay of the analysis tool in the software. **Results:** All 44 samples were identified as *Klebsiella pneumoniae* and all of them, were detected high level of resistance to imipenem and meropenem (MIC, ≥ 4 µg/mL) according to the CLSI, 2014 guidelines. The presence of the bla_{KPC} and $bla_{\text{CTX-M}}$ genes was detected in 44 and 33 samples, respectively. The rep-PCR analysis identified five different clusters (C1 to C5) and 11 patterns among the 44 isolates. Three of the five clusters (C3, C4 and C5) were identified in only one sample, each and represent three different hospitals (HA, HB and HF). On the other hand, cluster C1 showed two patterns and were identified in 15 isolates recovered from four hospitals (HB, HC, HD and HE). The cluster C2 showed six patterns and were identified in 26 isolates recovered from all six hospitals (HA, HB, HC, HD, HE and HF). The clusters C1, C2, C3, C4 and C5 showed a pattern of similarity of <75%. **Conclusion:** Our results reveal the prevalence and the spread of two main clusters (C1 and C2) of KPC in different hospitals and regions of São Paulo city. These findings reinforce the continued need for infection control, antibiotic management and application of an intra and inter-hospital surveillance system.

B-108

Comparing Plasma and Urine Parallel Samples for the Diagnosis of Zika Virus by Qualitative Real-Time PCR

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Background: Since May 2015, Brazil Ministry of Health confirmed a ZIKV outbreak in the regions where *A. aegypti* is widely distributed. Furthermore, sexual and blood transfusion-transmission have recently been reported, in addition to transplacental transmission. Several cases of the current newborn microcephaly outbreak in Brazil are potentially linked to ZIKV epidemics besides increased frequency of Guillain-Barré syndrome, also associated to ZIKV infection. However there are no specific symptoms for Zika fever, which may be confounded to other arboviroses affecting Brazil at the present moment. These concurrent outbreaks call for accurate diagnostic tools of limited availability. The best way to diagnose Zika infection is during the acute phase detecting viral RNA in plasma (0 to 5 days after symptoms onset) and urine (until the 15$^{\text{th}}$ day after symptoms onset) by reverse transcriptase PCR (RT-PCR). Serological diagnosis might be misleading due to cross-reactivity of ZIKV with other flaviviruses such as Dengue 1 - 4. **Objective:** The aim of this study was to compare ZIKV detection in plasma and urine samples from patients from a large Brazilian private laboratory using qualitative Real Time PCR. **Methods:** 371 Plasma samples and 177 urine samples were collected from patients, RNA automated extraction was performed using QIAsymphony® virus/bacteria mini kit (Qiagen), RT-PCR was performed with High Capacity cDNA Reverse Transcription Kit (Thermo Fisher) and the viral regions of interest were amplified with two different assays (Lanciotti RS et al, 2008) by TaqMan Real time PCR (Thermo Fisher). RNaseP mRNA was used as internal control. Samples were classified as "detectable" when both primers were amplified, "undetectable" when there was no amplification for both primers and "inconclusive" when amplification was observed for only one assay or if the result was below the limit of detection of the test (plasma: 820 copies/ml; urine: 160 copies/ml). Real time PCR for inconclusive plasma samples was also performed in urine to assure the result. **Results:** From the 548 samples, 116 samples of plasma and urine were collected simultaneously, in the other 432, urine was solicited on demand to confirm plasma result. A total of 44 samples were considered reactive (8.02%). Among the detectable samples, ten were undetectable or inconclusive in plasma but detectable in urine (22.7%), five were detectable in plasma and undetectable or inconclusive in urine (11.4%) and six were undetectable in plasma and inconclusive in urine (13.7%) while 5 showed concordance in between the two biological fluids (11.4%). **Conclusion:** According to our results, the use of urine samples increased the rate of molecular detection of ZIKV and should be considered when blood collection is difficult to be assessed. Furthermore, despite urine diagnosis may not be of clinical

relevance for adult patients, it may be of particular interest in pregnant women, possibly reflecting viral replication in fetal tissues.

Lanciotti et al. Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. Emerging Infectious Diseases Vol. 14, No. 8, 2008.

B-109

Detection of NDM-1 and CTX-M-type producing *Enterobacteriaceae* strains in Brazil

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Background: *New Delhi Metallo β-Lactamase* (NDM) has become one of the main globally described carbapenemases. The first report of NDM happened in 2009 in India. Four years later, in 2013, Brazilian researchers reported the first description of NDM in our country in a *Providencia rettgeri* samples. Since then, the emergence of this mechanism of resistance has been described in different species of Gram-negative samples in Brazil. The objective of this study was report the detection of NDM-1 producing *Enterobacteriaceae* clinical isolates recovered at Brazilian hospitals. **Methods:** In January 2015 and in January 2016 five carbapenem-resistant *Enterobacteriaceae* strains were isolated from five patients hospitalized in two tertiary Brazilian hospitals. These strains were isolated from Urine (n=2), catheter tip (n=1) and rectal swab (n=2). The bacterial identification was performed using mass spectrometry (Vitek-MS, bioMerieux) and the minimal inhibitory concentration (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux), as well as E-test method, according to CLSI guidelines. The screening test of carbapenemase was performed by phenotypic assay using commercially available disks containing carbapenems with and without EDTA (0.1 M), cloxacillin (75 mg/mL) or phenylboronic acid (40 mg/mL), as recommended by the Brazilian National Health Surveillance Agency (ANVISA). Then, the detection of the carbapenemase ($bla_{KPC,}$ bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{GES} and $bla_{OXA-48-like}$) genes and ESβL genes ($bla_{TEM,}$ bla_{SHV} and bla_{CTX-M}) was determined by PCR followed by Sanger sequencing analysis of the genes. Genetic relatedness of the strains was characterized by Pulsed-Field Gel Electrophoresis (PFGE) using the *Spe*I restriction endonuclease. **Results:** Among the five *Enterobacteriaceae* studied, three isolates were identified as *Providencia rettgeri* and two as *Klebsiella pneumoniae.* In all of them, high-level of resistance were detected to ceftazidime (MIC, >16 µg/mL), ceftriaxone (MIC, >4 µg/mL), cefepime (MIC, >16 µg/mL), ertapenem (MIC, >2 µg/mL), imipenem and meropenem (MIC, >4 µg/mL). One of *K. pneumoniae* strains was also resistant to amikacin (MIC, 32 µg/mL) and colistin (MIC, >16 µg/mL). All strains showed the diameter-zone difference >5 mm between the carbapenem/ EDTA and carbapenem disks, screened as possible metallo-β-lactamase (MβL) producers. The presence of bla_{NDM} and bla_{CTX-M} genes was detected in all strains. All *P. rettgeri* strains showed an identical PFGE pattern. The PFGE protocol to *K. pneumoniae* strains is running. **Conclusion:** An early detection by screening methods could be very helpful for surveillance and infection control measures. In addition to that, the findings of the identical PFGE pattern among *P. rettgeri* suggest the occurrence of a horizontal dissemination and an emergence of this important mechanism of resistance in Brazil.

B-110

Comparison of DiversiLab System for Microbial DNA Typing with Pulsed Field Gel Electrophoresis (PFGE)

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Background: Hospital-acquired infections due to bacteria that are resistant to multiply antibiotics have led to a worrying situation in many parts of the world. The control of nosocomial infections is based, in part, on tracking the spread of isolates potentially responsible for outbreaks. Due to its high discriminatory power, pulsed-field gel electrophoresis (PFGE) is considered as the reference method for molecular typing of many bacterial. However, this technique has major limitations: it is time-consuming, labor-intensive, technically variable, which affects reproducibility and provides subjective-interpretable results. DiversiLab® System (Biomerieux) is rapid and semi-automation technique for typing microbial strains based on repetitive-PCR technology (Rep-PCR), which primers are complementary to non-coding repetitive sequences found in microbial genome. Biomerieux portfolio presents different species specific-kits.

Objective: The aim of this study was to assess the DiversiLab (DL) system for typing same bacterial pathogens causing hospital-associated outbreaks by evaluation and comparing its performances with PFGE results. **Methods:** 4 strains of *Acinetobacter baumannii* and 4 *Klebsiella pneumoniae* were evaluated by DL (generic) kit and species specific kits. 2 strains of *Stenotrophomonas maltophilia* and 4 *Pseudomonas aeruginosa* were evaluated only with DL Bacterial generic kit. DNA from bacteria cultures was extracted with MoBio Ultra Clean Extraction Kit (MoBio) and rep-PCR was performed with the respective DL system kit including positive controls. Chips (Biomerieux) containing rep-PCR products were inserted into the Bioanalyzer (Agilent) and the results analyzed by DL software. **Results:** Results by DL Bacterial Kit agreed to those generated by PFGE for typing of *S. maltophilia* and *P. aeruginosa* strains, suggesting that *S. maltophilia* samples were indistinguishable from each other and *P. aeruginosa* were different between then. When PFGE was compared to DL Bacterial (generic) kit and DL *Acinetobacter* kit, our data showed that PFGE results were similar to DL *Acinetobacter* kit results, suggesting that these strains were indistinguishable. PFGE results also corroborated with those generated by DL *Klebsiella* kit, showing that 2 strains were similar to each other, and the other 2 *K. pneumoniae* were different. In both cases, DL Bacterial (generic) kit generated different results from PFGE. Our data show that DL results were in accordance to PFGE results, validating DL system with as reliable to perform bacteria typing. **Conclusion:** In summary, DL is a useful and fast tool to help identify hospital outbreaks of *A. baumannii*, *S. maltophilia*, *K. pneumoniae* and *P. aeruginosa.*

B-112

Clinical Performance of Elecsys® Anti-HCV II in Subjects with Increased Risk of Hepatitis

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A multicenter clinical performance study of liquid Elecsys® Anti-HCV II immunoassay on **cobas e** 601 analyzer was recently completed. Study population consisted of adult and pediatric subjects at risk for hepatitis (sexual practice, behavior, medical status or occupation). Elecsys® assay is an automated sandwich immunoassay based on the chemiluminescence principle where complexes of sample anti-HCV antibodies, biotinylated-/ ruthenylated-antigens (core, non-structural NS3/NS4), and streptavidin-magnetic microparticles are captured on an electrode. The primary objective was to evaluate percent agreement between Anti-HCV II and reference assay. The secondary objectives included evaluation of hepatitis A/B co-infections, non-HCV Flavivirdae viruses, anti-HCV antibody genotype recognition, seroconversion sensitivity, and determining imprecision.

Specimens (serum, sodium citrate plasma) were tested in four US sites using Elecsys® Anti-HCV reference assay. Final HCV interpretation was based on a testing algorithm for reference reactive samples: two comparator immunoassays, Abbott Architect Anti-HCV and ORTHO VITROS a-HCV, and HCV RNA determination (Roche AMPLICOR Hepatitis C assay) for comparator-indeterminate samples.

Positive /negative percent agreements with 95% confidence limits are listed below.

Cohort	Positive		Negative	
	n	Percent Agreement, CL	n	Percent Agreement, CL
Adult[A]	560	99.64% 98.72-99.96%	1683	98.81% 98.17-99.27%
Pediatric[B]	32	100.00% 89.11-100.00%	197	98.48% 95.61-99.68%
Pregnant[C]	63	98.41% 91.47-99.96%	207	99.52% 97.34-99.99%

[A]Agreements were comparable in these prospectively-collected asymptomatic/symptomatic cohorts and ranked risk subgroups. No discrepant results were observed in co-infected subjects: 43 acute/chronic hepatitis B, 3 acute hepatitis A.
[B]192 subjects were US prospectively-collected; 37 specimens were acquired ex-US retrospectively.
[C]205 subjects were US prospectively-collected; 65 specimens were acquired US retrospectively.

Twenty-nine Flaviviridae (West Nile Disease, Dengue Fever, Murray Valley Encephalitis, Kunjin Fever) and 289 other specificity specimens representing 28 diseases were concordant between both assays.

Seroconversion sensitivities in both assays were equivalent in ten commercial panels; Elecsys® Anti-HCV II converted earlier in six panels and later in one panel.

Both assays demonstrated equivalent genotype recognition in three genotype panels.

Imprecision (CLSI EP5-A2) was evaluated using three reagent lots and two operators in three US sites. Three replicates imprecision pools were tested in two runs/day for

five days. All %CVs for Repeatability and Reproducibility were <3.1% and <12.0%, respectively. The C5–C95 (CLSI EP12-A2) interval ranged from 0.97-1.06 COI.

Evaluation of the Elecsys® Anti-HCV II assay / **cobas e** 601 analyzer demonstrated acceptable clinical/analytical comparison between lyophilized first generation and second generation liquid reagent.

B-114

Recombinant chimeric IgG/IgM mouse-human antibodies for the manufacture of calibrators and controls for diagnostic test kits.

D. Shan, R. MacDonald. *CTK Biotech, San Diego, CA*

Recombinant chimeric antibodies containing murine variable regions and human constant regions are good candidates as calibrators or controls in immunoassays when positive human samples are difficult to obtain. Dengue was selected as a model system. We chose to use a diagnostic kit, the *OnSite* Dengue IgG/IgM Combo Rapid Test (R0061C), which is designed to detect and discriminate the IgG/IgM subtypes against the Dengue virus envelope protein in human serum or plasma. A monoclonal antibody against a specific site in the dengue envelope protein which is conserved in all four types of the dengue virus was developed from murine. This antibody was used to generate humanized, chimeric antibodies carrying the target binding site. First, heavy-and light-chain variable-region genes were cloned from the hybridoma cells and transferred into immunoglobulin expression vectors containing human kappa and IgG1 or IgM constant regions. The constructs were then used to transiently transfect COS cells, and the chimeric antibodies that were expressed were tested by ELISA for target-binding activity. After confirmation of the binding activity, CHO cells were stably transfected with these constructs. Chimeric IgG/IgM antibodies purified from the CHO cell culture media were tested using the OnSite Dengue IgG/IgM Combo Rapid Test kit. A strong test line density was observed for both chimeric IgG and IgM antibodies, which was comparable to the results using positive patient samples. The immunoreactivities of both chimeric IgG and IgM antibodies were also confirmed by RecombiELISA Dengue IgG and IgM ELISA tests. These data demonstrate that chimeric mouse-human antibodies are a feasible alternative to high-titer positive human samples for the manufacture of calibrators and controls for diagnostic test kits.

Wednesday, August 3, 2016

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

Lipids/Lipoproteins

B-115

Detection and Identification of Phosphatidylcholine Hydroperoxides in Plasma and Triglyceride-rich Lipoproteins using Orbitrap Mass Spectrometry

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Background: A growing body of evidence supports the association of triglyceride-rich lipoproteins (TRL) with atherosclerosis and coronary heart disease (CHD). Oxidized lipids in the TRL may be one of the promoting factors for its atherogenicity. We previously reported that TRL, including very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), carries several molecular species of cholesteryl ester hydroperoxides and triglyceride hydroperoxides. Since the lipid peroxidation can induce chain-peroxidation reaction to the adjacent lipid molecules, it is likely that TRL may also carry phosphatidylcholine hydroperoxides (PCOOH). Furthermore, phospholipids exist on the surface of lipoproteins and thus are relatively more susceptible to the peroxidation. Therefore, analysis of PCOOH can be valuable in overall evaluation of future coronary diseases and oxidative injury. In light of this importance, this study was carried out to identify and characterize PCOOH in plasma and TRL.

Methods: We developed a novel approach for the identification and characterization of PCOOH from the lipid extract of plasma and lipoprotein fractions, using reversed-phase liquid chromatography coupled with a hybrid linear ion trap-Orbitrap mass spectrometer (LC-LTQ Orbitrap). A fasting EDTA blood sample was collected from 9 healthy human volunteers. VLDL (d <1.006) and IDL (d =1.006-1.019) were isolated from the plasma (n=6) by sequential ultracentrifugation. The purity of isolated lipoproteins was assessed by its molecular composition, mobility in polyacrylamide gel electrophoresis (LipoPhor) and apolipoproteins study using SDS-PAGE. Total lipids were extracted from plasma and lipoprotein samples, and subjected to the LC-LTQ Orbitrap analysis equipped with an electrospray ionization source. The mass spectrometry was operated in a positive-ion mode, extracted ion chromatogram was drawn with the mass tolerance set at 5.0 ppm, and PCOOH were detected as $[M+H]^+$ ion. Authentic synthetic standards of PCOOH were used for equivocal identification.

Results: On the basis of specific elemental composition, m/z mass spectra, and retention time in LC, we detected several molecular species of PCOOH that exist in human plasma and TRL. PC16:0/18:2-OOH and PC16:0/20:4-OOH were detected in all plasma samples. PC 16:0/18:2-OOH, PC 18:0/18:2-OOH, PC 16:0/20:4-OOH, PC 18:0/20:4-OOH, PC 16:0/22:6-OOH, and PC 18:0/22:6-OOH were the most predominant PCOOH detected in TRL. The distribution of PCOOH is higher in VLDL compared to IDL with consistent detection of PC16:0/20:4-OOH and PC16:0/22:6-OOH in all samples. PC16:0/20:5-OOH and PC18:0/22:6-OOH were not present in any of plasma samples.

Conclusion: We identified and characterized 12 molecular species of PCOOH in native lipoproteins and plasma. The existence of PCOOH in TRL is possibly associated with its atherogenicity; therefore, can serve as markers for early prediction and risk assessment of CHD. Further work is needed to reveal the association of these PCOOH in the atherosclerotic process.

B-116

Prevalence and Pattern of Dyslipidemia in Nepalese Individuals with Type 2 Diabetes

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Background: Atherogenic dyslipidemia is an important modifiable risk factor for cardiovascular disease among patients of type 2 diabetes mellitus. Its timely detection and characterization helps clinicians estimate future risk of CVD and take appropriate preventive measures. We thus aim to study the prevalence and pattern of dyslipidemia in a cohort of Nepalese patients with type 2 diabetes.

Patients and methods: We conducted a cross-sectional study on 497 diabetic patients aged 30-74 years at Manipal Teaching Hospital, Pokhara, Nepal. The relevant personal, medical, demographic and anthropometric data were collected. Blood samples were analyzed for plasma glucose, HbA1c and lipid profile. The National Cholesterol Education Program Adult Treatment Plan III guidelines were used to define prevalence of dyslipidemia. Statistical analyses of the data were performed using SPSS, with level of significance set at $p<0.050$.

Results: We found mixed dyslipidemia as the most prevalent (88.2%) and isolated dyslipidemia (10.1%) as the least prevalent forms of dyslipidemia in our patients. The most prevalent form of single dyslipidemia was high LDL-C (73.8%) and combined dyslipidemia was high TG, high LDL-C and low HDL-C (44.7%). Prevalence of all single and mixed dyslipidemia was higher in patients with poor glycemic control and hypertension. The glycemic status of patients correlated with their fasting serum lipid profile. Dyslipidemia was associated mainly with male gender, poor glycemic control and hypertension.

Conclusion: The prevalence of dyslipidemia is very high in Nepalese diabetic patients. Hence, urgent lifestyle intervention and aggressive lipid lowering treatment plans are needed to minimize their future risk of cardiovascular disease.

B-119

Serum amyloid A increases the antioxidant ability of high density lipoprotein

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Background: Atherosclerosis is associated with chronic inflammation. Sustained inflammation is the essential feature of atherosclerotic lesions as well as the accumulation of cholesterol. Although many study reported that the activation of inflammatory cells and the release of various cytokines contributed to the progress of atherosclerosis, it remains to be completely elucidated how the inflammation impacts on lipid metabolism. Serum amyloid A (SAA) is one of the acute phase proteins and its concentration in plasma is also elevated modestly in chronic inflammatory disorders. Furthermore SAA is notably increased at inflamed regions with a higher level than that in plasma under chronic inflammatory conditions. Elevated SAA becomes the major apolipoprotein component of high density lipoprotein (HDL) due to a displacement of apolipoprotein A-I (apoA-I). Consequently, this remodeling could affect HDL metabolism, however there is almost no report about the direct influences of SAA on the function of HDL. In this study, we evaluated the effects of SAA on antioxidant ability, which is one of various anti-atherogenic properties of HDL using both SAA protein and SAA-containing HDL.

Methods: LDL (d = 1.006-1.063 g/mL) and HDL (d = 1.063-1.210 g/mL) were isolated by ultracentrifugation. Reconstituted SAA-HDL was prepared by incubating recombinant human SAA (rhSAA) with HDL isolated from serum of normal subjects. HDLs obtained from various patients serum were divided into three groups by SAA levels (Low: ≤ 8 μg/mL, n=11, Middle: 8-100 μg/mL, n=10, and High: > 100 μg/mL, n=20). The antioxidant ability was estimated by the effect of each protein (SAA or apoA-I) or HDL on LDL oxidation, which was monitored as the formation of conjugated dienes at 234 nm. Antioxidant ability was defined as relative prolongation of the lag time and as relative decrease of the maximum velocity (V_{max}) compared to LDL alone.

Results: The relative lag time (mean ± SD) of rhSAA was 1.33 ± 0.05 whereas that of apoA-I was 1.22 ± 0.02 ($p < 0.05$). The relative V_{max} was 0.79 ± 0.03 and 0.86 ± 0.02, respectively ($p < 0.05$). As for HDL particles, the relative lag times of rhSAA-HDL and normal HDL were 1.83 ± 0.20 and 1.46 ± 0.27 ($p < 0.05$), and the relative V_{max} were 0.54 ± 0.15 and 0.66 ± 0.09, respectively. With regard to results in patients, the relative lag times of HDL in each group (Low, Middle, and High) were 1.24 ± 0.06, 1.25 ± 0.06, 1.36 ± 0.14 ($p < 0.05$), the relative V_{max} were 1.18 ± 0.08, 1.11 ± 0.15, and 1.06 ± 0.08, respectively ($p < 0.01$).

Conclusion: rhSAA had higher ability for inhibition of LDL oxidation than apoA-I. Similarly, rhSAA-HDL and HDL obtained from the patients with high SAA levels prolonged the relative lag time of oxidation profile. These indicate that SAA increased antioxidant ability of HDL. It might mean that SAA, whether the conformation such as lipid-free or HDL bound, plays an atheroprotective role as an antioxidant agent both at tissues and in the circulation.

B-120

Effect of Differentiation and Foam Cell Formation on Cholesterol Efflux Capacity of Apolipoprotein A-I.

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Backgroud: Cholesterol efflux capacity is one of the atheroprotective functions of apolipoprotein A-I (apoA-I). In recent years, a number of studies have suggested a relationship between cholesterol efflux capacity and cardiovascular disease. However, the assay method of cholesterol efflux capacity has not been optimized. It might be one of the reasons why the different results have been given by the similar experiments. The purpose of this study is to investigate the effect of different states of cells on the cholesterol efflux capacity of apoA-I and *N*-homocysteinylated apoA-I (*N*-Hcy apoA-I), known as one of the risk factors for cardiovascular disease.

Methods: Cholesterol efflux capacity was determined by measuring a rate of ^{3}H-cholesterol removed by apoA-I from THP-1 (a human monocytic leukemia cell line) macrophages. We estimated the cholesterol efflux capacity under various cell conditions as follows: 1) THP-1 cells were differentiated with phorbol myristate acetate (PMA) for different terms (1 to 5 days) and the degree of cell differentiation was evaluated by detecting the expression of ATP-binding cassette transporter A1 (ABCA1) and CD11b, an adhesion molecule, with flow cytometry and western blotting analysis. 2) After differentiation with PMA treatment for a fixed term, THP-1 macrophages were incubated with acetylated low density lipoprotein (acLDL) for different terms (1 to 5 days) and the lipid droplet accumulation in foam cells was determined by oil red O staining. 3) Under these conditions, cholesterol efflux capacity was compared between *N*-Hcy apoA-I and normal apoA-I.

Results: 1) When foam cell formation was fixed for 1 day, cholesterol efflux capacities were the highest and the second highest at day 2 and day 1 differentiation respectively, and decreased after day 3. The amount of ^{3}H-cholesterol took inside the cells was also the highest at day 2, but the lowest at day 1, and decreased after day 3. ABCA1 expression was decreased on a parallel with PMA treatment period ($p < 0.05$). 2) The amount of lipid droplet accumulation was lineally increased during acLDL loading ($p < 0.05$). The amount of ^{3}H-cholesterol took inside the cells was also lineally increased for 3 days but then almost steadied. Cholesterol efflux capacity was the highest at day 1 loading with acLDL and decreased in a loading time-dependent manner. ABCA1 expression did not change significantly. 3) Cholesterol efflux capacity of *N*-Hcy apoA-I was significantly lower than that of apoA-I by differentiation and foam cell formation for 1 or 2 days. However, no significant difference was observed under the states of excessive differentiation or excessive foam cell formation.

Conclusion: We found that cholesterol efflux capacity was greatly affected by the state of differentiation and foam cell formation. Actually, the different cell conditions did not always produce the fixed result that *N*-homocysteinylation influenced on cholesterol efflux capacity of apoA-I. These results suggest that cholesterol efflux capacity varies depending on the

state of cells and the assays should be performed using the defined protocols including stimulation period.

B-121

LDL particle size associates with apoE-containing HDL in patients who undergo coronary computed tomographic angiography

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Background: Patients with coronary artery disease have several lipoprotein abnormalities, such as increased triglyceride (TG) and LDL-cholesterol, and reduced HDL-cholesterol (HDL-C). Small-dense LDL, with increased TG and reduced HDL-C levels, constitute the atherogenic lipoprotein phenotype. However, associations of LDL particle size (LDL-size) with HDL-associated apolipoproteins and their subclasses have not well been understood. We compared LDL-size with several HDL variables, including apoE-containing HDL-C.

Methods: We studied 52 consecutive patients (age: 66.4 ± 11.9 years, male 50.0%) who underwent coronary computed tomographic angiography. Plasma samples were analyzed by a gel-permeation liquid chromatography system, equipped with a Superose 6 column (GE healthcare), and major lipoprotein cholesterol levels and LDL-size were calculated from each of peak area and elution times on the chromatographic cholesterol patterns, respectively. HDL-associated apolipoproteins (apoA-I, A-II, C-I, C-III and E) were determined by sandwich ELISA on the HDL fractions separated from whole plasma with the polyethylene glycol (PEG) precipitation method. For measurement of apoE-containing HDL-C, the isolated HDL fraction was applied into a heparin affinity column (HiTrap Heparin, GE healthcare) equilibrated with 10 mM MOPS (pH 7.0) containing 50 mM sodium chloride, and bound HDL (apoE-containing HDL) was eluted by 10 mM MOPS (pH 7.0) containing 1.0 M sodium acetate, followed by an on-line enzymatic detection for cholesterol. Pearson's correlation coefficients were used to evaluate the correlation between two variables. A stepwise multiple regression analysis was used to identify HDL variables influencing for LDL-size.

Results: Total cholesterol, HDL-C, and TG levels of the studied subjects were 223 ± 53 (mg/dL), 68.6 ± 20.5 (mg/dL), and 145 ± 61 (mg/dL), respectively. LDL-size was significantly correlated with plasma TG ($r = -0.683$) and HDL-C levels ($r = 0.542$), consistent with previous reports. Moreover, we found a significant positive correlation between LDL-size and apoE levels in the HDL fractions (HDL-apoE) ($r = 0.510$, $P<0.001$). ApoC-I and apoC-III levels in the HDL fractions were also associated with LDL-size, but their correlation coefficient values were smaller than HDL-apoE levels. In contrast, apoA-I and apoA-II, which are major protein components of HDL particles, were not significantly associated with LDL-size. ApoE-containing HDL-C levels and the ratio of apoE-containing HDL-C to total HDL-C were significantly correlated with LDL-size ($r = 0.606$, $P<0.001$, $r = 0.355$, $P<0.05$, respectively). There was a positive correlation between apoE-containing HDL-C and HDL-apoE levels ($r = 0.853$, $P<0.001$). Stepwise multiple regression analyses selected only apoE-containing HDL-C (beta coefficient = 0.606, $P<0.001$) that significantly correlated with LDL-size (adjusted $R^2 = 0.355$), suggesting apoE-containing HDL-C as independent predictor of LDLs.

Conclusion: We found a significant linkage of LDL-size with apoE-containing HDL-C levels in patients with or without various degree of coronary stenosis. These findings may contribute to understanding of lipoprotein metabolisms involved in apoE-containing HDL.

B-123

Cholesterol uptake capacity, a new concept of HDL functionality, for risk stratification in coronary artery disease

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Background: Recent studies have shown that the capacity of high density lipoprotein (HDL) to stimulate cholesterol efflux from lipid-laden macrophages is a better predictor of cardiovascular disease status than overall HDL cholesterol concentration. However, the standard procedures for measuring efflux capacity involve radioisotope-labeled cholesterol and cultured macrophages, thus more simplified method to measure HDL functional levels is strongly demanded for its clinical application.

Methods: We hypothesized that the efficiency of HDL-induced cholesterol efflux from macrophages is mainly dependent on the capacity of HDL to accept cholesterol, which we named "cholesterol uptake capacity". In order to test the hypothesis, we constructed a cell-free plate assay system to evaluate cholesterol uptake capacity of HDL using a fluorescence-labeled cholesterol and an apolipoprotein A1 (apoA1) specific antibody. Briefly, apoB depleted serum was incubated in reaction buffer containing the labeled cholesterol, followed by their capture to the immobilized anti-apoA1 antibody, and then fluorescence signals were detected from the labeled cholesterol incorporated into HDL or apoA1. To investigate the feasibility of cholesterol uptake capacity for coronary risk assessment, we quantified cholesterol uptake capacity of serum samples from 210 patients with coronary artery disease who had previously undergone revascularization.

Results: The assay system had high reproducibility (CV<10%) and a short processing time (<6 hours). Steroid structure of the labeled cholesterol, not fluorescence moiety itself, was essential for its incorporation into HDL and apoA1. Cholesterol uptake capacity declined with the myeloperoxidase-mediated oxidation of HDL or in the presence of lecithin-cholesterol acyltransferase. Cholesterol uptake capacity correlated significantly with cholesterol efflux capacity ($r=0.82$, $P<0.0001$, $n=29$). There was a significant inverse association between requirement of revascularization and cholesterol uptake capacity ($p=0.023$). In patients with the optimal control of low-density lipoprotein cholesterol ($n=125$), only cholesterol uptake capacity remained significant in multivariable analysis adjusted for age, sex, smoking history, HbA1c level, blood pressure, and concentrations of LDL-C and HDL-C (odds ratio 0.53, 95% CI 0.29-0.96, $p=0.0037$).

Conclusion: Cholesterol uptake capacity assay can evaluate functionality of HDL and lipid-free apoA1 in a sensitive and high-throughput manner without using radioisotope-label and cells. The assay system could be applicable for assessing CVD risk in the clinical setting.

B-124

Development of a New Biochip Array for ApoE4 Classification from Plasma Samples Using Immunoassay Based Methods

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Background: Apolipoprotein E (APOE) plays a key role in lipid metabolism and is recognised as one of the most powerful genetic risk factors for dementia and other neurodegenerative diseases. It has become one of the most widely studied gene variants in Alzheimer's disease and constitutes a major consideration for preventive medicine. ApoE exists in three common isoforms (ApoE2, ApoE3 and ApoE4) which are coded by three co-dominant alleles (e2, e3, e4). As such six common ApoE phenotypes exist within the general population E2/E2, E3/E3, E4/E4 (homozygous) and E2/E3, E2/E4, E3/E4 (heterozygous). The presence of the ApoE4 isoform is recognised as a major genetic risk factor for development of Alzheimer's disease. The availability of analytical methods for rapid and reliable ApoE4 classification is therefore advantageous.

Relevance: Biochip Array Technology (BAT) enables the determination of multiple analytes from a single sample. This technology has been successfully applied to a new biochip array to directly identify from a plasma sample whether patients are ApoE4 heterozygous, homozygous or null through simultaneous detection of both total ApoE levels and specific ApoE4 levels.

Methods: Simultaneous chemiluminescent biochip-based sandwich immunoassays for measurement of ApoE4 and total ApoE directly from plasma samples were employed and applied to the Evidence Investigator analyser. An initial cohort of 272 plasma samples of known genotype were used to establish initial assay parameters. A ratio was calculated using total ApoE ApoE4 protein levels to classify samples as ApoE4 heterozygous, homozygous or null. A further cohort of 112 plasma samples of unknown genotype were utilised to verify performance characteristics established employing the initial cohort. Genotype concordance was further investigated by genotyping these same 112 plasma samples from circulating cell free DNA (cfDNA) through the use of another biochip array platform, based on a combination of multiplex PCR and biochip array hybridisation, which allows simultaneous detection of APOE specific single nucleotide polymorphisms (SNPs). Receiver Operating Characteristics (ROC) curve was used to establish the sensitivity and specificity of the assay using the combined cohort of 384 plasma samples.

Results: From the initial cohort of 272 samples with known genotypes, 100% were correctly identified as null, heterozygous or homozygous for ApoE4 by the biochip array. From the additional 112 plasma samples, analysed using BAT for protein and SNPs detection, 100% concordance was found between both approaches. ROC analysis showed that patient samples could be identified as APOE4 positive or negative with 100% sensitivity and 100% specificity, all in approximately 3 hours.

Conclusions: An individual's APOE status has been shown to affect pre-symptomatic risk, diagnosis, prognosis, and treatment response for a variety of diseases, in particular Alzheimer's disease. The results show that BAT can be successfully applied to provide a platform to rapidly and accurately detect an individual's APOE4 status directly from a plasma sample. In combination with medical and family history, medication and lifestyle, this can deliver valuable information for personalised medicine approaches.

B-125

Performance of Calculated Low Density Lipoprotein Cholesterol in a Pediatric Population

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Background: The Friedewald equation is the most widely-used method for determining low-density lipoprotein cholesterol (LDL-C) concentrations. Recent evidence suggests that this formula is constrained by a significant negative bias when LDL-C falls below 70 mg/dL; an effect which is amplified with increasing triglyceride levels.

Objective: The goal of this study was to examine the bias of Friedewald and Hopkins-calculated LDL-C in pediatric samples with high triglycerides (150-399 mg/dL) and low LDL-C levels (≤ 100 mg/dL) relative to the reference method (ultracentrifugation (UC)).

Subjects and Methods: LDL-C concentrations in 69 pediatric plasma samples were measured by UC (LDL-UC) and calculated using Friedewald (LDL-C_F) and Hopkins (LDL-C_H) equations. Bias was assessed by difference plots and regression analysis produced using EP Evaluator software (Data Innovations).

Results: As seen in the table below, LDL-C_F performed well when LDL-UC levels >100 mg/dL (-0.4%), however a significant mean negative bias was observed with LDL-UC ≤100 mg/dL and LDL-UC ≤70 mg/dL (-15 and -16%). The Hopkins formula produced a bias of approximately 5% when LDL-UC>70 mg/dL and this increased to about 11% for LDL-UC ≤70mg/dL. To address this bias, we performed a modification to the Friedewald formula using a mean TG: VLDL ratio of 6.3 (derived from calculation), which negated most bias in low LDL-UC concentration samples (≤100 mg/dL = -1.3% and ≤70 mg/dL = 0.9%).

Conclusions: A modified Friedewald equation, using a TG: VLDL of 6.3, may improve the concordance of calculated results to the reference method in pediatric samples with low LDL-C and increased TG levels. Larger studies are underway to confirm these novel findings in the pediatric populations.

	Average % Bias		
Method	**LDL-UC ≤ 70mg/dL (n = 22)**	**LDL-UC ≤ 100mg/dL (n = 54)**	**LDL-UC >100 mg/dL (n = 15)**
LDL-C_F	-16.2	-15.4	-0.4
LDL-C_H	10.6	4.7	5.2
TG: VLDL = 6.3	0.9	-1.3	6.3

B-126

Detailed Faecal Fat analysis Using FT-IR Spectroscopy: Exploring The Possibilities

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Background: Fourier transform infrared (FT-IR) spectroscopic determination of faecal fat has been reported to be a simple and elegant alternative for the classical Van De Kamer approach. Next to quantification of the fat content, it allows detailed analysis of the chemical nature of fats by molecular fingerprinting. Analysis of lipase hydrolysis efficiency (fatty acid/triglyceride ratio; FA/TG ratio), fatty acid chain length and trans-unsaturated fatty acids could provide better monitoring of dietary treatment in patients with (non-)pancreatic malabsorption. We performed an in-depth exploration of these possibilities.

Methods: Stool samples were prepared for analysis according to the method described by Jakobs et al. (Ann Clin Biochem, 2000). Analyses were carried out on a Perkin Elmer Spectrum Two® spectrometer in the range 3500-450 cm^{-1}, using chloroform for background substraction. Human stool samples (24hrs collection, stored at -20°C, n=96) from patients on which routine fat analysis was requested (n=41) or from patients with known pancreatic and non-pancreatic steatorrhea (n=55) were used. Samples with a fat content < 1g/100g faeces were excluded (n=24), due to limited clinical value and loss of interpretability of the calculations. **A)** FA/TG ratio could be determined using the ratio of absorbance at resp. 2855 and 1746 cm^{-1}. To estimate hydrolysis efficiency of lipase (%), sample ratios were compared with the ratio of butter (containing 82% total fat) and pure free FA (i.e. stearic acid, palmitic acid and mixture of stearic:palmitic acid 65:35). **B)** Mean FA chain length could be calculated using the ratio of absorbance at resp. 2855 and 1709 cm^{-1}. **C)** To determine the specific absorbance peak of trans double bounds, trielaidine (a monoacid trans-unsaturated TG) was analyzed. The area of the peak at 966 cm^{-1} was used to trace the presence of trans-type unsaturated FA.

Results: A) Butter showed a low FA/TG ratio (1,21) and free FA a high FA/TG ratio (6.76). The mean sample ratio was 4,61 (i.e. 61% efficiency), with values ranging from 1,05 (7%) to 7,19 (108% efficiency, similar with ratio of pure FA).

B) The amount of absorbance was correlated with the mean acyl chain length of stearic acid (C18) or palmitic acid (C16)). The relative absorbance contribution per C-atom was calculated (ratio 1,06 for C18-standard and 0,91 for C16-standard), indicating an absorbance-contribution of 0,06 per C-atom. The mean ratio of the samples was 1,12 (i.e. mean acyl chain length of C19), with values ranging from 0,73 (C12) to 1,68 (C28).

C) Two samples from pancreas-insufficient patients contained traceable amounts of trans-type unsaturated FA.

Conclusion: For the analysis of faecal material, FT-IR provides unique information difficult to obtain using other techniques. It allows to elucidate the efficiency of fat digestion by pancreatic lipase, the determination of fatty acid chain length and the detection of trans-unsaturated fatty acids in stool samples. These findings offer new perspectives for diet monitoring in patients with (non-)pancreatic malabsorption.

B-127

Coupling of PAF receptor and modified LDL receptor LOX-1 to transduce oxidized LDL-induced cell signaling.

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Aim: To investigate the potential synergism between LOX-1 and PAF receptor in intracellular signal transduction in response to oxLDL.

Background: Lectin-like oxidized low density lipoprotein (LDL) receptor 1 (LOX-1) is the major receptor for oxidized LDL (oxLDL) in endothelial cells and LOX-1-mediated oxLDL actions induce endothelial dysfunction. Recently, we have reported that the angiotensin II type 1 receptor (AT_1) promotes oxLDL-induced cell responses through interaction with LOX-1 (Yamamoto K., FASEB J 2015). On the other hand, some reports suggested that PAF receptor (PAFR), also a member of G-protein coupled receptor, might be involved in mediating the signal transduction of oxLDL.

Methods: ***Detection of phosphorylation of ERK1/2:*** The cells seeded on 12-well dishes were starved for serum for 24 h, and then incubated with oxLDL (10 μg/ml) for 10 min at 37°C. ERK1/2 phosphorylation of the cell lysates was analyzed by immunoblotting with the antibodies: anti-phosphoERK1/2 (Thr202/Tyr204) antibody, anti-total-ERK1/2 antibody, anti-V5 antibody, and anti-HA antibody.

Luciferase reporter assay for NF-κB and SRF: LOX-1 and PAFR expressing cells seeded in a 24-well plate were transiently co-transfected with 500 ng of NF-κB or SRF promoter Firefly luciferase reporter vectors, pGF1-NF-κB or pGF1-SRF, together with pRL-CMV Renilla luciferase control reporter vector, and cultured for 6 h. Then, the cells were starved for 24 h, and stimulated with oxLDL for 20 h. The luciferase activity was measured with the Dual-Luciferase Reporter Assay Kit.

Detection of DiI-labeled oxLDL: LOX-1 and PAFR expressing cells were treated for 60 min with 3 μg/ml of DiI-labeled oxLDL on ice. Nuclei of the cells were stained with DAPI (1 μg/ml). Quantitative fluorescence cell image analysis was performed using the INCell analyzer 2000 system.

Co-immunoprecipitation: Cell lysate were prepared with a lysis buffer containing 1%Triron- X100. Proteins were precipitated with anti-FLAG-M2 affinity gel and eluted with FLAG peptide. The purified proteins were then analyzed by immunoblotting with antibodies against HA and V5.

Results: OxLDL-induced cell responses, ERK, NF-κB and SRF activation, were promoted by additional expression of PAFR compared with cells expressing solely LOX-1 or PAFR. In this condition, the binding of DiI-labeled oxLDL to the cells expressing both LOX-1 and PAFR was not significantly different from the cells expressing solely LOX-1. In addition, PAFR antagonists, ABT-491, suppressed oxLDL-induced ERK phosphorylation in endothelial cells. Furthermore, immunoprecipitation analysis showed that LOX-1 was co-immunoprecipitated with PAFR from the cells transfected with both PAFR and LOX-1, while Dectin-1, which has highest homology to LOX-1 in C-type lectin-like protein family, was not. These results suggested that LOX-1 and PAFR expressed physically proximal to each other in cell surface might cooperatively strengthen the signal of oxLDL action.

Conclusion: Coupling of LOX-1 and PAFR, as the case of AT_1, might be of importance in oxLDL-induced biological reactions.

B-128

Influence of triglycerides on LDL-C measured by direct homogeneous method and estimated by the Friedewald equation and a novel calculation

B. Leindecker, K. Roberts, J. Hornseth, L. Donato, N. Baumann, J. Meeusen. *Mayo Clinic, Rochester, MN*

Background: Low-density lipoprotein cholesterol (LDL-C) is the primary biomarker for assessing cardiovascular risk. The gold-standard LDL-C method (beta-quantification; LDL-C_{BQ}) involves ultracentrifugation and lipoprotein specific precipitation and is not influenced by triglycerides (TG). Due to the expense and expertise requirements of LDL-C_{BQ}, estimation of LDL-C by the Friedewald equation (LDL-C_F) is routinely used. Alternative LDL-C methods with purportedly superior performance across TG concentrations include direct homogeneous measurement (LDL-C_D), and the novel estimation formula (LDL-C_N). **Objective**: To assess the influence of TG on performance of LDL-C_F, LDL-C_D, and LDL-C_N compared to LDL-C_{BQ}. **Methods**: Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG, and LDL-C_D were measured using Roche Cobas c501 analyzers. LDL-C_{BQ} was performed using Beckman LE-80K ultracentrifuge and dextran sulfate-Mg/Cl precipitation. LDL-C_F was calculated as TC - HDL-C - (TG/5); LDL-C_N was calculated as TC - HDL-C - (TG/X) where X is an empirical value determined based on TG, TC and HDL-C as described in by Martin, et al (JAMA, 2013). Bias was assessed by comparison to LDL-C_{BQ}. Clinical categories were <100, 100-129, 130-159, 160-189, and ≥190 mg/dL LDL-C. **Results:** The median LDL-C_{BQ} for all samples was 97 mg/dL and did not significantly differ with TG. As TG concentrations increased, LDL-C_F significantly underestimated LDL-C_{BQ}, LDL-C_N overestimated LDL-C_{BQ}, and LDL-C_D remained unchanged (**Table 1**). Variability (indicated by SD of bias) increased significantly with TG for LDL-C_F and LDL-C_N and a lesser extent for LDL-C_D. LDL-C_F was most concordant, followed by LDL-C_N and finally LDL-C_D. LDL-C_N was significantly less concordant among samples with TG >300 mg/dL, while LDL-C_F and LDL-C_D were not significantly impacted by TG. **Conclusions**: LDL-C_F was most concordant with LDL-C_{BQ} overall and when TG >400 mg/dL. TG strongly influenced bias and variability for both estimated LDL-C methods. LDL-C_D was least influenced by TG, however, there appeared to be a systematic bias.

Table 1. LDL-C performance across TG

Triglycerides, mg/dL	<150	150-199	200-299	300-399	≥400	Any
N	51	53	51	47	52	254
LDL-C, mg/dL; median (IQR)						
Beta-quantification	91(47)	106(39)	106(46)	88(41)	95(55)	97(46)
Friedewald	93(39)	107(41)	106(54)	85(42)	80(62)	93(52)
Direct	101(43)	118(36)	118(62)	103(44)	103(58)	108(52)
Novel	92(37)	112(38)	116(52)	108(36)	119(52)	108(47)
Bias, mg/dL ; mean±SD						
Friedewald	-1±14	-2±13	-2±13	-6±16	-17±41	-6±23
Direct	8±13	12±11	14±13	15±12	10±26	12±16
Novel	-1±14	4±12	10±13	16±14	23±33	10±21
Concordance						
Friedewald	80%	87%	84%	81%	75%	81%
Direct	69%	51%	51%	57%	58%	57%
Novel	76%	83%	71%	55%	44%	66%

B-129

Prevalence of Lipoprotein X in Samples with Extremely Low High Density Lipoprotein Cholesterol

J. M. Hornseth, J. W. Meeusen, A. S. Jaffe, N. A. Baumann, L. J. Donato. *Mayo Clinic, Rochester, MN*

Background: A hallmark abnormal lipoprotein produced during cholestatic liver disease is lipoprotein X (LpX). LpX is rich in unesterified cholesterol and phospholipids and the protein component is high in albumin. Despite a lack of apolipoprotein B, LpX has similar density to low-density lipoprotein (LDL) making LpX cholesterol indistinguishable from LDL-C by most LDL-C methods. The presence of LpX in patient sera is detected as a reverse migrating band on lipoprotein electrophoresis methods. In late stages of primary biliary cholestasis, hypercholesterolemia is common; however, concentrations of high density lipoprotein (HDL-C) are severely decreased. Other causes of extremely low HDL-C values include various inherited disorders, malignancy, or androgen use. The prevalence of Lp-X in serum from patients with extremely low HDL-C is unknown. **Objective:** To establish the prevalence of LpX among patients with extremely low HDL-C (<10 mg/dL), and determine if other laboratory values such as apolipoprotein A1 (apoA1), total cholesterol, LDL-C, or serum icterus index correlate with the presence of LpX. **Design and methods:** Sera from 554 consecutive samples with HDL-C <10 mg/dL measured by automated direct method (Roche Cobas c501) were collected between 12/2011 and 12/2015. The presence of LpX was determined by lipoprotein electrophoresis (Helena SPIFE 3000

system using SPIFE Vis Cholesterol Kit). Additional measures included apoA1 and total cholesterol (Roche Cobas systems), serum icterus indices (I-index; Roche Cobas systems). The presence of LpX precludes the ability to measure or estimate LDL-C, however, for purposes of this study LDL-C was estimated by the Friedewald formula in all samples. **Results:** LpX was identified in 125 (22.6%) of 554 specimens. The average (interquartile range (IQR)) HDL-C was 6 (4-7) mg/dL and apoA1 was 40 (24-50) mg/dL in the entire cohort; values were not significantly different in samples with or without LpX ($p > 0.10$). The mean (IQR) I-index for LpX positive samples was 14 (8-18.5) and was significantly higher than LpX negative samples at 7 (1-8) $p<0.0001$). ROC curve analysis for LpX as a function of I-index found an optimal cutoff of 8 (AUC 0.776, sensitivity/specificity = 76%/73%). Samples with LpX had significantly higher serum concentrations for total cholesterol (248 mg/dL vs. 103 mg/dL, cutpoint 128 mg/dL, AUC 0.868, sensitivity/specificity = 87%/74%) and LDL-C (190 mg/dL vs. 55 mg/dL, cutpoint 84 mg/dL, AUC 0.871, sensitivity/specificity = 78%/82%). When using laboratory values obtained from a basic lipid panel to predict the presence or absence of LpX, 40 of 41 samples with an I-index of zero were negative for LpX. Conversely, 55 of 60 samples with an I-index >2 and LDL-C ≥160 mg/dL were positive for LpX. **Conclusion:** LpX is present in 1 of 5 samples (20%) with extremely low HDL-C (<10mg/dL) submitted for standard lipid panel analysis. The presence of LpX in samples with HDL-C <10 mg/dL can be nearly always ruled-out by undetectable I-index, while the presence of LpX is strongly suggested by elevations of both I-index (>2) and LDL-C (≥160 mg/dL).

Wednesday, August 3, 2016

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

Management

B-130

Sigma Metrics as Performance Indicator Contributes to Effective Cost and Man-hour Saving in Chemical Pathology Laboratory

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Backgrounds: Sigma metrics as performance indicator allows analyzing control materials (IQC) in a flexible manner according to analyte performance, thus avoids repeated testing of IQC in a period when the system was performing stably, consequently minimizes un-necessary cost expenditure and man-hours wastage.

Methods: Starting from 1 January 2010 our laboratory applied sigma metrics in internal quality control plan. Instead of analyzing IQC for 29 chemistry and 15 immunoassay analytes, in Cobas 6000 (Roche Diagnostic), every eight hours, frequency became analyte performance specific. Performance of each analyte was reviewed every 8 months according to IQC data with sample size ranged 200 to 700 per analyte. During which more than one lot of reagent, calibrator and control were involved. Sigma metrics of each analyte was calculated using the formula [Sigma metric = (TEa-bias)/ CV]. TEa, bias and CV were obtained from respective defined allowable limit of performance of the Royal College of Pathologists of Australasia Quality Assurance Program, in-house observed inaccuracy and imprecision respectively. Performance of each analyte was assessed on the sigma scale. Frequency of analyzing IQC for analyte of sigma >=5, 4 and 3 was once, twice and thrice respectively.

Results: Among 44 analytes, daily IQC analysis frequency of 31 (sigma>=5), 7 (sigma=4) and 6 (sigma=3) analytes was once, twice and thrice respectively. These changes contributed to over 50% reduction in control materials and reagents consumption, which accounted for an annual cost saving of over HKD 1,200,000. Time reduction in IQC preparation, analysis and reviewing stably performed analytes contributed to an annual man-hour saving of 0.3 full time employee which was valuable for new service development while facing manpower constraint.

Conclusions: Applying sigma metrics as performance indicator, our laboratory executed an analyte performance specific internal quality control plan. While maintaining overall service quality, the plan contributed to an effective cost and man-hour saving.

B-131

Determining the utility of creatinine delta checks: a large retrospective analysis

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Background:

Delta checks use two successive test results to detect changes greater than expected for physiological variation. A flagged delta check holds the result for further review by laboratory staff – a long-standing practice for identifying errors that are not detected by other routine quality control measures in the lab. However, now with relatively fewer errors since the introduction of modern automated clinical chemistry analyzers and laboratory information systems (LIS), excessive or "false alarm" delta checks can increase workload, inefficiency of staff, and turnaround times. As a result, the utility of delta checks in detecting true error is unclear. In our laboratory, we experienced a small number of erroneously high creatinine results over a 6 month period that were determined to be analytic, or instrument-related errors. Therefore, the objective of this study was to perform a retrospective analysis of creatinine results to determine whether establishment of a creatinine delta check would be an effective means for capturing true laboratory error going forward.

Methods:

All patients with a minimum of two creatinine results during March of 2015 were selected for preliminary review (n = 23,410). Of the analytic errors previously confirmed in the lab, the minimum percent change was 58%; therefore it was decided to review all results that changed by ± 50% (n = 234) to determine the utility of establishing a delta check of this magnitude. Result review entailed thorough examination of patient medical records, including provider notes, ancillary studies, and other laboratory tests at the time of the delta check results. Based on this review, the etiology of creatinine value change was categorized as laboratory error, physiologic change, or inconclusive. Physiologic change was further classified as secondary to pre-renal, renal, post-renal, dialysis/end stage kidney disease (ESKD), transplant, or mixed (i.e. renal and pre-renal) etiologies.

Results:

Out of the 234 delta checks reviewed, 1.3% (3/234) were determined to reflect 2 instances of true laboratory error that went unrecognized by laboratory staff. In both of these cases, the clinical teams immediately recognized that the results were erroneous and ordered a re-draw. 91.0% (213/234) of the delta checks were determined to reflect a physiologic change in creatinine levels. The remaining 7.7% of delta checks (18/234) were deemed inconclusive. The most common etiology for physiologic change in creatinine was pre-renal at 49.3% (105/213), compared to dialysis/ESKD (22.1%, 47/213), transplant (11.3%, 24/213), mixed (10.8%, 23/213), renal (5.2%, 11/213), and post-renal (1.4%, 3/213).

Conclusion:

This retrospective analysis identified two instances of laboratory error reflected by 3 delta checks (1.3%). The vast majority (91.0%) of creatinine results that changed by ± 50% were due to physiologic etiologies. Our analysis clearly demonstrated that establishment of a ± 50% delta check for creatinine would overwhelmingly flag true biological change and would not be an efficient means for identifying rare laboratory errors. Thus, large-scale, retrospective data analysis with medical record review can serve as a powerful tool to determine the efficacy of current or proposed delta checks.

B-132

Verification of the target values established using robust statistical method

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Background: External quality assessment (EQA) schemes are important for laboratory quality management. In EQA, results are usually assessed against a target value. Therefore, the establishment of reliable target value is the premise of effective assessment. **Methods:** Analytical results were grouped according to the analytical method. Data distributions of the original results were tested using Shapiro-Wilks (n<50) or Kolmogorov-Smirnov Z test (n>50). Outliers in each set of analytical results were deleted using a robust statistical method, which involved establishing a Tukey fence, namely Q_1 -1.5 IQR to Q_3 +1.5 IQR. Data distributions of trimmed analytical results were tested using the above statistical methods. The mean was used as a target value if the trimmed analytical results were normally distributed; otherwise, the median was used. Percent difference between the target value and Roche's calibration value was calculated. **Results:** The original analytical results were not all normally distributed. Outliers were deleted at 0-14.6%. The trimmed analytical results were all normally distributed. The target values are shown in the Table. Percent differences between target values and Roche's calibration values were -0.55-1.48. **Conclusion:** Target values established using a robust statistical method are close to the calibration values provided by Roche.

Analyte, no. of results and outliers, method, calibration value, target value and % difference						
Analyte	No. of results	No. of outliers	Analytical method	Calibration value	Target value	% difference
P	54	0	Molybdate UV	1.70 mmol/L	1.70 mmol/L	0.00
GLU	52	2	HK	11.0 mmol/L	11.0 mmol/L	0.00
UA	58	1	Enzymatic colorimetric test	308 umol/L	307 umol/L	-0.32
TP	59	2	Biuret	54.2 g/L	54.0 g/L	-0.37
ALB	58	1	BCG	37.9 g/L	38.1 g/L	0.53
TC	59	2	CHOD-PAP	4.14 mmol/L	4.19 mmol/L	1.21
TG	58	2	GPO-PAP	1.46 mmol/L	1.46 mmol/L	0.00
ALT	61	2	IFCC w/ or w/o pyridoxal phosphate	96.4 U/L	97.7 U/L	1.35
AST	61	1	IFCC w/o pyridoxal phosphate	97.0 U/L	98.2 U/L	1.24
ALP	58	1	IFCC	203 U/L	206 U/L	1.48
AMY	41	6	IFCC liquid	181 U/L	180 U/L	-0.55
LDH	53	2	IFCC liquid	242 U/L	242 U/L	0.00
FE	37	1	FerroZine	35.4 umol/L	35.6 umol/L	0.56
HBDH	40	1	DGKC	254 U/L	255 U/L	0.39

B-133

A National Survey of Critical Value Reporting Procedures for Chemistry and Haematology Analytes in Clinical Laboratories across Nigeria

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Background: Critical value (CV) reporting is a vital quality indicator of the post-analytical phase of the total testing process. Several international healthcare regulatory agencies have specified requirements for the process of critical value notification (CVN). Despite these requirements and the implications of CVs on patient safety, the practice of CV reporting is not well entrenched across laboratories, particularly, in developing countries, where laboratory medicine is at a budding stage. A national survey on CV reporting for chemistry and haematology analytes was conducted to obtain baseline information on the practice of CVN in laboratories across Nigeria.

Methods: Selected public and private laboratories serving secondary and tertiary healthcare institutions in the six geo-political zones in Nigeria, were enrolled in the study. Questionnaires were distributed by electronic mail and physical dispatch. General information such as the type of laboratory and the level of healthcare serviced, were collected for each laboratory. Specific information regarding the handling of CVs, practice of CVN and the existence of a written policy for CV were also collected. Data analysis was performed using SPSS version 22.0.

Results: The response rate was 46.5% (eighty six laboratories). Most (75.6%) laboratories were government (public) laboratories and 82.6% of the surveyed laboratories serviced tertiary healthcare institutions. Over half (53.5%) of the laboratories did not practice CVN and 52.5% of the laboratories which did practice CVN, did not do so all the time. There were no CV limits or CV lists in 60.0% of the laboratories and no written policy for CV handling in 67.5% of the laboratories. The most frequent analytes on available CV lists were haemoglobin, platelet count, serum potassium, sodium, calcium, glucose, creatinine, and bilirubin. The CV limits for paediatric and adult patients were similar in 57.5% of the laboratories. Telephone call was the means for CVN in 45.0% of the laboratories but only 11.1% of these laboratories had a 'read-back' policy to ensure accurate reception of the notification. All the laboratories practising CVN also repeated the assay of every CV obtained, prior to reporting it, 30.0% however, had no specified number of times to repeat an assay for a CV. In 25.0% of the laboratories, there was no laid-down rule for which of the critical values to report, amongst the repeated assays. More than half (62.5%) of the laboratories had no specifications on the time-frame for which a CVN must occur after a CV is obtained. Only 17.5% of the laboratories had an algorithm in place for situations in which the patient's caregiver is unreachable.

Conclusion: Many laboratories serving higher level healthcare institutions do not practice CVN. Remarkable variability exists amongst the laboratories that do practice CVN with only a few having CV limits and CV lists, and even fewer laboratories having written policies for CV reporting. Repeat-testing of CVs is a unanimous practice amongst the laboratories that practise CVN. There is an urgent need to develop locally applicable guidelines for CVN in order to foster uniform and regular practice of CVN among clinical laboratories in Nigeria.

B-134

Utilization and characteristics of STAT whole blood lactate measurements associated with a newly-implemented sepsis early management program

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BACKGROUND: Elevated lactate (>2 mmol/L) is a condition included among criteria used to diagnose severe sepsis. Identification of severe sepsis leads to initiation of a sepsis protocol, according to specifications of the CMS Core Measure, SEP-1 Early Management Bundle, Severe Sepsis/Septic Shock, implemented in October, 2015. In order to facilitate rapid diagnosis of severe sepsis and timed sample specifications of the Core Measure, STAT whole blood lactate (BL) was made available from the central laboratory for this purpose. Our objectives in this study were to review rates of utilization of BL, and to evaluate turn-around-times and results distributions for BL in comparison to those for conventional STAT serum lactate (SL). METHODS: To meet SEP-1 definition of elevated lactate, the upper limit of our reference interval for lactate was changed system-wide to 2.0 mmol/L (from 2.2 mmol/L). For BL, whole blood samples were delivered to the laboratory as individual samples accompanied by a BL order form. BL was performed using Radiometer 837 analyzers. Elevated BL (>2.0 mmol/L) was reported by telephone. Primary data for ordering locations, results distributions and turn-around-times (TATs) were from LIS reports (Sunquest) for BL and SL (performed by Roche Cobas c500 analyzers) covering a period of 84 days after implementation of BL. RESULTS: Results distributions for BL (n=851 (10.2/day), 548 patients) were slightly right-shifted compared to SL (n=8305 (98.9/day), 3784 patients): medians (BL/SL=1.8/1.6 mmol/L); 95%-iles (BL/SL=6.7/5.7 mmol/L); %positive (>2.0 mmol/L; BL/SL=41.5%/34.2%); %critical (>4.0 mmol/L; BL/SL=14.5%/9.4%). ED orders for BL comprised 42.0% of total BL and 21.6% of total SL. Accrual rates vs. time-of-day for BL followed a pattern reflecting ED admission rates. Turn-around-times (medians, min) were significantly less for BL compared to SL: collect-to-receive (BL/SL=8/16 min); receive-to-report (BL/SL=6/36 min); collect-to-report (BL/SL=15/56 min). Receive-to-report TATs had numerous unexplained outliers (95%-ile=24 min). Among all BL, 184 (21.6%) were first-time identifications of elevated lactate (14.3% of all first-time identifications among BL and SL). Among these, 67 (36.4%) had follow-up lactates either by BL or SL within a 6h interval. In comparison, hospital monitoring of core measures indicated a 91% completion rate for 6h follow-up of initial lactates given diagnosis of severe sepsis (per SEP-1 bundle requirements). Thus, most elevated lactates obtained initially by BL measurement were apparently not associated with a diagnosis of severe sepsis. Last, it was found that a small fraction (5.2%) of BL were submitted from intensive care units (ICUs) from which BL should have instead been performed using available point-of-care testing (POCT). CONCLUSIONS: BL showed significantly reduced turn-around-times compared to SL, meeting administrative objectives in establishment of laboratory-based BL for use in the severe sepsis/septic shock protocol. There was modest preselection for elevated lactate among BL compared to SL. Low follow-up measurement rates for initial elevated lactates obtained by BL indicated that most of these cases were not associated with final diagnosis of severe sepsis. Areas identified for improvement were to minimize outlier receive-to-report intervals for BL, and to limit BL submissions to laboratory from locations where POCT BL is available.

B-135

"Accurate Results for Patient Care:" The Role of Traceability in Laboratory Medicine

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Clinical laboratories require global metrological standardization to produce equivalent patient test results across space and time. Standardization is required to use evidence based laboratory medicine (EBLM) practice guidelines and eliminate the need for local or method-specific reference intervals/decision cut-offs with the goal of improving e-healthcare and patient safety. Healthcare providers and patients take for granted all test results are accurate, comparable and interchangeable, and

clinical practice guidelines assume results are independent of assay methodology. Due to lack of standardization, currently all results are not equivalent and assay method-specific reference intervals and medical decision points are required. The European Union's In Vitro Diagnostics Directive (IVDD) mandates metrological traceability for calibrators and trueness controls to promote assay standardization. The Joint Committee for Traceability in Laboratory Medicine (JCTLM), formed in 2002, promotes standardization in the clinical laboratory. It was founded by the BIPM (Bureau International des Poids et Mesures), the IFCC (International Federation for Clinical Biochemistry and Laboratory Medicine), and ILAC (International Laboratory Accreditation Cooperation). JCTLM now has 28 member organizations, including AACC that are committed to traceability in laboratory medicine. JCTLM promotes the use of proven metrological principles to support equivalence of measurements in the clinical laboratory through metrological traceability to appropriate reference materials and methods. Standardization is achieved when all routine assay results for test are traceable, with an unbroken metrological chain of comparisons, to reference materials and methods of a "higher order," with a sufficiently small uncertainty such that results may be validly compared. The JCTLM has developed a database of such higher order reference materials and methods and reference measurement services (http://www.bipm.org/jcltm/). Entry in the database is determined by review by experts using ISO standards and approval by the JCTLM Database Working Group and Executive Committee. In 2015 the database contained listings for 295 materials for 162 measurands, `70 methods for 79 analytes and 130 reference measurement services for 39 analytes. Implementation of traceability requires action by many bodies: national measurement institutes and other organizations that prepare materials and develop methods; reference measurement service laboratories; IVD manufacturers that prepare calibrators/trueness controls for field assays following appropriate traceability chains and provide traceability information to users; clinical laboratories that select and use traceable assays; EQA/PT providers that confirm claimed traceability; and guideline committees that base recommendations on traceable results. To promote these activities the JCTLM formed a Working Group on Traceability: Education and Promotion (WG-TEP) in 2015 to produce and use educational materials demonstrating the value of traceability in laboratory medicine. Its sixteen members represent the JCTLM Executive Committee, the wider international membership, and individuals with skills and experience in creating educational materials. WG-TEP provides key traceability educational material at professional society meetings and maintains a traceability website containing information and resource materials about traceability and standardization in laboratory medicine and links to the

JCTLM database and member organizations. WG-TEP also provides the clinical laboratory industry with recommendations for calibration traceability statements and supporting documentation that provides metrologically appropriate and clear assay standardization descriptions which are the responsibility of IVD manufacturers.

B-136

An Assessment of Why Physicians' Electronically Ordered Add-on Laboratory Tests Are Not Performed in a Large, University Hospital Laboratory

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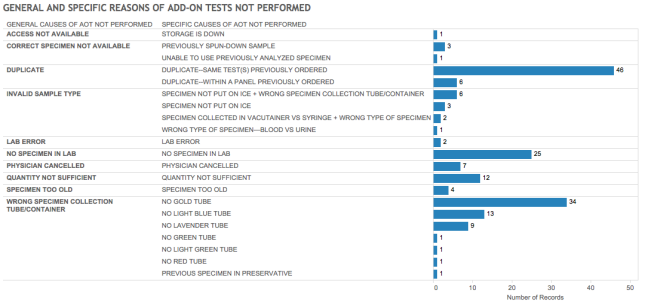
With the universal adoption of the electronic medical records systems in hospitals, physicians often tend to abuse the features that allow for unrestricted electronic laboratory tests orders for so called "add-on" testing. An add-on test is a physician order for a test to be performed on a specimen that has already been collected for other testing purposes. We have observed that having physicians electronically do add-on laboratory orders does not work well since there are many issues involved that may make the add-on ordered test impossible for the laboratory to perform. This leaves the laboratory staff having to contact the physician by telephone to let them know there is a problem. To address this issue, we monitored add-on test orders for a seven day period and observed the following: 1) a total of 1,062 add-on tests were electronically ordered, 2) 883 (83%) of add-on tests were performed and 3) 179 (17%) of add-on tests were not performed. We investigated the major reasons why these add-on tests could not be performed and summarized our findings into 10 major categories which can be broken down into 21 subcategories. As shown in the figure, 34 % (60/179) of add-on tests not performed were specimens collected in the wrong tube/container, 29% (52/179) of tests not performed were duplicate orders, while for 14% (25/179) of add-on tests not performed, no specimen was available in the laboratory. We conclude physician add-on orders require either 1) a sophisticated rules-based assisted expert physician order entry system to alert the physician when an add-on test is possible or 2) a more collaborative approach with the laboratory....such as physicians calling the laboratory and placing the order verbally. Unfortunately, many hospitals including ours do not have the level of IT technology required to do expert system assisted physician add-on order management.



B-137

Clinical Chemistry Education in Medical Students

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Background: Marmara University Medical Faculty currently applies an integrated educational program for medical students enriched with interactive instructional activities based on problem-based sessions and experiential learning. Following the evaluation by "The National Accreditation Council for Undergraduate Medical Education" considering 70 different standards based on the international standards in medical education, the education in our Medical School has been accredited until 2017.

Methods: A 2.5 day introductory education program was implemented within the adaptation to clinical courses module at the beginning of 4th grade, to give the medical students the principles and skills of basic laboratory procedures and introduce them to the principles of evidence based laboratory medicine which is an important part of clinical decision making. In this study, we aimed to evaluate the effectiveness of the clinical chemistry part of this education program. Fourth grade medical students of Marmara University Medical Faculty (n=134) were enrolled in the study. The students were divided into 8 small groups and each group had a lecture on pre-analytic variables, a clinical chemistry laboratory visit, 2 practices (peripheral smear and urine sediment examinations), and a case discussion session focusing on evidence based decision-making according to test results. The course was evaluated at 2 levels: (1) assessment of knowledge transfer by a quiz (10-questions, either multiple choice or correct/incorrect or descriptive type) which was administered at the beginning and end of the course and (2) student satisfaction assessed by a survey.

Results: The median score was 18 for the pre-test and 84 for the post-test over 100 points Improvement was statistically significant for total scores and each question scores (P<0.001). Students' overall feed-back was satisfactory.

course

Q.N.	Question Content	Quiz Time	Median (25th-75th)
1	Differences of anticoagulant types	Pre-test score	0 (0-10)
		Post-test score	10 (10-10)
2	Peripheral blood smear preparation	Pre-test score	4 (4-6)
		Post-test score	8 (6-8)
3	Peripheral blood smear examination	Pre-test score	0 (0-0)
		Post-test score	5 (5-10)
4	Peripheral blood smear faults	Pre-test score	4 (4-6)
		Post-test score	8 (6-10)
5	Pre-analytical errors in clinical laboratory	Pre-test score	0 (0-0)
		Post-test score	10 (10-10)
6	Heel prick blood sampling in neonates	Pre-test score	0 (0-0)
		Post-test score	10 (10-10)
7	Urine sediment preparation	Pre-test score	0 (0-0)
		Post-test score	10 (7-10)
8	Urine sediment examination	Pre-test score	0 (0-0)
		Post-test score	5 (5-10)
9	Urine sediment examination	Pre-test score	0 (0-0)
		Post-test score	10 (10-10)
10	Urine sediment examination	Pre-test score	0 (0-0)
		Post-test score	10 (10-10)
Total Score		Pre-test score	15 (8-25)
		Post-test score	84 (75-88)

Conclusion: Medical student knowledge regarding the foundations of laboratory medicine was improved through this 2.5-day curriculum. Similar courses could be implemented by other medical schools to successfully impart laboratory medicine concepts to medical students.

B-139

Application of a series of robust statistical methods in EQA scheme

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Background: Outliers occur constantly in external quality assessment (EQA) scheme to cause the data are not normally distributed. Robust statistical methods should be applied to deal with these data. **Methods:** Analytical results were grouped according to the analytical method. Outliers in each set of analytical results were deleted using a robust statistical method, which involved establishing a Tukey fence, namely Q_1-1.5IQR to Q_3+1.5IQR. The values outside this fence were considered outliers and were removed. The mean was used as a target value if the trimmed analytical results were normally distributed; otherwise, the median was used. Robust between-laboratories z-score (ZB) and within-laboratory z-score (ZW) were calculated using the formulas: ZB = S-median$_{(s)}$/NIQR$_{(s)}$ and ZW = D-median$_{(d)}$/NIQR$_{(d)}$. A robust Youden plot was constructed based on the robust statistical parameters, which were calculated using the formulas: a (major radius) = 2.448NIQR$_{(S)}$ and b (minor radius) = 2.448NIQR$_{(d)}$. Acceptable and unacceptable results fall inside and outside the Youden ellipse, respectively. Questionable results are located on or near the Youden ellipse. **Results:** The robust target values of the 1st and 2nd measurements were P, 1.70 and 1.70 mmol/L; GLU, 11.1 and 11.0 mmol/L; Urea, 16.8 and 16.8 mmol/L; UA, 307 and 307 µmol/L; TP, 53.9 and 54.0 g/L; ALB, 38.1 and 38.3 g/L; TC, 4.19 and 4.19 mmol/L; TG, 1.46 and 1.46 mmol/L; ALT, 98.1 and 97.7 U/L; AST, 98.2 and 98.5 U/L; ALP, 206 and 206 U/L; AMY, 179 and 180 U/L; LDH, 242 and 243 U/L; GGT, 115 and 115 U/L; FE 35.5 and 35.6 µmol/L; and HBDH, 255 and 255 U/L, respectively. The ranges of the robust ZB and ZW absolute values and the robust Youden plots were shown in the Figure. **Conclusions:** It was reasonable to choose robust target value, ZB, and ZW as assessment indexes and the robust Youden plot can reasonably illustrate EQA data.

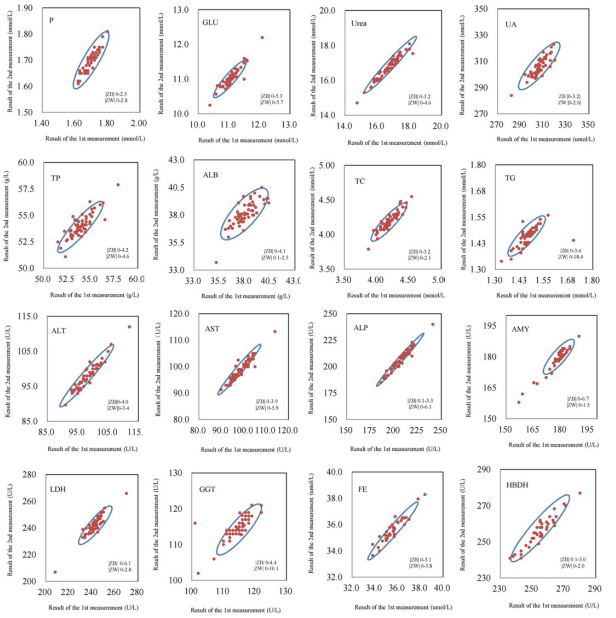

Fig. Robust Youden plots of the measurement results of P, GLU, Urea, UA, TP, ALB, TC, TG, ALT, AST, ALP, AMY, LDH, GGT, FE, and HBDH concentrations

B-140

Analysis of the pre-analytical phase for parathyroid hormone (PTH) measurement in renal patients.

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Background: The parathyroid hormone (PTH) is an important key regulatory hormone in calcium homeostasis and bone mineralization. The balance of calcium levels is achieved through the tight regulation of several mechanisms:intestinal absorption of calcium; calcium and phosphorus mobilization from bone tissue; and renal tubular reabsorption of calcium and phosphorus excretion. Measurement of PTH plasmatic levels is very important for the correct diagnosis of several diseases, including primary or secondary hyperparathyroidism and hypoparathyroidism, and kidney diseases. PTH determination represents a paradigm of quality in laboratory medicine as many variables in the pre-, intra-, and post-analytical phases strongly affect the value of the clinical information. In recent years, clinical laboratories have accepted the evidence that errors in the pre- and post-analytical phases occurred much more frequently than in the analytical phase. **Objective**: To evaluate the frequency of pre-analytical errors related to PTH measurement in clinical samples from renal patients. **Methods**: Data were collected from Hermes Pardini Institute database (Vespasiano, Minas Gerais, Brazil) during the period of 3 months (October to December) in 2015. **Results**: Among 286 analyses, 81 (28%) pre-analytical errors were confirmed, of which 8.74% were problems associated with sample storage. **Conclusion**: The results indicated a significant number of inadequately stored samples, and it is related directly sample collection, generating a delaying in reporting the results. These issues in the pre-analytical phase usually originate from high turnover of laboratory professionals, negligence, and lack of training of good laboratory practices. In spite of the technological improvements in laboratory routine, the pre-analytical phase is still the main responsible for laboratory errors.These errors can be prevented by identifying their causes and by understanding their impacts.

B-146

Sometimes less is more! Managing wisely laboratory tests among hospitalized patients

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Background: Interest in the subject of choosing of medical tests and treatments wisely is growing. The underlying principle is the desire to use evidence-based medicine (EBM) and the need to decrease excess medicine in general and the unnecessary ordering of repeated tests in particular. Consequences of overuse include patient exposure to infections, pain and stress, as well as extra work for the laboratory staff and unnecessary costs. It is relatively easy to set standards and monitor special treatments and tests. However, it is more difficult to determine standards for routine laboratory tests performed on hospitalized patients.

Objective: Decrease excessive blood tests among in-patients by developing a method to map the extent of repeated routine tests in all departments, and to intervene when necessary.

Methods: Five Internal Medicine departments (similar in patient mix and size) were piloted. We focused on blood count and chemistry tests and developed a computerized report of tests ordered. An index was created to determine the number of repeat tests for each patient and for each test in every department. It is expressed as an index that provides a measure of the number of repeated tests. An intervention was implemented in the department with strikingly excessive orders, data were collected and on-going follow-up was conducted.

Results: One department was reordering tests at a 30% higher rate than the rest of the pilot departments (index 2.0 vs. 1.6). As a result, interventions included appointing a departmental representative, collecting data for six months with regular feed-back to the department along with periodic discussions on the progress and improvement plan. After the intervention, repeat tests ordered by the department decreased to 1.5 (compared to an average 1.6 in other departments).

Conclusions: Awareness of the phenomenon of excessive use of routine laboratory tests led to the development and implementation of a quantitative method to estimate the rate of repeated tests and to compare across departments and institutions. This method allows management to detect variances and encourages departments to establish clear criteria for ordering routine laboratory tests.

B-148

A Process Improvement Project Based on the Updated Guidance for the Management of Myocardial Infarction/Acute Coronary Syndrome: A Significant Reduction in Unnecessary Orderable Testing and Laboratory Costs

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Background: In 2014, the American College of Cardiology (ACC) and the American Heart Association (AHA) updated guidelines for the evaluation and management of acute coronary syndrome (ACS). Measurement of creatine kinase MB fraction (CK-MB) now carries a Class III recommendation (No benefit or may cause harm) based on A-level evidence (data derived from multiple randomized clinical trials or meta-analyses). The guidelines specify the troponin assay as the preferred diagnostic test for the evaluation of ST and non-ST elevation myocardial infarction and unstable angina.

Objective: In order to align standard practice with consensus guidelines and optimize resource utilization, we collaborated with the Department of Cardiovascular Medicine to remove the CK and CK-MB assays from our Cardiac Panel and develop educational materials to explain the change to providers. CK and CK-MB remain available as individually orderable tests in the Laboratory Information System (CHCS); however, their routine use in the setting of suspected ACS is discouraged.

Methods: A letter reviewed and approved by the Cardiology Department Chief at Walter Reed National Military Medical Center (WRNMMC) and the Army's Cardiology Consultant to the Surgeon General describing the proposed modifications was prospectively sent to providers at all medical treatment facilities (MTFs) and outlying clinics within the National Capital Region (NCR). Once consensus acceptance was received from all MTFs and outlying clinics, a second letter was sent to all NCR client laboratories indicating the changes and effective date (July 1st, 2015). Workload data for CK and CK-MB was collected from July 2015 to January 2016 and compared to the data from July 2014 to January 2015.

Results: We observed a stepwise reduction in CK and CK-MB orders from July 2015 to January 2016. While total tests ordered decreased from 2690 to 1865 for CK-MB (31% reduction) and from 4650 to 3539 for CK (24% reduction) for this 6 month period; January 2016 saw a 78% reduction in CK-MB orders and a 43% reduction in CK orders as compared to January 2015. This change in ordering practice resulted in a savings of $12,000 over a four month period.

Conclusion: We highlight how a collaborative partnership between Cardiology and the Laboratory led to changes in clinical practice that improved adherence to standard of care as well as utilization of laboratory resources in the management of ACS. These findings should encourage laboratories to investigate and implement similar collaborative efforts.

B-149

Pre-analytical nonconformities in immunophenotyping of hematological malignancies

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Flow cytometric immunophenotyping is essential part of the laboratory diagnosis, prognostic classification and treatment effectiveness of hematological diseases.This method has become the preferred to assess the immunophenotypic features of cells present in bone marrow (BM), peripheral blood (PB) and other types of samples suspected of containing neoplastic cells. The list of clinically useful antibodies has progressively increased and this facilitates more precise identification and characterization of specific populations of tumor cells. The quality of the samples and the selection of a suitable panel of antibodies are essential for diagnosis of leukemias and lymphomas. There are some pre-analytical requirements for this test in our laboratory:(I) EDTA-anticoagulated PB or BM samples within 48 hours after withdrawal, (II) the corresponding smear for morphological analysis, (III) clinical information and (IV) hemogram information containing white blood count. In this context, the aim of this study was to assess the pre-analytical nonconformities in immunophenotyping analysis of hematological malignancies in our institution. We retrospectively evaluated 697 immunophenotyping tests conducted at Progenetica Laboratory - Hermes Pardini Institute, between January and July2015.These samples were received from partner laboratories across the country. In our study were identified ten kinds of nonconformities in 44.2% (308) of the tests analyzed.The nonconformities identified and its frequencies were respectively:absence of smear for morphological analysis (23.1%), absence of results of leukocyte differential count blood and smear for morphological analysis (22.1%), absence of clinical information (20.1%), absence of results of leukocyte differential count blood (17.5%), absence of clinical information and results of leukocyte differential count blood (6.5%), samples receivedover48 hours afterwithdrawal (5.5%), absence of clinical information, smearformorphologicalanalysis and results of leukocyte differential count blood (2.6%),inadequate samples (1.3%) and absence of clinical information and smear for morphological analysis (1.3%). As with all diagnostic modalities, an adequate and representative sample is necessary for meaning ful analysis. Three of 17samples received more than 48 hours after with drawal could not be analyzed, due to low viability of the cells in the sample.One of four inadequate samples could not be analyzed due to the presence of clots. The careful correlation of the patient's clinical history and other diagnostic details are necessary to ensure accurate diagnosis. In this study, five of 62 cases without clinical information had inconclusive results. Three of eight cases without clinical information, smear for morphological analysis and leukocyte count also had inconclusive results. These pre-analytical requirements are essential to choose the panel of antibodies. The correct data interpretation and diagnosis is also based on appropriate panel of antibodies. The absence of these requirements only allows selecting a general panel, which explains inconclusive results. The Pre-analytical requirements should be familiar to laboratory personnel and partner laboratories, since many factors may influence the technical preparation and a variety of causes can result in misinterpretations.The knowledge of pre-analytical nonconformities helps avoiding potential sources of errors, ensuring quality in analysis and accurate diagnosis.

B-150

Laboratory labor and cost efficiency improvement with the implementation of six-sigma statistical quality control management

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Introduction: Accurate and reproducible test results are critical as 70% of medical decisions are influenced by laboratory results. Quality control programs are an integral

part of laboratory operation to monitor and ensure accuracy. Six-Sigma metrics applied to quality control programs can help identify waste and redundancy, while verifying analytical performance quality. Adopting a Six Sigma quality control program enable the laboratory to have a standardize method to quantify laboratory quality and improved laboratory efficiencies by eliminating redundant procedures. **Methods**: Sigma metrics were evaluated for 29 chemistry assays on the Abbott's ARCHITECT c8000 chemistry analyzer using the equation: Sigma metrics = (TEa - %Bias) / %CV. The choice of TEa was selected based on the recommendation of the Westgard Verification Program. % Bias and % CV were calculated with QC data collected over a 3 months period. QC optimization of control rules and frequency were based on the Sigma metrics achieved for each of the 29 chemistry assays. Laboratory operation and cost efficiencies were determined after implementing the Six Sigma quality control management program. **Results**: 26 out of 29 chemistry assays (90%) were of world class and excellent performance achieving Sigma metrics >5. The remaining 3 assays were between 3-5 Sigma metrics. Importantly, no assays were <3 Sigma metrics. QC optimization based on the Sigma metrics achieved for each of the results, resulted in reduction of QC frequency from 8x per day to 2x per day for those assays >5 Sigma metrics and 4x per day for those assays between 3-5 Sigma. With the reduction of the QC frequency, this enabled a direct QC material cost reduction of 78% annually for an estimated annual saving of US$5,300. Assay reagent savings of US$36,000 annually, were correspondingly achieved to achieve a total annual operation cost saving of over US$40,000. Importantly, laboratory operation efficiency was dramatically improved with a labor saving reduction of ~78% man hours, from 240 man hour to 52.5 man hour per month.**Conclusion**: By adopting and implementing Six Sigma quality control management, we were able to have a standardize method to quantify our laboratory quality control practice. This provided us with an international benchmark to guide us in optimizing our QC operation that lead to significant labor and cost savings without compromising patient care. Participation in the Westgard Sigma Verification program further validated our laboratory quality control management system while providing excellent quality health care for its patients.

B-151

Change management in continuous quality improvement program implementation

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Introduction: Laboratories are continuously striving to improve their quality standards in order to deliver excellent health care. To fully benefit from any quality improvement program, fast and successful implementation of the program is critical and this would require alignment and buy-in from all levels of the laboratory staffs. Careful change management is an essential component for successfully implementing new practices to the clinical laboratory. **Methods**: To ensure alignment and buy in of laboratory staff during quality improvement changes, a committee comprising laboratory staffs of all levels were formed to evaluate and determine the project(s) to implement. Various programs of consideration included 1. Six Sigma Statistical Quality Control Management; 2. Improvement of timeliness of report inspection; 3. Reduction of sample rejection etc. Careful gap analysis was undertaken to determine the challenges for program implementation. Stepwise incremental program development activities, individual roles and responsibility, timing and milestone goals were established and aligned. **Results**: Based on consensus agreement from the quality improvement committee, the Six Sigma Statistical quality control management program was evaluated to be of most importance and urgency to ensure the laboratory achieved high quality standards to meet patient health care demands more effectively. Furthermore, the program was also considered to be all encompassing, requiring participation of all laboratory staff. Through educational activities, such as quality control expert visits and lectures, it not only enhance and strengthen staffs quality control concept and knowledge such as problem solving capability and communication skills, but also improved team work and spirit as measured by employee engagement surveys. **Conclusion**: By involving and getting buy in and alignment at all levels of the laboratory staff in the choice of program and implementation plan, this ensured fast and successful implementation of the Six Sigma statistical quality control management program as part of the laboratory goal to continuously strive for quality improvement. In addition to breaking old habits and achieving quality improvement, through a world class quality control system validated by the Westgard Sigma Verification program, staff moral, sense of achievement, and engagement were also improved as a result

B-152

Optimalisation of the turnaround time of Borrelia antibodies determination

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Background: Ticks occur very frequently in the Central Europe and much of their population is a carrier of Borrelia burgdorferi causing Lyme borreliosis. Determination of the Borrelia antibodies is therefore a very important examination for possible infection diagnosing and the success of the antibiotic therapy monitoring.

Methods: Testing for Borrelia antibodies classes IgG and IgM was performed by enzyme linked immunosorbent assay (ELISA) using BioRad Evolis processor batchwise twice weekly. The average turnaround time for both of these tests was 96 hours, the median turnaround time was also 96 hours. When confirming the positive results using immunoblot , the response time extended by another 24 hours. In terms of clinical importance of these investigations, it was necessary to shorten the response times significantly. To solve this problem it was chosen to install these methods for the automated immunoassay analyzer Diasorin Liaison XL. Diagnostic kits LIAISON Borrelia IgG and LIAISON Borrelia IgM II were used for the determination of the Borrelia burgdorferi antibodies by indirect chemiluminescent immunoassay (CLIA).

Results: Using this system, the turnaround time for determination of IgG antibodies was reduced to 190 minutes on average with a median of 93 minutes. The turnaround time for IgM antibodies was 186 minutes on average with a median of 90 minutes. The capacity of the analyzer with such determinations was utilized in 44% (22% IgG and 22% IgM). The rest of the analyzer capacity was used for further diagnosis of infectious diseases - CMV, EBV, Rubella, etc. This significant reduction of the response time plays an important role also in the overall operation of the laboratory, because the general part of these tests comes in very narrow time frame - 40% of the samples between the 10 and 11 o'clock, 25% between 8 and 9 am and 20% between 1 and 2 o'clock in the afternoon. There is also a seasonal effect noticeable, the difference between the number of examinations in the summer and winter months is about 20%.

Conclusion: The response time is positively reflected in the perception of the usefulness of this test by the customers of the laboratory, which showed a rise in the number of assays of Borrelia burgdorferi antibodies by an average of 35%.

B-153

Contribution of ESEAP - The Greek Proficiency Testing Scheme for Clinical Laboratories in the improvement of analytical performance of participating laboratories.

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The Greek Proficiency Testing Scheme for Clinical Laboratories (ESEAP) has been operating continuously since 1994. At the beginning the number of participants was about 100 laboratories, but today, the number of participants has reached 320, including almost all public hospital laboratories in Greece an increasing number of diagnostic centers and private laboratories all over Greece and 50 laboratories of the public and private sector in Cyprus. The wide impact and acceptance of our schemes is due to the fact that, they operate in Greek language, cover the most frequently ordered tests in Laboratory Medicine and, although there are very friendly and easy to use, they provide laboratories with an objective assessment of their own performance as well as in relation to that of other laboratories. Furthermore they provide information on the relative performance of the available methods and analyzers, identify factors associated with good and poor performance via the Youden plot and improve the inter-laboratory agreement.

A cycle of the clinical chemistry program involves twelve distributions and covers a two year period. Twenty five analytes are statistically processed on the overall results, regardless of the methodology. After the elimination of outliers (elimination in two passes of all results > or < 2.5SD of the consensus mean value), the "consensus" mean, namely the mean from all individual results is used as target value. At the end of each two-year cycle, the performance of each participant is assessed through a standard scoring and ranking system.

A totally new, web enabled, software was implemented at 2008 which allows participants to send their own results and obtain reports and information about their performance through the Internet. New software features permit further grouping of methods and analyzers, the enhancement of the statistical evaluation of the results, as also as the evaluation of reproducibility of the measurments using 4 samples during each year, (two replicates of the same sample and two other samples, derived from the initial sample by dilution or concentration by p.ex. 10% and 8% respectively,

modifying accordingly the volume of serum to be lyophilized per vial in order to avoid the possibility that participants can detect the replicated samples and to report already known target values. Obviously, two of the samples (the replicates) are evaluated as received and the other 2 after correction with the appropriate factors for the dilution or concentration.

ESEAP has considerably contributed to the improvement of performance for the majority of the laboratories, as the mean CVs for all analytes showed a significant decrease from cycle-1 (1994-1996) to cycle-3 (1998-2000) of the program. Afterwards, and until the latest fully completed cycle (cycle-10 2012-2014) the CVs remained stable. The CV per cycle for each analyte was calculated as average between control A and control B mean CVs over the 12 distributions of each cycle (inter-laboratory CV). This reduction ranges from 1 to 2% (electrolytes), from 2 to 4% (substrates) and from 4 to 8% (enzymes). In this calculation we included only the 100 laboratories that participated continuously in all the above cycles.

B-154

Evaluation of the viability of the decentralization process tests carried out on a large laboratory support for regional technical operational centers

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Background: The Turnaround time (TAT) of a test is used as a laboratory efficiency indicator and the existing high competitiveness in today's clinical laboratory market becomes an extremely important factor when acquiring new customers, being they patients, doctors or laboratories. As conventional ways to reduce the TAT are already present in several laboratories, such as automation at all stages of the process, pre-analytical issues and barcode tube labeling. The Laboratory X, which operates in the private market (patients) and support (laboratories), sought a new way to decrease TAT, by carrying out the examinations in regional technical operational centers (TOC) previously performed only in the headquarters located in western Parana, to verify the possibility of increasing the number of examinations and laboratories attended.

Methods: Thus they were created 3 TOCs: TOC1, TOC2 and TOC3, where the six highest tests in demand (TSH - Thyroid stimulating hormone, VIT25 - Vitamin D - 25 Hydroxy, T4L - free thyroxine, HBGLI - Glycated hemoglobin, FERRI - Ferritin and T4 - thyroxine) were evaluated for 7 different client labs for each TOC, comparing the first 4 months of the years 2014 and 2015.

Results: There was a considerable decrease in the TAT, with an average reduction in hours of TAT for TOC3 of 21:20:57; TOC1 15:40:47 and TOC2 of 12:44:08 (table 1). There was an increase in the number of tests of 582.691, as well as the number of clients that went from 8141 customers in April of 2014 to 8835 in April of 2015.

Conclusion: The test execution process in regional TOCs was highly favorable, and can give the Laboratory X a great competitive advantage in the clinical laboratory market.

Table 1: Difference of TAT in each TOC between the years 2014 and 2015.

TOC	Jan/14	Fev/14	Mar/14	Abr/14	TAT Medium	Difference between TATs
TOC1	25:38:08	33:51:24	25:32:05	22:12:13	26:48:27	15:40:47
TOC2	30:30:59	39:47:52	32:50:51	27:55:04	32:46:12	12:44:08
TOC3	33:31:53	37:28:49	30:51:31	25:38:54	31:52:47	21:20:57
	Jan/15	Fev/15	Mar/15	Abr/15	TAT Medium	
TOC1	14:53:19	12:04:30	9:03:47	8:29:02	11:07:40	
TOC2	18:36:29	16:47:36	18:21:44	26:22:27	20:02:04	
TOC3	11:31:30	11:20:04	10:55:17	8:20:28	10:31:50	

B-155

Automation of a High Volume High Complexity LC-MS/MS Clinical Laboratory

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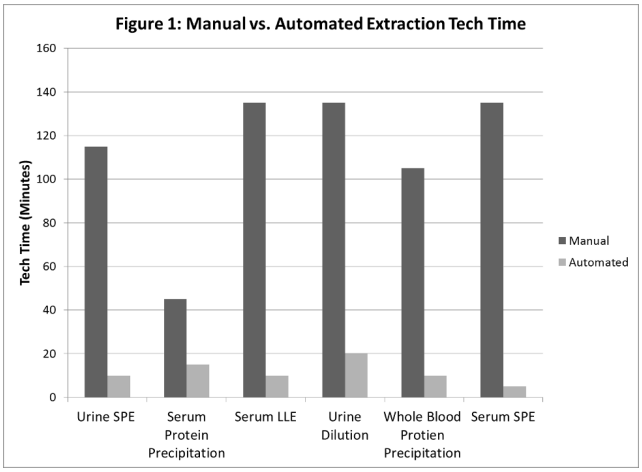
Background: Testing in a high complexity liquid chromatography tandem mass spectrometry (LC-MS/MS) clinical laboratory typically involves a complicated and highly manual workflow. An example of a workflow is as follows: samples received → manual work list created → technologist numbers work list → technologist manually extracts samples → manually enters work list into instrument → samples analyzed → manual review of data → manual entry of results. There are many inherent issues and risks with a highly manual workflow including increased tech time, decreased efficiency and capacity for growth, misidentification and data entry errors, and increased risk of failed runs. **Objective:** The objective of this project was to automate the laboratory through the use of an automated sample preparation instrument (ASI) and electronic interfaces for result upload to the laboratory information system (LIS) in order to reduce the risk of potential errors, increase efficiency, and decrease tech time. **Results:** Several different ASIs were evaluated prior to the selection. Once implemented the workflow of the laboratory is greatly simplified and is as follows: samples received → loaded on to ASI → ASI scans barcodes, creates work list, and extracts samples → Work list electronically transferred to LC-MS/MS instrument → samples analyzed → manual review of data → result electronically transferred to LIS. This greatly simplified workflow has the potential for a ~20% reduction in the number of full time employees (FTE) needed to operate the laboratory. Figure 1 demonstrates the potential tech time savings for a representative six assays. A financial analysis showed that the potential return on investment (ROI) after 5 years is >200%. There is also the potential of increased patient safety and care due to the reduced risk of possible manual errors. **Conclusion:** This significantly improved laboratory workflow potentially has a considerable positive quality and financial impact on an LC-MS/MS laboratory.



B-156

Microbiology Proficiency Testing: Usa and Improvements Based on a National Survey of Laboratory Professionals

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Background: In the U.S., proficiency testing (PT) is required by the Clinical Laboratory Improvement Amendments (CLIA) of 1988 and accrediting agencies as an external performance assessment of clinical laboratory testing. However, PT can have many benefits beyond regulatory requirements. In 2013, the Centers for Disease Control and Prevention, in collaboration with the Association of Public Health Laboratories, evaluated the use of PT and the perception of its value by laboratory professionals through a voluntary survey. A section of the survey contained questions specifically targeted to microbiology PT to assess opportunities for improvement.**Methods:** Survey questions were based upon results from focus groups previously convened to discuss PT uses. Survey participants were recruited from all laboratories in the U.S. certified by CLIA to perform nonwaived testing. A brochure was mailed to the laboratories in July 2013 followed by a reminder postcard in September 2013. Additionally, a one page advertisement was included in the August, September, and October 2013 issues of a professional journal. Only one respondent per laboratory was included in the analysis.**Results:** Of the 679 respondents that answered the question, "Does your laboratory perform microbiology testing?" 401 (59%) responded affirmatively; these were prompted to answer a series of questions about microbiology PT. When asked if the laboratory reported PT results to the same level as reported for patient testing, 86% responded 'yes,' while 12% answered 'yes,' but occasionally report to a lower level, 1% sometimes report PT at a higher level and 1% answered 'no.' Specimen source/type/site, Gram stain results, and patient symptoms, age, and sex, were all considered necessary information to process and analyze PT samples appropriately by a majority of respondents. Additionally, 75% of all respondents agreed that changes should be made to microbiology PT grading to allow for monitoring performance over time

for a particular type of test or examination. When asked to rate the importance of specific proposals to improve PT, a majority of respondents indicated that improving the quality of photographs (55%) and improving the quality of stained slides (61%) were important. Including fewer susceptibility testing challenges (66%), including fewer emerging or less common organisms (67%), and increasing the use of slides or photographs instead of digital images (59%) were not important. Requiring direct antigen testing in mycology (62%) or parasitology (53%), or requiring susceptibility testing in mycology (64%) or virology (64%) were not applicable to most of the respondents. Free text contributions reinforced the need for better quality for Gram stains, listed problems due to preservation/lyophilization of microorganisms, and suggested presumptive identifications be reported without a penalty in cases where confirmation is needed.**Conclusions:** While survey results are not representative of all clinical laboratories in the U.S., it is clear that respondents thought that changes should be made to microbiology PT. Areas of importance included grading, the quality of photographs and slides, and the need to reduce difficulties due to preservation of the samples.

B-157

A Theoretical Basis for Establishing Acceptance Limits for Proficiency Testing Based on Clinical Needs as Reflected in Biological Variability

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Objective We explored the potential to link proficiency testing (PT) acceptance limits (ALs) with goals for total allowable error (TE_A) derived from estimates of biological variation.

Methods Using published data for within-individual coefficient of variation (CV_I), and between-individual CV (CV_G), we calculated the TE_A using a previously published model*, using the achieved factor (f_{AL}) as a variable describing suitability of the TE_A. We examined the current AL's for representative PT analytes required by the Clinical Laboratory Improvement Amendments (CLIA) regulations. We determined the f_{AL} for TE_A goals that are "optimal" (f_{AL}<0.125; Code O in the table), "desirable" (0.125 < f_{AL}<0.25; Code D), "minimal" (0.25 < f_{AL}<0.375; Code M), "marginal" (0. 375 < f_{AL}< 1.0; "R") or "excessive" (f_{AL}>1.0); "E"). We calculated $f_{AL} = CV_B / (AL - k \times CV_A)$ *with* k = 1.65 for 95% CI, one-sided.

Results Few ALs meet optimal clinical needs, but for some analytes current ALs have f_{AL}s in the minimal or desirable ranges. Some analytes have f_{ALs} that are marginal and for these further AL tightening may achieve the minimal error goal (f_{AL}<0.375) or move the AL closer to optimal. For other analytes, the analytical goals may not be achievable with current technology.

Conclusion It is possible to calculate the AL necessary to achieve minimal, desired and optimal TE_A. For some analytes it will be possible to decrease ALs to enhance the ability of PT to identify laboratories that cannot provide testing accuracy necessary for clinical needs; for other analytes different approaches might be needed to determine AL. These findings are consistent with the models for analytical goals proposed by the recent Milan Conference, recommending analytical goals based on: (1) ability to distinguish disease states, (2) the capability of measurement systems, or (3) biological variation.

*Miller et al, Arch Pathol Lab Med. Vol 132:838, 2008.

Suitability of CLIA PT Acceptance Limits

Analyte	CLIA AL	CV_I	CV_G	CV_B	f_{AL}	Code	AL for f_{AL} <0.375
ALT	20%	19.4	41.6	45.9	0.09	O	
Alk.Phos.	30%	6.5	26.1	26.9	0.92	R	15%
Chol., Tot.	10%	6.0	15.3	16.4	0.31	M	
Cortisol	25%	15.2	38.1	41.0	0.30	M	
IgG	25%	4.5	16.5	17.1	1.24	E	10%
Iron, Total	20%	26.5	23.2	35.2	-0.05	O	
Leuk. Cnt	15%	11.4	21.3	24.2	0.23	D	
Magnes.	25%	3.6	6.4	7.3	3.00	E	5%
Protein, T.	10%	2.8	4.7	5.4	1.42	E	4%
Trigs	25%	19.9	32.7	38.3	0.22	D	
Uric Acid	17%	8.6	17.5	19.5	0.51	R	14%

OPTIMAL, O f_{AL} < 0.125
DESIRABLE, D 0.125 < f_{AL} < 0.25
MINIMAL, M 0.25 < f_{AL} < 0.375
MARGINAL, R 0.375 < f_{AL} < 1.0 ALs should be reduced
EXCESSIVE, E f_{AL} > 1.0 current technology may not support ALs tight enough to meet clinical needs

B-158

Glycine's affinity to a cation-exchange resin offers potential treatment for glycine encephalopathy

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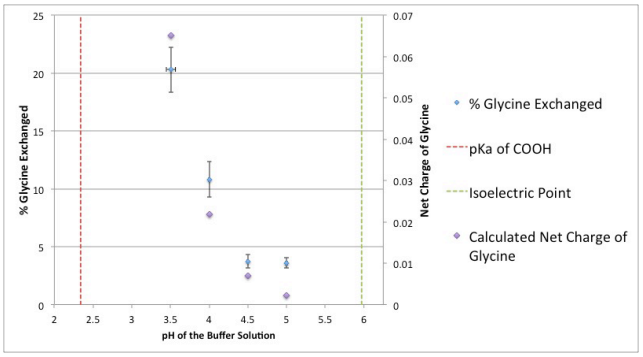
Background: Glycine encephalopathy(~ 1 in 60,000 births) is a rare autosomal recessive disorder characterized by high cerebrospinal fluid and plasma glycine concentrations. Glycine accumulation leads to NMDA receptor hyperactivity, resulting in hypotonia, apnea and death in neonates, and mental retardation and seizures in infants. The only existing therapy is the sodium benzoate, which is toxic and poorly penetrates the CSF. Plasmapheresis using a cation exchange resin may reduce circulating glycine, depending upon the affinity of the glycine-resin interaction. Glycine's anionic carboxylic acid (COOH) moiety reduces its affinity to cation resins, but neutralization of COOH by lowering the pH may increase affinity and improve glycine's removal.

Objective: To determine whether varying pH alters glycine's affinity to cation-exchange resins as a possible removal method.

Methods: We used potassium hydrogen phthalate solutions to alter pH of a glycine solution before passing it through 2mL of cation-exchange resin (Sigma-Aldrich 50WX8 hydrogen) embedded with a sulfonic acid functional group. Glycine removal was calculated after 5 passes through the resin at each pH with similar eluent volumes. pH was titrated to values between 3.50 to 5.25, between the pKa of the COOH group in glycine and the isoelectric point (5.97) to induce protonation of glycine. Affinity was quantified as percent glycine exchanged from solution. The theoretical net charge of glycine in each pH environment was additionally calculated.

Results: Decreasing pH resulted in a significant increase in glycine's affinity to the resin (ANOVA, $p < 0.05$). Highest affinity to the resin occurred at pH 3.50 with 20.30±1.96% of glycine exchanged (Figure). Calculated charge of glycine correlated to the affinity to the resin by Pearson correlation (R^2=0.98).

Conclusion: Protonation of glycine in low pH increases affinity to cation exchange resins. At physiological pH (7.4), extracorporeal exchange resins within an acidic environment (pH ~4) may be used to treat glycine encephalopathy.



B-159

Comparing Instrument Performance using Bio-Rad Mission: Control Software

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Objective: Evaluate and compare performance of different clinical diagnostic instruments using Bio-Rad Mission: Control software.

Relevance: Clinical diagnostic instrument performance is critical to producing reliable patient results that enhance patient care and reduce patient risk. We investigate the value of Mission: Control software to evaluate and compare instrument performance and help assess the need for new instruments in our laboratory.

Method: We are interested in two assays; 25-OH Vitamin D and HbA1c.

25-OH Vitamin D: We compared our current laboratory instrument to a new instrument being considered for acquisition. Our 25-OH Vitamin D allowable total error limits are ±15%, we use a 1:3s/2:2s/R:4s QC rule with 2 QC concentration levels, and we average 61 patient examinations between QC events. We used manufacturer's information for means and SDs for the new instrument.

HbA1c: We recently changed instruments after moving to a new buying group. We wished to compare our previous instrument's performance to the new instrument. We used an allowable total error of 15%, 1:3s/2:2s/R:4s multirule with 2 QC concentration levels, and we average approximately 200 patient examinations between QC events.

Results: Mission: Control software was used to estimate the worst-case expected number of unreliable final patient results, max E(Nuf), and average sigma values. Assuming no bias in the measurement procedures, the max E(Nuf) for our instruments is higher compared to the new Instruments.

Comparing Instruments on 25-OH Vitamin D Assay		
	Current Instrument	**New Instrument**
Max E(N_{uf})	137.7	<1
Average Sigma	1.7	5.1
Comparing Instruments on HbA1c Assay		
	Previous Instrument	**New Instrument**
Max E(N_{uf})	2.4	1.2
Average Sigma	4.3	4.6

Conclusion: The Bio-Rad Mission: Control software helped us evaluate and compare the performance of different instruments. We used this analysis for our business plan to support the need for a new instrument. However, budget constraints prevented the purchase of the new instrument for 25-OH Vitamin D. Our move to the new instrument for HbA1c was justified as it turned out to be a less expensive alternative with better performance characteristics.

B-160

Defining Performance Metrics and Workload Capacity in a High Throughput Immunological Cellular Function Testing Clinical Laboratory

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Rationale: To remain competitive in today's crowded clinical reference laboratory business environment, productivity and turnaround times (TAT) that are the most noticeable signs of laboratory service and performance must be measured. Here we describe the development and implementation of novel key performance indicators (KPI) for our lymphocyte proliferation testing (LPT) laboratory that have helped us measure and predict laboratory staffing needs and meet turnaround time expectations, thereby improving our laboratory's productivity, efficiency and overall quality of testing.

Methods: To assess process, we defined average capacity per performing technician for a number of testing processes based on historical average test volume, ergonomic considerations, key performance benchmarks for the various steps of the assay, published TAT, and laboratory needs, amongst other things. We then implemented manual input mechanisms outside of our Laboratory Information System (LIS) to measure test volume and productivity in the laboratory for individual steps in the testing process. The very manual nature of the LPT test is a limiting factor. The test takes 14 days to complete and consists of 82 individual non-interfaced steps. In the 14 day LPT testing process our leading indicator for TAT is a step after cellular proliferation has occurred, but before measurement of proliferation can begin called harvesting. We chose to measure this harvesting process as our KPI to most accurately predict the TAT and resulting of the LPT test, because it occurs after day 7 but before day 13, giving us a 6 day window of opportunity to make improvements to meet TAT. Metrics for the various assay processes were then charted and measured against incoming sample volumes to predict TAT and future staffing needs.

Results: Over the 18 months that these measurement tools were implemented, we noticed a dramatic decrease in the laboratory LPT testing TAT. Before implementation, the TAT was a weekly average of 18 days, 4 over our contractual agreement to our clients. After implementation, our performance metrics and the definition of our new KPI we were able to allocate resources to compensate workloads and improve TAT back to a monthly average of 9 days for January of 2016.

Conclusion: Laboratories that do not have automated and customizable reporting metrics would benefit from creating their own. In a competitive and quality driven clinical testing environment, it is necessary to define and monitor of KPIs as they apply to the individualized test. In the LPT Laboratory at National Jewish Health, we improved our processes by redefining them and what they needed to succeed. Measuring the manual and previously unmeasured harvesting process as a KPI and then adjusting staffing and delegation of tasks accordingly improved patient care through improved TATs on clinical testing.

B-161

Improving Quality Patient Care by Strengthening Relations

B. Moua, P. Simpson, R. Harbeck. *National Jewish Health, Denver, CO*

Rationale: Translating quality between nursing staff and the clinical laboratory is very important. The two areas need to work hand in hand to ensure smooth transition of samples and patient information. Appropriate collection, handling, and analyzing patient samples correctly the first time and every time should be the goal of every clinical environment. The most common issues between nursing staff and clinical laboratory staff involve poor communication, and lack of understanding of both sides about their processes and requirements. Realizing this, we reached out to our nursing staff and implemented a program between the two groups with an ultimate vision to improve communication through mutual respect and understanding that will foster the partnerships necessary to provide safe and high-quality patient care and testing to optimize the patient experience.

Methods: We created a proactive communication program between an established clinical laboratory and a new infusion clinic that was increasing not only in the number of patients served but in samples that required STAT handling as well. Meetings minutes were kept and followed up on over a 6 month period. We also analyzed sample volume, turnaround time (TAT), and critical reporting to make sure we were meeting the needs of the clinic. From these meetings and communications we made a number of policy and procedure changes that improved quality patient care and offered operational efficiencies across both areas.

Results: The program created discussion of specimen collection techniques and decrease rejection, as well as a better understanding of specimen results in relation to patient treatments. In addition, down time processes and procedures were improved to increase communication to the clinic staff which ultimately reduced patient testing TAT by allowing the clinics to better schedule around laboratory activities.

Conclusion: The program improved efficiencies and created improvements that would not be realized without good understanding of different workflow processes. Understanding the needs of the lab and of the clinical staff can better create a synergetic mindset for improving pre-analytical, analytical, and better patient outcomes. In the end, a proactive communication program seeks to accomplish the task of successfully facing a complex challenge that will allow all those included to feel and support a sense of shared power and collective competence, which will

improve and grow the organization. People in organizations want and need to work together effectively and productively. Individuals long to be part of a bigger picture that connects them to a larger purpose. That purpose at National Jewish Health (NJH) is "to heal, to discover and to educate as a preeminent health care institution". This change initiative will help to grow the mission of NJH by bettering communication and understanding throughout the organization by starting in the clinical laboratory and expanding collaboration from one area to another. This will benefit everyone by bringing us one step closer to quality patient care.

B-162

Intactness of medical nonsterile gloves against alcohol disinfectants

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Background: Every morning from 8 am to 10 am, the blood collection room in the outpatients department of our hospital becomes overly crowded. Patients should wait on long lines for blood collection. Healthcare workers wear gloves for their own protection, and wash hands (or apply alcohol disinfectants in pressing times) and change gloves between patients for patient safety. The current regulation prohibits alcohol disinfection when gloves are worn, since sanitary intactness of gloves may not be guaranteed by alcohol. However, when viewed as a time saver, alcohol disinfection with gloves between patient blood collections can shorten waiting times of patients.

Methods: Four kinds of medical gloves were used: 1) 3 types of powder-free non-sterile latex medical examination gloves, Top glove (Top Glove, Malaysia), Dowoo, (Siam Sempermed Corp., Thailand), and Maxter (Maxter glove manufacturing, Malaysia), and 2) 1 type of nitril gloves, DERMAGRIP Nitrile extended cuff examination gloves (WRP Asian Pacific, Malaysia). For disinfection, 2 kinds of ethanol 62% gel, Clesis hand sanitizer gel (Liebecos, Korea) and 3M Hand Instant Sanitizer(3M Korea, Korea), and an ethanol 83% disposable skin cleaner, Clean Swab A (Meditop, Korea), were used. For two types of latex gloves, and one type of nitril gloves, ethanol 62% gel was applied, rubbed and dried for 30 times. For another latex gloves brand, we used disposable ethanol 83%, skin cleaner. Five pairs of gloves for each brand of medical gloves, in total 40 gloves, were tested. Using Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, bacterial suspensions were prepared to match 0.5 McFarland turbidity standard. Glass slides were smeared with each inoculum and dried for 30 minites at room temperature. Glove fingertips were placed on the smeared surface for 1 minute. Fingertips were pressed into BAP and incubated for bacterial growth. **Results**: All the gloves were found intact after 30 times of rub-and-dry action with alcohol disinfectants. No significant bacterial growth was recognized on glove fingertips after ethanol disinfection. **Conclusion:** Gloves were found intact after 30 times of application of alcohol disinfection. Since blood collection for each adult patient takes less than 2 minutes, we recommend the use of alcohol disinfected gloves after 30 minutes or 15 patients if intact. The test results can apply only to adult outpatients in highly busy times.

B-163

Test utilization of serum free light chain assay in Northern Alberta

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Background: Inappropriate use of laboratory tests wastes valuable healthcare resources and places additional burden on the laboratory. Serum free light chain (sFLC) analysis is used as a prognostic indicator for the risk of progression from monoclonal gammopathies of undetermined significance (MGUS) to multiple myeloma (MM), and to detect and monitor monoclonal light chain diseases. Light chain escape is a recently described phenomenon which results from a shift in myeloma cell secretion of intact immunoglobulin to FLC only due to chronic and extensive treatments. Patients with light chain escape are especially vulnerable to renal impairment due to high level of FLC, and thus close monitoring of sFLC will be required to allow early detection of renal complications. The purpose of this study is to review sFLC assay utilization to determine if its use is appropriate in the context of current medical guidelines.

Methods: We completed a retrospective analysis of sFLC tests performed at DynaLIFEDX, Edmonton, Alberta, Canada from January 2014 to December 2015. De-identified data containing patient age, gender, interpretative results and ordering physician was extracted from the Laboratory Information System. Criteria to assess test utilization appropriateness include age, frequency, and the ordering physician specialty. Measurement of sFLC was performed in the Siemens Advia 1800 analyzer with reagents from BindingSite.

Results: The total number of sFLC assays requested increased by 41% from 2014 to 2015. A total of 10827 sFLC assays were performed in 2884 patients (57% male) in the study period of 24 months. There was a total of 307 sFLC assays performed for patients 40 years or under, with 41% of these tests having abnormal results as defined by free kappa/ free lambda ratio outside of the reference intervals (0.26 to 1.65). For patients older than 40 years where the majority of tests were performed (10522 tests), 63% of these tests had abnormal sFLC results. To determine the origin of the test orders, a list of top 20 physicians who ordered the most sFLC were generated. 87% of the test orders were from oncologists, while the remaining 13% were from internists, nephrologists, neurologists and family physicians. For monitoring the disease, 18% of the repeated sFLC tests were performed within 26 days, with the majority from oncologists.

Conclusion: Physician awareness of the Light chain escape phenomenon has led to a surge in sFLC testing in Northern Alberta. This study identified that approximately 1 in 5 serial sFLC tests were requested at intervals less than one month.

B-164

Most prevalent suspected diagnosis in primary care laboratory tests orders

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Background: The aim is to show the completeness of the request form regarding patient clinical question along years, and the most prevalent from primary care.

Methods: The laboratory located at a public University Hospital serves a population of 234 551 inhabitants, including nine different primary care centers (PCC). At least once a year, one meeting between laboratory professionals and General Practitioners (GPs) is held, to discuss the current strategies to improve laboratory service, and a median of 3 contacts each year through email.

Laboratory requests are made through Computerized Patient Order Entry that offers the GPs a field to be fulfilled regarding the reason for the laboratory request through International Classification of Diseases, Ninth Revision, Clinical Modification codes.

A retrospective observational cross-sectional study was conducted from January 1st 2009 to December 31th 2015. We counted the requests with clinical question and the number of every patient clinical question that was fulfilled in the request form and available in laboratory information system patient demographic data in absolute numbers and percentage (when percentage in 2015 was above 1%). The rest were grouped into a category called *other diagnosis*.

Results: The requests with patient clinical question increased over time (40% in year 2009)**,** achieving more than 80% in year 2015. Table shows annually the total annual number of requests from primary care and those with patient clinical information in the request through the 7 year period. It also shows the percentage of every diagnosis every year. Disorders of lipid metabolism, essential hypertension and diabetes mellitus were the most prevalent diagnosis.

Conclusions: The number of requests with patient clinical question augmented through a seven year period. Education and communication with GPs and new technologies could have contributed to this improvement along years.

	2009	2010	2011	2012	2013	2014	2015
TOTAL REQUESTS	89450	87056	91676	87424	94922	97438	49183
REQUESTS WITH CLINICAL QUESTION (%)	31296 (35.0%)	62028 (71.3%)	69018 (75.3%)	67556 (77.3%)	74701 (78.7%)	79057 (81.1%)	39914 (81.2%)
CIE-CODE (*)							
272	3,9%	7,7%	7,7%	7,9%	9,6%	9,8%	9,3%
401	2,7%	5,2%	5,2%	5,6%	6,2%	6,0%	6,2%
250	1,8%	3,7%	3,8%	4,1%	5,1%	5,4%	5,6%
595	1,0%	2,2%	2,3%	2,3%	2,7%	3,4%	3,0%
780	2,0%	3,8%	2,6%	2,5%	2,5%	2,7%	2,8%
244	0,7%	1,5%	1,6%	1,9%	2,3%	2,5%	2,7%
280	0,6%	1,1%	1,1%	1,3%	1,4%	1,5%	1,7%
790	0,5%	1,0%	1,1%	1,2%	1,3%	1,5%	1,6%
300	1,1%	2,0%	1,9%	1,9%	1,6%	1,6%	1,6%
285	0,5%	1,0%	1,2%	1,3%	1,5%	1,6%	1,6%
V72	0,3%	0,6%	0,8%	1,1%	1,1%	1,3%	1,5%
599	0,3%	0,6%	0,8%	1,3%	1,4%	1,6%	1,4%
536	0,4%	0,9%	1,1%	1,5%	1,2%	1,4%	1,3%
789	0,4%	1,1%	1,2%	1,3%	1,2%	1,3%	1,3%
719	0,5%	1,2%	1,2%	1,2%	1,2%	1,1%	1,2%
724	0,7%	1,3%	1,4%	1,4%	1,2%	1,1%	1,1%
788	0,4%	0,8%	0,9%	0,9%	1,1%	1,1%	1,1%
Rest of codes	17,1%	35,6%	39,1%	38,6%	36,1%	36,2%	36,4%

*** 272=Disorders of lipid metabolism; 401=Essential hypertension; 250=Diabetes mellitus; 595=Cystitis; 780=General symptoms; 244= Acquired hypothyroidism; 280=Iron deficiency anemias; 790=Nonspecific findings on examination of blood (Abnormality of red blood cells,Elevated sedimentation rate, Abnormal glucose, Excessive blood level of alcohol, Nonspecific elevation of levels of transaminase or lactic acid dehydrogenase [LDH], Other abnormal blood chemistry, Bacteremia, Viremia, Other nonspecific findings on examination of blood); 300=Anxiety, dissociative and somatoform disorders; 285=Other and unspecified anemias; V72=Special investigations and examinations (Examination of eyes and vision, Examination of ears and hearing, Dental examination, Gynecological examination, Pregnancy examination or test, Radiological examination, not elsewhere classified, Laboratory examination, Diagnostic skin and sensitization tests, Other specified examinations, Unspecified examination); 599=Other disorders of urethra and urinary tract; 536=Disorders of function of stomach; 789=Other symptoms involving abdomen and pelvis; 719=Other and unspecified disorders of joint; 724=Other and unspecified disorders of back; 788=Symptoms involving urinary system**

B-165

Extra technician tasks and turnaround time in a Stat Laboratory

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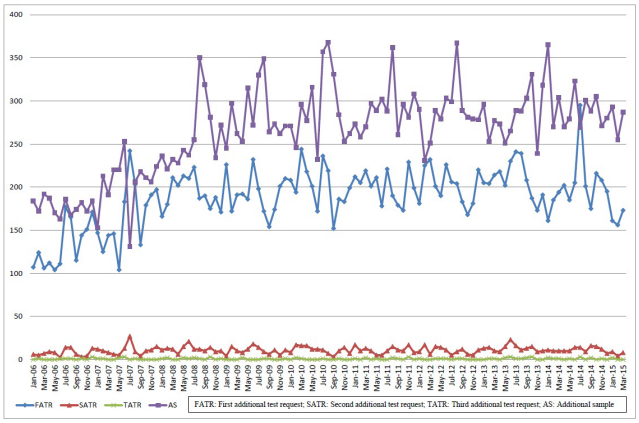
Background: The aim was to identify the main extra additional technician tasks carried out in a stat laboratory (SL) and its quantification and analysis over a ten year period through turnaround time (TAT) and test workload comparison.

Methods: In a meeting to identify and list the different extra additional tasks, to be collected its incidence in a daily basis, were decided as extra additional activities when a first, a second or a third additional test is requested. Also, we considered as extra additional task when an out of time sample is received. Technician would register in the Laboratory Information System (LIS), a specific "quality test" for each one of the four additional activities. The registers were collected automatically by the LIS using a data warehouse program. Every extra additional task was counted in absolute number in a monthly basis and referred to 1000 tests requested. Extra additional tasks, TAT results and tests workload in summer was compared to the rest of the year.

Results: In the 111 months studied, there were 51385 extra additional tasks. The monthly median was 475 extra additional tasks: 10.2 per 1000 tests requested. The main activity was "sample out of time" (271 per month), followed by "first additional test request" (191). The Figure shows in a monthly basis, the number of the four different extra additional activities along the period of study. In the summer period the workload and number of extra additional tasks were significantly higher. In spite of seasonal variations in workload and additional technician tasks, the TAT did not show this variation, complying always with our 30 minutes indicator target.

Conclusion: It is important to have the knowledge of the real SL workload, including extra additional tasks to improve the service provided to the ED patients.



B-166

The experience of setting up an advanced digital temperature monitoring system in the clinical laboratory and the application of log data

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Background: Accurate and reliable monitoring of temperature, humidity is crucial for the clinical laboratory to ensure that samples, materials and reagents are stored properly and compliance to laboratory quality control. It is recommended that the monitoring should be done continuously for 24 hours/every day. When a temperature control system fails, it is essential for appropriate personnel to be alerted immediately and actions should be taken properly to make sure the integrity of samples and reagents stored in the refrigerators/freezers. **Methods:** There are 451 sites located in four different buildings in which the temperature need to be monitored in our laboratory. We transformed the temperature monitoring system from manual recording, weekly and monthly reviewing and paper based archives to digital system that can record data automatically and data can be reviewed on line regularly since 2013. The continuous temperature log data collections from calibrated probes are compressed to one data point every five minutes and digital signals are transmitted to server by cable. Numeric data are stored in the structural database. **Results:** Besides all temperature data are kept in a manageable file structure that can be reviewed anytime and anywhere through web browser with authority control, alarms are notified through multi routes, including broadcast to GSM, e-mail, and alarm lights. The characteristics of these systems that are less mentioned previously but are much more important for the integrity of collected data are as follows: 1. mechanisms for detecting expected or unexpected shout down of the data collecting workstation, power cuts at any single probe, nodes or switches, disconnection of network or out of service of the database servers, 2. mechanisms for detecting any fails of the alarm system mentioned previously by dual separated system , 3. second backup of alarm system by Remote Computer Alarm Programs beside the work bench of the staffs who are in charge of the monitoring of temperature control equipments, 4. all the alarms of the temperature control equipments are logged automatically and the actions

taken and follow up can be documented by the linking of laboratory's document system. **Conclusion:** With the automatic digital laboratory temperature monitoring system, estimated more than fifteen thousand A4 sized papers and 540 working hours are saved from 2013 to 2015. The malfunction log of these equipments can be calculated routinely and the information from temperature log data can be extracted through data mining process by decision tree analysis. With the predicted results, the laboratory's managers/directors can evaluate the performance of the temperature control equipments in the basis of evidence and achieve prior risk management to prevent unexpected fails of the temperature control in the laboratory.

B-167

Reducing false quality control failure rate with implementation of six-sigma statistical quality control management program reduces laboratory operational cost.

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Introduction: The clinical laboratory is urged to produce high quality, timeliness results at a low cost. Laboratory Quality Control program has been used to detect analytical errors. Often to avoid missing any error, there is a tendency of the laboratory to maximize error detection with the selection of the QC rules with fairly high probability of error detection (P_{ed}) but this is often accompanied by a high probability of false rejection (P_{fr}) resulting in high false rejection rate. This will results in unnecessary use of resources, effort and time in performing troubleshooting for the false QC failure and results in high costs of failure. Aim of this study is to determine whether adopting a Six Sigma quality control management program can reduce false QC rejection rate and costs of failure of the laboratory, without affecting detection of errors. **Methods**: Sigma metrics were evaluated for 27 serum and urine chemistry assays across two units of Abbott's ARCHITECT c8000 chemistry analyzer using the equation: Sigma metrics = (TEa - %Bias) / %CV. % Bias and % CV were calculated with QC data collected over a six months period with the choice of TEa based on the recommendation of the Westgard Verification Program. QC optimization of control rules and frequency were based on the Sigma metrics achieved for each of the 22 assays. False QC failure rate and the amount of resources used on troubleshooting the false QC failure rate were evaluated before and after implementing the Six Sigma quality control management program. **Results**: QC rules optimization were achieved based on the assay's Sigma metrics performance whereby 22 of 27 assays (81%) were of world class and excellent performance achieving Sigma metrics >5 with the remaining 5 assays at >4 Sigma metrics. Changing from the standard 1-2s QC rules practice to QC rules optimized by Sigma metrics resulted in the reduction of 80% false QC failure rate. As a result of the reduction of the false QC failure rejection, the laboratory achieved an estimated annual operation efficiency saving of approximately 650 man hour (reduction from 820 man-hour to 170 man-hour) and direct estimated annual operating cost saving of approximately 18% comprising of reagent, control materials and labor cost saving, equating to MYR 45,000. **Conclusion**: By adopting and implementing Six Sigma quality control management, we were able to improve laboratory operation efficiency by reduction of false QC rejection rate, which in turn enable direct cost saving benefit without compromising the detection of any errors and enable us to continuously provide speedy, high quality service at low cost.

B-168

Increased demand in Primary Care in Spain. A Big Data analysis

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BACKGROUND: To compare Primary Care Requesting patterns between two different years in Spain, using appropriateness indicators, to try to ascertain tests demanding behaviours along years.

METHODS: Every Spanish citizen possesses the Individual Health Care Card, which let access to public health services as a healthcare user throughout the National Health System. Every Autonomous Community (17 in Spain) is divided into a number of Health Departments. Every Department covers a geographic area and its population and is composed by several primary care centers and usually a unique Hospital. The laboratory located at the hospital attends the needs of every Health Department inhabitant.

A call for data was posted via email. Spanish laboratories willing to participate in the study were invited to fill out an enrollment form and submit their results online. The dissemination of the questionnaire was addressed to the participants of previous studies of the REDCONLAB group that recommended to other laboratories to join the current edition. Numbers of 50 tests requested by all of the general practitioners for the year 2014 from laboratories at different hospitals from diverse departments across Spain were used. Each participating laboratory was required to be able to obtain patient data from local Laboratory Information Systems Patient's databases and also to provide data of the organization. The same study had previously been done in 2012.

After collecting the data, test-utilization rates were calculated by standardization with the population attended by each laboratory. Rates were expressed as tests per 1000 inhabitants. We considered inhabitants the residents in each public Health Departments. The differences in indicator results in both years were calculated by way of the U Mann-Whitney test analysis. A two-sided $p \leq 0.05$ rule was utilized as the criterion for rejecting the null hypothesis of no difference

RESULTS: In the 2012 study, 76 laboratories, on a voluntary basis, participated, corresponding to a catchment area of 17,679,195 inhabitants from 13 different communities throughout Spain (38% of the Spanish population) and 110 laboratories participated in the 2014 study, corresponding to 27,798,262 inhabitants from 16 different communities (59% of the Spanish population).

Significant increases in year 2014 were found in alanine aminotransferase, transglutaminase antibodies, calcium corrected for albumin, cholesterol, creatinine, folate, glucose, HDL-cholesterol, glycated hemoglobin, complete blood count, potassium, sodium, thyrotropin, triglycerides, urinalysis, vitamin B_{12} and 25-OH-vitamin D.

CONCLUSION: Overall, Primary Care requests in Spain have increased significantly in the most demanded tests in two years period. From the Laboratory is possible the use of management tools as Big Data, for the analysis of data with special characteristics of volume, variety, velocity, variability and veracity to get the knowledge regarding test demand in large geographical areas

B-169

Verify the Utility of a Simplified Model to Evaluate the Analytical Performance of Creatinine in the Medical Decision Points

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Background: Given that commercial materials of internal control do not generally cover all the concentrations that represent Medical Decision Points (MDPs), it is relevant to evaluate the analytical performance of the tests at these points. This work shows a simplified model (Model-2) of estimation of the analytical performance in the MDPs for creatinine in serum expressed like sigma metric using the results obtained from verification tests, and comparing them to a more robust model (Model-1).

Methods: A homogenous system was used in the analytical platform Architect ci8200. The method used for the determination of creatinine was kinetic alkaline-picrate. The theoretical MDPs (0.60, 1.60, 6.00mg/dL) were selected from the tables in Statland BE, Clinical Decision for Levels Laboratory Tests, Second Edition [Oradell NJ; Medical Economics Books, 1987.] In order to estimate the sigma metric in the MDPs, the ETa (Allowable Total Error) was selected from CLIA 88' (0.3mg/dL and 15%), and it was calculated with the formula Sigma = [ETa(%) - Bias(%)] / CV(%). Model-1: the bias that represents the systematic error of measurement was estimated as the absolute percentage difference between the theoretical and estimated MDP, where the estimated MDP was obtained by interpolating the theoretical MDPs in the Deming linear regression obtained by charting the creatinine concentrations from 36 external quality control surveys which ranged from 0.56 to 11.30mg/dL. The CV(%) that represents the random error was estimated in the MDPs through the equation obtained by charting different creatinine concentrations as a function of the coefficient of variation (precision profile); these data were collected by processing a pool of samples from patients with 16 different creatinine concentrations daily during a minimum of 30 days, the creatinine concentrations ranged from 0,052 to 6.65mg/dL. Model-2: the bias was estimated by using the linear regression from the linear verification test, and the precision in the MDPs was estimated from the results from the precision verification (EP15-A2) and the limit of quantification. **Results:** Model-1: the slope of the Deming linear regression was 1.013 (CI 95%: 1.002 to 1.024) and the y-intercept 0.0081 (CI 95%: -0.0411 to 0.0574). The estimated MDPs obtained from the Deming regression were 0.62mg/dL, 1.63mg/dL and 6.09mg/dL, the bias being of 3.3%, 1.9% and 1.5% respectively. The estimated CV(%) in the MDP

of 0.60mg/dL was of 2.17%, in 1.6mg/dL of 1.74% and in 6.0mg/dL of 1.29%. The sigma performance obtained in the MDP of 0.60mg/dL was of 21.5, in 1.60mg/dL of 9.7 and in 6.00mg/dL of 10.5. Model-2: the linear regression obtained from the linear verification was y=0.929x+0.075 and the estimated MDPs were 0.63mg/dL, 1.56mg/dL and 5.65mg/dL, the bias being of 5.0%, 2.5% and 5.8% respectively. The estimated CV(%) in the MDP of 0.60mg/dL was of 4.5%, in 1.6mg/dL of 1.98% and in 6.0mg/dL of 0.87%. The sigma performance obtained in the MDP of 0.60mg/dL was of 10, in 1.60mg/dL of 8.2 and in 6.00mg/dL of 10.6. **Conclusion:** The utility of the simplified model to estimate the sigma performance in the MDPs was verified.

B-170

An Audit Of Critical Result Reporting In A New Regional Hospital

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Background: Accurate and timely transmission of critical results to the appropriate caregiver ensures patient safety, and is one of the criteria that regulatory bodies worldwide requires clinical laboratories to meet for licensing and accreditation purposes. Our new regional hospital, Ng Teng Fong General Hospital, began operations in phases from July 2015. Our objective is to perform an audit of critical result reporting from July to December 2015, to appreciate our new patient profiles, and to ensure our protocols and service levels meet international standards.

Methods: Critical results triggered from July to December 2015 were obtained from our Laboratory Information System. The following were analyzed: total number of tests reported, number of critical results, the most common critical results, and the laboratory sections and time of day with the most number of critical results (categorized as 8am - 4pm, 4pm - 12am, 12am - 8am). Pertaining to notification, information on response time, number of failed responders (defined as TAT more than 60 minutes) and main reasons for failure were studied.

Results: The total number of critical results reported each month showed a steady increase from 63,692 in July to 141,288 in December. This correlated with the increasing number of patient beds being opened in phases. The proportion of critical results over all tests was similar ranging from 0.54% to 0.67%. The top 5 critical results triggered were positive blood culture (21%), followed by platelets (10.9%), sodium (10.7%), glucose (9%) and potassium (9%). As a section, biochemistry had the most number of critical results (54%), followed by microbiology (27%) and hematology (19%). The 8am - 4pm period had the highest number of critical results (49%), followed by 4pm - 12am period (26%) and 12am - 8am period (25%). Most of the critical results were electronically communicated to the requesting physician via Healthcare Messaging System (HMS) within 11 - 30 minutes (53%), followed by within 10 minutes (35%), between 31 - 60 minutes (11%) and beyond 60 minutes (1%). The proportion of failed responders, defined as more than 60 minutes, ranged from 0.6% to 1.4%. Main reason was delayed closure of the case by the call center operator. Our further investigation revealed no compromise in patient safety as appropriate management plans were already in place based on prior clinical suspicions.

Conclusion: Majority of critical results are communicated to the appropriate clinician without delay via automated notification systems, thus helping save time and manpower. Coupled with the information above, more effective manpower allocation can be achieved, which hopefully translates into improved quality and efficiency of laboratory processes, and ultimately patient care. Frequent monitoring and review of critical results management processes and feedback to all stakeholders should be a good standard of practice that all clinical laboratories adopt.

B-173

Laboratory Utilization Analysis as a Clinical Service: A Pilot Study Using Dashboards with an Intensive Care Unit

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Background: The laboratory utilization literature suggests that both overutilization and underutilization are significant problems. Past work also demonstrates that some physicians are not comfortable using some laboratory tests, have not received adequate training on concepts of laboratory medicine, and are not aware of the costs for even common laboratory tests. Rather than test a single intervention to improve laboratory utilization, we piloted a collaborative process with an intensive care unit to assess needs and jointly develop solutions to improve appropriate test use.

Methods: The first step in the process was an analysis of laboratory utilization patterns for a pilot ICU, which included test volumes, frequencies of testing for individual tests, and direct cost and charge information. We also developed an interactive utilization dashboard that displays testing frequency and proportion of tests duplicated within 24 hours by specific test for individual patients, in addition to summary views of general utilization. We then met with senior physicians and administrative leadership to discuss general patterns of utilization and identify areas for improvement, and provided clinicians access to dashboards to drill down on outliers and determine appropriateness of testing. Based on their feedback, we came to consensus on which tests were the best candidates for improvement and identified interventions with target goals.

Results: Point of care testing (POCT) was the highest volume test category in the pilot unit. POCT utilization was driven by lack of awareness of the optimal uses of POCT and a lack of standard ordersets to guide ordering of routine tests. Residents in particular were not aware that POCT results can be less reliable than central laboratory testing for some analytes and are costlier, nor were they aware of the rapid turnaround times for blood gases performed in the central laboratory. After discussion and review of dashboard data, there was agreement that at least 50% of the POCT performed could either be shifted to blood gases, other equivalent testing, or eliminated altogether. Awareness of the issue decreased the overall rate of utilization from a baseline of 0.98 tests/patient/day over 12 months (n=1539 patients) to 0.55 tests/patient/day over 4.5 months (n=617 patients). The effect was expected to be transient so the unit also created an orderset to better standardize ordering and placed POCT testing below blood gases and other equivalent tests. This change further decreased POCT use to 0.39 tests/patient/day over a 2-month period (n=368 patients), or a 60% decrease in daily utilization from the original baseline. Even after accounting for the increase in blood gas utilization, these changes are projected to decrease yearly direct costs by $60,000 for the pilot unit alone. Furthermore, after instituting the orderset, utilization of other labs besides POCT and blood gases decreased by 12%, from 7.7 tests/patient/day to 6.7 tests/patient/day.

Conclusions: Utilization of laboratory testing is highly variable between practice settings, so implementing systemic changes to improve utilization can be challenging. We demonstrate that a process targeted by clinical unit that includes data analysis and partnership with clinicians improves laboratory utilization.

B-174

Comparative Study of Six Sigma Assay Performance on VITROS Systems

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Objective: Evaluate long term manufacturing release data to determine process capability within a tightly controlled laboratory and compare those results to external laboratory data using the six sigma statistic, where a sigma greater than 4 indicates good performance.

Methodology: Perform a retrospective analysis of manufacturing data from 3 years of precision and uniformity testing on a single level of QC material, across 10 different VITROS® Systems to quantify the total error (%bias and %CV) relative to total allowable error (TEa). CLIA proficiency testing limits[1] were used as the total allowable error (TEa) criteria; for those not graded or where a definitive unit or percentage was not given (example: +/- 3sd), the TEa was based on biological variation.[2] Percent bias was estimated using the rolling average (defined as the average difference in measured values between reagent lots) divided by the QC fluid target value X 100. The SD across reagent lot data collected was used to determine the %CV; (fluid target mean/SD) X 100. The total error was determined by subtracting the %bias from the TEa expressed as a percentage and divided by the CV% [(%TEa - %bias)/%CV]. A separate analysis was performed using within-lab QC data collected using e-Connectivity® Technology from two facilities, with 2 VITROS® 5600 Integrated Systems each, that utilize the Bio-Rad quality control materials. The within-lab grand mean was calculated to estimate %bias within each lab and the %CV was established by calculating the SD over a minimum of 30 days of stable operation divided by the within-lab grand mean X 100. The sigma for each assay was calculated for each QC level in use.

Results: Long term manufacturing data demonstrated sigma levels of 6 or better for the 17 assays evaluated. Data pulled electronically from the two external laboratories across two levels of assayed control materials demonstrated > 6 sigma on both levels, all analyzers, for AST, ALKP, GGT, Crea, Gluc, K, Cl, Ca, Chol, Trig, HDL, and LDL. The remaining assays were >5 sigma (Urea, TP, Alb, TropI, TSH). **Conclusions:** Choice of TEa as well as the concentration or activity level assessed will impact the sigma calculated, as will the timeframe for data collection. Evaluation of manufacturing release data indicate that all assays assessed exceeded 6 sigma using CLIA limits or, in the absence of distinct limits, limits for biological variation. External laboratories achieved >6 sigma for 70% of the assays - across all analyzers and QC levels. The remaining 30% demonstrated >5 sigma. Manufacturing release

data are collected under tightly controlled conditions, limiting variation that may be more prevalent in external laboratories. While sigma values were higher under these conditions, the external laboratories demonstrated excellent performance, both within and between analyzers.

[1] Federal Register February 28, 1992;57(40):7002-186.

[2] Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. Database updated in 2014.

B-175

Frequency that laboratory tests influence medical decisions

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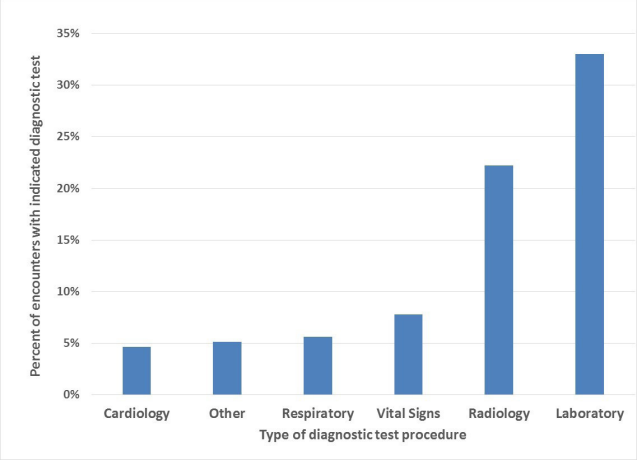
Background: Among the variables that influence medical decisions, laboratory tests are considered to be among the most important and frequently utilized. The influence of laboratory tests on medical decisions have been difficult to estimate. The goal of this study was to estimate the number of patient encounters that had an associated laboratory test.

Methods: We extracted information for 71,201 patient encounters from one-week intervals each quarter of a year from our comprehensive academic medical center electronic medical record. We determined for which encounters laboratory and other orders existed.

Results: Of the encounters examined, 33% had one or more laboratory tests ordered. The figure shows the frequency that different types of diagnostic procedures were ordered. Note that a single patient encounter may have more than one type of diagnostic procedure. For inpatient, emergency department and outpatient populations, ≥87%, 50% and 29%, respectively, had one or more laboratory tests ordered. The influence of laboratory tests on inpatients was dependent on the length of stay with 1, 2 or >2 days stay having laboratory orders 87%, 96% or 98% of the time, respectively.

Conclusion: Overall 33% of patient encounters had laboratory tests ordered. Meaningful differences in laboratory utilization were observed with almost all inpatient, half of emergency department and nearly one-third of outpatient visits informed by laboratory tests.



B-176

Notification for recollection in a large clinical laboratory: is there a need to review the process?

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Background: Assay interferences may occur in variable degrees with many laboratory tests; therefore the reliability of a specific result may need a call for recollection. The notification process for that purpose should be well designed and effective, to guarantee proper medical intervention. The aim of this study was to access the dimension and efficiency of the established process for outpatient call for recollections. **Methods**: To understand the actual performance of the recollection call process demanded by "highly altered results", we evaluated LIS data from June to November of 2015, from a large clinical laboratory, where about 6 million laboratory tests are performed monthly. Medical staff, remotely based outside the central lab, daily access a list of necessary recollection calls of outpatients through LIS feature. Patient contact is performed within 24 hours after demand input on the LIS from central lab. Primary contact is tried by telephone; if not achieved, a telegram is sent. The 5 tests with highest recollection demand rates were selected for performance analysis of contact efficacy and results on the new sample, compared to the primary result. **Results**: Laboratory quality processes resulted in 11,202 calls for recollection demanded by "highly altered test result". Telephone notification succeeded in 91.5% of recollection calls, and 947 telegrams were sent. The 5 most prevalent tests were serum vitamin C (VitC) (3,382; 30.2%), serum aluminum (Al) (288; 2.6%), serum selenium (Se) (185; 1.7%), serum copper (Cu) (173; 1.5%), and serum vitamin B6 (VitB6) (84; 0.75%). The rate of no show for recollection was 63.8%, 74.0%, 59.5%, 64.7% and 67.9%, respectively. Among patients that returned, the result of the new sample remained in the same range of previous result in 78.9%, 22.6%, 65.3%, 72.1% and 48.1%, respectively. Results of the new sample fell in normal ranges and in the opposite result range on 19.5 and 1.6% for VitC, 49.3% and 28.0% for Al, 16.0% and 18.6% for Se, 13.1% and 14.7% for Cu and 7.4% and 44.4% for VitB6. **Discussion**: Even with a consistent mechanism of notification, the majority of patients did not return for recollection. In our current model of recall, we do not know if the patient was effectively reached when telegram is needed, due to possible errors in registered address. Phone call is the main form of contact, but the use of other medias could possibly increase the rate of return. Still, among patients that return for recollection, the same result is maintained for the majority of cases of VitC, Se, Cu and VitB6, but the minor rate of normalization reflects the importance of the repetition for its clinical implications. On the other hand, Al results in the new sample were found normal more often after repetition than for the other analytes, showing how patient's return can prevent unnecessary treatments. **Conclusion**: This process review shows that patient's compliance with recollection is important and rates of no show must be reduced. Therefore, the investment in new media such as SMS, email alert, and app developments for smartphones could be appropriate in large clinical laboratories.

B-177

Implementation and Performance Characteristics of a Weekly Patient Pooled Quality Control Specimen in a Networked Healthcare System.

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Background: Traditional quality assurance programs usually consist of daily quality control (QC) runs, quarterly proficiency testing, and at times, periodic with-in site instrument comparisons. These programs tend to use third party control materials that serve as surrogates for patient serum and can miss potential issues related to the sample matrices. This poster defines an implemented protocol for using pooled patient specimens as supplemental QC within a hospital network and includes specimen preparation, test ordering, data capture and interpretation, and corrective action communications when necessary. The quality assurance program also highlights the advantage of standardized equipment and the performance characteristics of these assays over time.

Methods: A team of 4 people from the laboratory identified the steps needed to move a pooled patient specimen, and the results, around 4 hospital sites comprising 6 instruments in total, without introducing additional work for the technologist at each site. To minimize manual test ordering at each site, a protocol was developed, generating site specific labels with preordered lab tests, to be performed when the specimen was received. Upon arrival, the technologist incorporated the specimen into the routine workload. The laboratory results automatically downloaded from the instrument and into the LIS, Sunquest v. 7.1. Data was retrieved from the LIS and populated in an excel worksheet and interpreted. Weekly, summarized, emails were sent out to the chemistry departments. Cumulative variances were also calculated and tracked over time for trending. All chemistry or immunoassay testing was performed on the Abbott ARCHITECT c4000, ci4100, or c8200 systems per the manufacturer and laboratory procedures.

Results: Implementation of the protocol requires no more than 3 key resources , avoiding additional steps at off-site labs. Procedural steps for ordering tests at the core lab, generates specific bar codes for each specimen that is unique to the site. Weekly email communication by a supervisor has led to more proactive involvement by lab leadership, with specific directives each week for specific assays. Weekly monitoring for approximately one year indicates very little variability across the 32 assays monitored.

Conclusion: This semi-automated quality control strategy for utilizing a pooled patient specimen for quality performance has been successfully implemented across 5 sites, with sample prep and data analysis occurring at the core lab. As a result, the

weekly quality metric has led to earlier interventions through email communication to each site. In addition, involving a supervisor to summarize the findings each week has also facilitated more collaborative communication with the techs and overall knowledge of how the systems are performing. Matrix matched patient controls can help identify assay or instrument errors that may have been masked by third party materials. Minimizing the steps needed by the off-site labs has contributed to the overall success of the program. Finally, the small variances observed week to week illustrate the advantages of having standardized high quality equipment and assays.

B-179

Does essential human resource to mix primary blood tubes before to assay HbA1c by turbidimetric inhibition immunoassay?

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Background: Pre-analytical phase is considered the most vulnerable phase in laboratory diagnostics. Presently, accurate mixing of primary blood tubes to assay HbA1c is claimed to be important and recommended by laboratory managers. This procedure directly impact on laboratory costs since need expressive human resource. We aim to evaluate whether it is really necessary to mix the primary blood tubes immediately before to assay HbA1c by turbidimetric inhibition immunoassay.

Methods: Blood from 20 diabetes patients were collected directly into K2EDTA evacuated tubes. The collection of all diagnostic blood specimens were performed by a single, expert phlebotomist, following the international standard from Clinical Laboratory Standard Institute. All diagnostic blood specimens were kept in vertical, closure-up position, and hand carried by our laboratory personnel in an appropriate biohazard container at room temperature from the phlebotomy service to the core laboratory. The mean transport time was 7 min. All specimens were manually mixed by inversion ten times, as recommended - front of the cobas c501 (Roche Diagnostics) - then HbA1c were immediately assayed using properly reagent Tina-quant Hemoglobin A1c Gen.3 (Roche Diagnostics). Subsequent the same blood specimens were left in upright position at room temperature, without mixing afterwards, and re-assayed at 2, and 4 hours after blood collection. The instrument was calibrated against appropriate proprietary reference standard material and verified with third-party control material (independent from calibrator material). Differences between samples were assessed by Wilcoxon ranked-pairs test. The level of statistical significance was set at $P < 0.05$.

Results: Main results are showed in Table 1.

Table 1. Impact of no-mix blood tubes before to assay HbA1c by turbidimetric inhibition immunoassay.

Parameter	Basal	2h	4h
HbA1c (%)	8.34 [6.74-9.62]	8.30 [6.78-9.51] P=0.556	8.40 [6.77-9.63] P=0.435

Values expressed as median [interquartile range].
P values represents significance by Wilcoxon ranked-pairs test.

Conclusion: We considered unnecessary to mix primary blood tubes before to assay HbA1c by turbidimetric inhibition immunoassay, since no statistical significant differences were observed between results from sample immediately mixed, and sample no-mixed till 4 hours after blood collection. We also strongly encourage all laboratory managers to perform similar verification to save both laboratory costs and human resource.

B-182

To Add-on or not to Add-on - Analysis of the Add-on testing in the context of TAT and volume for 2 major clinical hospitals

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Background: Request for additional testing to existing specimens is frequently encountered in the clinical setting. Add-ons can theoretically reduce unnecessary blood collection and save costs. Despite these advantages, the processing of add-on requests is challenging for many laboratories and creates substantial issues for workflow and processing. To better understand add-on trends the authors retrospectively evaluated ordering trends to identify possible areas for improvement. Data from the LIS was used analyze add—on volume, TAT, for both ED and non-ED locations.

Methods: The authors retrieved add-on data over a period of six months retrieved from Beaker LIS. The data was then sectioned into ED and non-ED locations. The daily add-on volume for the Santa Clara Valley Medical Center (SCVMC) averages around 300-400 different test add-on requests peaking 20-30 order an hour. The order shows up on the pending log and is followed up by the MLA. Then the specimen has to be located and linked to the specimen if enough sample is left and then added on the appropriate analyzer. This process takes about 4-5 minutes for each specimen.

Results: The authors made a detailed TAT and volume analysis showing 54% of the add-on volume during the dayshift, 39% during the evening shift and 7% night shift. The emergency department accounts for about 40% of the total add-on volume with an evenly distribution of the order volume. The most frequent chemistry add-on tests are liver function tests (7.1%), magnesium (5.4%) and basic metabolic panel (4.3%). The observed TAT for STAT troponins was drastically prolonged when ordered as add-on. There about 11% of all STAT troponin add-ons required more than 60 minutes between order and add-on by the MLA, which is above the cut-off of 60 minutes between order to result.

Conclusion: Frequently, physicians added different tests on the same specimen with just minutes apart. While this might be due to an interrupted order pattern, this increases the workload for the lab. Add-ons are not included by the auto-cancel policy implemented in the EMR. That leads to unnecessary test duplication on the same specimen. This investigation noticed that often multiple different provider request the same add-on tests on the same specimen. It also became very apparent that the information system department has a big problem with pulling a comprehensive and accurate report. The observed reporting problems include add-on ordering provider vs ordering provider of the primary tests, add-on ordering test vs primary tests, add-on ordering time and add-on time.

Wednesday, August 3, 2016

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

Molecular Pathology/Probes

B-183

Association between Plasminogen Activator Inhibitor-1 4G/5G Promotor Genotype and its Circulating Levels in Middle Eastern Population

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Background

Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA) and, as such, plays an important role in the regulation of fibrinolysis. Elevated (PAI-1) levels are associated with increased venous thromboembolism (VT) risk in the general population. The relation of PAI-1 4G/5G promotor genotype [NM_000602.4] (c.-817dupG) to venous thrombosis has been investigated primarily in case-control studies, which have produced inconsistent findings. Most studies, however, have reported higher PAI-1 plasma levels in individuals with 4G/4G. the evidence regarding the relationship between an elevated PAI-1 plasma level or PAI-1 genetic polymorphism and the risk of VT is conflicting. There is insufficient information to recommend use of either PAI-1 levels or genotype in evaluating risk of thrombophilia. The aim of the present study was to investigate the frequency of the 4G/5G PAI-1 promoter genotype in Middle Eastern population and the relation of the genotype to circulating PAI-1 levels.

Method

A total of 1930 individual (837 men and 1093 women; 18 to 76 years old) with no personal history of VT from 4 different middle eastern countries (Egypt (833), Saudi Arabia (512), Qatar (373) and United Arab Emirates(212)) included in the study. PAI-1 4G/5G promotor genotype was determined using validated reverse hybridization polymerase chain reaction (PCR) derived from ViennaLab Diagnostics GmbH Vienna, Austria. PAI-1 level was measured quantitatively using a validated solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA) derived from Invitrogen Corporation, CA, USA.

Results

There were 285 subjects with the 4G/4G genotype (14.7%), 885 with 4G/5G genotype (54.85%), and 760 with 5G/5G genotype (39.38%). PAI-1 levels were significantly higher homozygotes for the PAI-1 gene deletion allele (4G/4G) (P<.001) and heterozygous PAI-1 gene deletion allele (4G/5G) (P<.001), while the 5G homozygotes allele had the lowest levels of PAI-1.

Conclusion:

There is a significant correlation between gene variants of PAI-1 and circulating PAI-1 antigen levels in Middle Eastern individuals without clinical evidence or history of venous thromboembolism and thus the PAI-1 genotype and levels could be used along with clinical data and other laboratory findings in evaluation risk of thrombophilia.

B-185

Genotype distribution of high risk human papillomavirus in women from the state of Jalisco, Mexico

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Background: The majority of cervical cancer (CC) diagnoses are attributed to persistent infection of high risk HPV (Human Papillomavirus). Particularly, HPV 16 followed by HPV 18, are responsible for approximately 70% of cervical cancers. In 2015, the Public Health Laboratory *(Laboratorio Estatal de Salud Pública-LESP)* implemented quantitative real-time polymerase chain reaction (PCR) molecular technology in order to increase accuracy of HPV testing. Our aim was to identify high risk genotypes: HPV 16, HPV 18 and a 12 VPH genotype pool: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 in women from the 13 sanitary regions of Jalisco, Mexico. **Methods:** In this cross-sectional study from April 14 to October 15, 2015, a total of 44,349 cervical cell samples of women ages 35 to 64 years attending health centers, mobile health caravans, regional hospitals and dysplasia clinics of the 13 sanitary regions of the state of Jalisco, were assessed for HPV genotype distribution by real-time PCR (Cobas ®4800 HPV; clinical sensitivity: 92.9%, specificity: 71.0%). Cervical cells were obtained from ectocervix and endocervix of the uterus of every woman and maintained in PreservCyt® solution.

Results: From the 44,349 cervical cell samples, 3,791 were positive to some type of HPV. From these, we found a total of 457 positive samples (12.05%) for HPV 16 and a total of 179 (4.72%) positive samples for HPV 18. In addition, 10 samples were positive to HPV 16+18 (0.26%) and 3,145 (82.97%) samples to the HPV 12 pool. Table 1 shows the genotype distributions of each region in Jalisco.

Table 1. HPV genotype distribution in women from the 13 sanitary regions of Jalisco

SANITARY REGIONS IN THE STATE OF JALISCO	HPV-POSITIVE CERVICAL CELL SAMPLES				
	TOTAL (n)	HPV 16	HPV 18	HPV (16+18)	HPV 12 POOL
COLOTLAN	77	12 (15.6 %)	1 (1.3 %)	1 (1.30%)	63 (81.80%)
LAGOS DE MORENO	162	18 (11.1 %)	7 (4.3 %)	-	137 (84.60%)
TEPATITLAN DE MORELOS	163	22 (13.5 %)	5 (3.1 %)	-	136 (83.40%)
LA BARCA	422	46 (10.9 %)	27 (6.4 %)	1 (0.24%)	348 (82.46%)
TAMAZULA DE GORDIANO	60	3 (5.0 %)	4 (6.7 %)	-	53 (88.30%)
CIUDAD GUZMAN	213	36 (16.9 %)	4 (1.9 %)	1 (0.47%)	172 (80.73%)
AUTLAN DE NAVARRO	252	21 (8.3 %)	15 (6.0 %)	1 (0.40%)	215 (85.30%)
PUERTO VALLARTA	179	23 (12.9 %)	7 (3.9 %)	-	149 (83.20%)
AMECA	315	36 (11.4 %)	17 (5.4 %)	-	262 (83.20%)
ZAPOPAN	376	57 (15.2 %)	14 (3.7 %)	1 (0.27%)	304 (80.83%)
TONALA	313	31 (9.9 %)	14 (4.5 %)	-	268 (85.60%)
TLAQUEPAQUE	342	37 (10.8 %)	18 (5.3 %)	1 (0.29%)	286 (83.61%)
GUADALAJARA	917	115 (12.5 %)	46 (5.0 %)	4 (0.44%)	752 (82.06%)

Data are expressed as the total number of HPV-positive cervical cell samples (n) and HPV genotypes as the total number of cases (n) and percentage (%); HPV: Human papillomavirus.

Conclusion:: : Quantitative real-time PCR technology for HPV testing has allowed rapid, accurate and reproducible results for CC early detection. In Jalisco, the primary high risk VPH genotypes were VPH 16 followed by VPH 18, both of which have an important clinical significance in CC.

B-186

Comparison of Genefinder HPV Liquid Beads Microarray PCR Kit and Hybrid Capture 2 Assay for Detection of HPV Infection

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Background: Along with advances in methodological technologies, various assays for detecting high-risk human papillomavirus (HR HPV) have been introduced. The Genefinder HPV liquid beads microarray PCR kit is one of the recently developed. Our aim was to compare the performance of Genefinder to Hybrid Capture 2 for detection of HR HPV.

Methods: A total of 900 cervical swab specimens were obtained. We submitted all specimens for HR HPV detection with HC2 and Genefinder, and then additionally analyzed the discordant or both positive results using restriction fragment mass polymorphism (RFMP) genotyping analysis.

Results: HC2 detected 12.8% cases and Genefinder detected 15.8% cases with 13 HR HPV types. Also, Genefinder detected 27.4% cases for the 32 detectable HPV types. The overall agreement rate was 93.2% with 0.724 kappa coefficient. Discordant results between these two assays were observed in 56 cases. HC2 showed sensitivity of 83.5% and specificity of 95.9%, while Genefinder showed sensitivity of 85.4% and specificity of 91.9%. For HPV 16 or HPV 18 detection, Genefinder showed 95.0% or 66.7% of sensitivity and 99.2% or 100%, respectively. Overall coinfection rate was 15.4% (38/247) in Genefinder analysis.

Conclusion: Considering the high agreement rate with HC2, high sensitivity and the ability to differentiate 32 HPV genotypes including HPV 16/18, Genefinder could be used as a laboratory testing method for the screening of HPV infections. The use of Genefinder may also contribute to future research associated with the significance of various HPV types and multiple coinfections.

B-188

Copy number variation results of intellectually disabled patients using agilent and affymetrix chromosomal microarrays

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Background: Chromosome microarray analysis can detect causative genetic abnormalities in up to 15% of patients with intellectual disabilities who had a normal G-banded karyotype.The ability of these assays to detect chromosome imbalances is influenced by the microarray design, coverage and probe density. AgilentTechnologies designs chromosomal arrays with longer oligonucleotides and uses hybridization of patient and reference DNAs while Affymetrix designs arrays with shorter probes and compare patient with an ideal sample in virtual database. Aims: The aim of this study was to carry out the verification of two microarray techniques in our laboratory using Agilent and Affymetrix platforms, and compare the concordance across platforms concerning copy number variation (CNV) results. Patients and Methods: A blind genomic analysis was performed retrospectively in 12 samples from patients with intellectual disabilities who had been previously tested by a reference laboratory. DNA was extracted with DNeasy Blood and Tissue kit (QIAGEN) and quality was assed using Nanodrop2000 (Thermo Scientific). Two comparable arrays with resolution of approximately 100 kb and higher probe densities in ClinGen regions were chosen: Cytoscan 750k (Affymetrix) and SurePrint G3 CGH ISCA v2 180k (Agilent) and experiments were performed according to the manufactures´s instructions. Affymetrix data were analyzed with Chromosome Analysis Suitv3.1software; 25 probes, 50Kb and -0,45log2 ratiowere defined for losses callings and 50 probes, 100Kband 0,30log2 ratiofor gains. Agilent data were analyzed with Cytogenomics v2.9.2.4 software with a three-probe minimum aberration call, log2ratio ≥0,25 for gains and ≤ 0,25 for losses. CNVs were classified in accordance with the guidelines from the American College of Medical Genetics. Results: Taking together, the reference reports(based on an Agilent 180k array) showed three aberrations classified as pathogenic, five variants of uncertain significance (VOUS) and fourteen benign CNVs longer than 200kb. In our analyses, all the pathogenic and VOUS callings were detected in both Agilent and Affymetrix assays. CNVs considered benign, on the other hand, were more frequently reported when using Agilent. Five of the benign CNVs were not detected by Cytoscan 750k either because there were no probes in those specific chromosome regions, or because few probes (<20) were altered. Among these CNVs, four were located in pericentromeric regions and one was non-coding located in the long arm of chr22. Conclusion: Using Cytoscan 750k and SurePrint G3 ISCA 180k arrays we were able to define correctly all the pathogenic and VOUS previously reported in 12 patients.One third of the CNVs classified as benign, though, were detected using SurePrint ISCA 180k only, mainly due to differences in the array designs. Nevertheless, the regions that are not covered by Cytoscanbear CNVs common in the population according to the Database of Genomic Variants (DGV) and do not affect the calling of causative aberrations. Therefore, we concluded that the results were highly concordant. There is evidence that the methodologies are equally efficient for the detection of CNVs with clinical importance, although because of the small number of cases studied, further investigation will be needed.

B-189

Utilization and reliability of genetic testing by analysis of repeat measurements from a large data repository

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Background: Repeat testing for the same genetic allele or mutation is typically unnecessary. However, this may occur from lack of awareness about prior testing, unavailability of previous results or desire to retest for confirmation due to concerns about reliability of results or clinical consequences to patients and their family members. This study examined the frequency of repeat genetic testing to assess utilization and reproducibility of results. **Methods:** Hemochromatosis (HFE), factor V Leiden (FVL), and *HLA-B*57:01* (B5701) test information was collected from the Veteran Affairs (VA) Healthcare Corporate Data Warehouse for results reported for up to 15 years since 2014. The frequency of cases undergoing repeat testing was evaluated. Cases with incomplete or missing information were excluded from analysis involving specific genotypes. Statistical analysis involving differences between proportions of specific genotypes were performed by the chi-square method.

Results: A total of 46,929 HFE, 47,050 FVL, and 9,358 B5701 cases were validated for evaluation from 118, 120, and 94 VA healthcare facilities respectively. One or more repeat tests were observed from 3,530 (7.5%) HFE, 3,762 (8.0%) FVL, and 704 (7.5%) B5701 cases of which 712 (20.2%) HFE, 753 (20.0%) FVL and 282 (40.1%) B5701 were retested at another facility. Frequency of discrepant results after retesting were 27/2,827 (0.96%) for HFE, 24/3,786 (0.63%) for FVL, and 0/675 for B5701. Retesting was more frequent for homozygous C282Y/C282Y 283/2,608 (10.9%) and H63D/H63D 93/1,099 (8.5%) genotypes compared to compound (C282Y/H63D) 135/1,758 (7.7%), C282Y 301/4,477 (6.7%), and H63D 467/7,506 (6.2%) heterozygotes and wild genotypes, 1,505/22,626 (6.7%), *P*<.001. Among FVL tests, homozygous, heterozygous and wild type retesting rates were 22/197 (11.2%), 488/5,596 (8.7%) and 3,205/40,456 (7.9%), respectively, *P*=.032. There was no difference in retesting frequency between negative 680/8,767 (7.8%) and positive 23/410 (5.6%) B5701 genotypes, *P*=.16. **Conclusion:** These results show that HFE, FVL and H5701 measurements are reliable based on low rates of discrepant results when retested at different times and locations on the same patient . These findings provide supporting evidence that retesting is generally unnecessary to confirm results. Causes for the few discrepancies observed were not evaluated but may have been due to problems associated with mislabeling, analytical errors or clerical mistakes. Since many repeat tests were performed at another facility, it is likely that retesting was, in part, caused by lack of awareness of previous results. Retesting was more common for abnormal HFE and FVL genotypes, possibly due to higher likelihood of clinical findings that prompted testing as well as possible intent to confirm abnormal results due to impact on clinical management and patients' family members. In summary, the three genetic tests evaluated were shown, in practice, to be reliable among a large and diverse group of laboratories but sometimes inappropriately repeated.

B-190

Compound heterozygous mutations identified in a Brazilian infant with glycogen storage disease type Ia

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Background: The liver is the largest internal organ in the body and it is associated with more than 100 different forms of diseases that affectpeople of any age. The diagnostic of liver diseases can be difficult since its symptoms can be unclear, non-specific and easily confused with other health problems. Liver diseases can be caused by a variety of factors, such as defective genes,viruses, medications and drugs. **Objective:** To identify, by whole exome sequencing (WES), the genetic cause of liver disease in an infant who had typical clinical features of lysosomal acid lipase deficiency (LAL-D) but who was LAL-D negative based on the measurement of LAL enzyme level and activity. **Case report:** A 6-month-old male infant born from healthy and non-consanguineous parents presented a poor weight gain, watery stools, steatorrhea, hepatomegaly with hepatic steatosis, hypertriglyceridemia and hypoglycemia. Family history was negative. LAL-D was proposed and discarded based on the measurement of LAL enzyme level and activity in the blood. The patient presents a good response oflow-fat diet. **Methods:** Genomic DNA was extracted from blood sample, following standard protocols. WES was performed using the Illumina Nextera technology according to the manufacturer's instructions and sequenced on the Illumina HiSeq 2500 platform. About 200 thousands exons of 20,500 genes were captured. A total of 158,193,788 sequences were obtained. The average coverage was 98-fold, with 96.0% of the target bases being covered at least 10X. **Results:** Two heterozygous mutations were identified on *G6PC* gene: a known pathogenic mutation in exon four (rs80356482; ENST00000253801:c.562G>C; p.Gly188Arg) and a previously undescribed mutation in exon three (ENST00000253801:c.510delA; p.Ile171Serfs*62).This mutation results in a frameshift and stop signal 62 codons downstream, causing a premature truncation of the G6PC protein at amino acid position 233. Online prediction program Mutation Taster suggested that this variant is a disease causing mutation with a probability value of 1.0 due to the truncated protein. The two mutations were identified in trans configuration, defining this individual as a compound heterozygote. **Discussion:** Glycogen storage diseases (GSD) type Ia is associated with abnormalities in the *G6PC* gene. Mutation in this gene result in a deficiency in the glucose-6-phosphatase (G6Pase) enzyme. GSD are a group of disorders in which stored glycogen cannot be metabolized into glucose to supply energy for the body. GSD type I (GSDI) or von Gierke's disease is the most common of the GSD and it is inherited as an autosomal recessive genetic disorder. Although its recessive nature, compound heterozygous mutations can cause genetic disease in the heterozygous state, since both alleles are defective. Others compound heterozygous mutations have already been described in patients with GSD Ia.**Conclusios:** WES revealed an important, sensitive and efficient tool for the molecular diagnosis in genetic and phenotypic complex diseases, where the phenotype is not suggestive of a particular candidate gene or set of genes, such as liver diseases. Furthermore, it has important implications for prognosis, carrier testing, genetic counselling and prenatal diagnosis.

B-191

Molecular cytogenetic characterization of a add(13)(q32) detected in a boy with multiple abnormalities

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Array comparative genomic hybridization (array-CGH) is a molecular analysis designed to detect gains and losses of genetic material in clinically significant regions of the human genome. Array-CGH detects the presence of microdeletions and microduplications that would be undetectable by conventional cytogenetic technique.We report here the cytogenetic investigation of one-year-old male with cardiovascular disease, optic atrophy, microcephaly, hypotonia, low weight, short stature, dimorphisms and global developmental delay.The initial standard chromosome study revealed 46,XY, add(13)(q32) karyotype. Array-CGH was performed to delineate the origin of additional genomic gain in chromosome 13. Array-CGH analysis additionally revealed a 29.1 Mb pathogenic duplication in the 2p25.3-p23.2 region and confirmed a 7.3 Mb deletion in the 13q33.3q34 region (arr [hg18] 2p25.3p23.2 (20,341-29,172,594) ×3, 13q33.3q34 (106,815,039-114,114,568) ×1). This suggests the additional chromosomal segment attached on chromosome 13q has originated from chromosome 2p with breakpoints in 2p25.3p23.2.The duplication in 2p25.3p23.2 region encompasses more than 100 genes and the deletion in chromosome 13q33.3q34 includes more than 40 genes.There are few reports of patients with pure duplication in 2p. The clinical findings are associated to variable phenotypes as cardiac anomalies, facial dimorphism and psychomotor delay. The 13q33.3q34 deletion has been associated with intellectual deficit, growth impairment and microcephaly. There is a report that described a patient with similar chromosomal rearrangement, but not characterized at the molecular level, that presented a clinical phenotype oflow birth weight , convulsions, neuroblastoma, low-set malformed ears, hypertelorism, micrognathia, micropenis and polydactyly with conventional karyotype 46, XY , der (13) t (2; 13) (p23 ; q34) dn. Moreover, we found in Decipher database a case of patient with coloboma, global development delay, mild intellectual deficit, intrauterine growth retardation, microcephaly, micrognathia, valgus foot deformity and ventricular septal defect, carrying a 13.55Mb terminal duplication in the short arm of the chromosome 2, as well a 5.87Mb terminal deletion in the long arm the chromosome 13. Parental karyotype analysis could help to characterize this chromosomal rearrangement and evaluate the recurrent risk. However, Array-CGH is a useful method for identifying unknown additional and unbalanced rearranged chromosomes that are not detected by conventional cytogenetic analysis.

B-192

Validation of fluorescence in situ hybridization assay for detection of the BCR/ABL rearrangement

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The BCR/ABL1 gene rearrangement is generated by a t(9;22)(q34;q11) reciprocal translocation. In most cases, it is cytogenetically visualized by the Philadelphia (Ph) chromosome. The Ph chromosome is the typical hallmark in chronic myeloid leukemia (CML), but can also be present in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Ph chromosome detection by conventional cytogenetic can be hampered by low quantity and quality of metaphases. Furthermore, BCR/ABL1 rearrangements may be hidden due to cryptic rearrangements or complex aberrations. About 5-10% of CML patients lack cytogenetic evidence of the Ph translocation but show BCR/ABL fusion by fluorescence in situ hybridization (FISH). FISH can be performed in dividing and non-dividing cells, which is important when dealing with leukemia cells with low proliferation. Although manufacturers evaluate the performance of most FISH probes prior to commercialization, it is necessary to revalidate all probes before the implementation of assay for clinical diagnostic. Therefore, clinical laboratories must adopt protocols for verify the performance of the assay. In this context, this study aimed to validate FISH assay for detection of BCR/ABL1 translocation following recommendations from the American College of Medical Genetics (ACMG). It was used the BCR/ABL1 translocation, dual fusion probe manufactured by Cytocell®. Metaphase cells obtained from five 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), it was estimated the false positive rate from 10 uncultured normal bone marrow samples and 10 uncultured normal blood samples. Two analysts scored 500 interphase cells (250 per analyst). All probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function available in Microsoft Excel software. The BCR/ABL1 probe presents the ABL1 (9q34) and BCR (22q11.2) probes labeled with red and green fluorophores, respectively. A normal result using this probe should show two green and two red signals (2G2R). Two fusion signals in addition to one green and one red signals (2F1G1R) indicate the presence of classical translocation. The probe demonstrated 100% specificity and analytical sensitivity. After analysis, three and seven atypical signal patterns were respectively identified in bone marrow and blood samples. . It was not observed change in the cutoffs with increasing cell count. The signal patterns and its cutoffs for bone marrow samples were 1F1G1R (3.5%), 2G1R (6.0%) and 1G2R (2.5%). The signal patterns and its cutoffs for blood samples were 1F1G1R (1.5%), 1F2G1R (1.5%), 1F2G2R (2.5%), 2G1R (2.5%), 1G2R (3.5%), 2G3R (1.5%) and 1G1R (1.5%). The cutoffs obtained with BETAINV function were validated using a sample of 200 cells. The analyses of normal and abnormal samples by FISH were in agreement with the karyotype analysis. It showed high reproducibility and quality in different hybridizations. The probe specificity and sensibility was higher than recommended by the ACMG.

B-193

Developing and evaluating a new *in-house* genotyping test for *CYP2D6* gene

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Background: The ability to metabolize drug is influenced by genetic variability. Interindividual differences result in a wide variation in drug response, leading to adverse drug reactions, therapeutic failure, and affects patient well-being and survival. Most of the commercialized drugs are metabolized by polymorphic enzymes and mainly by one or more cytochrome P450 (CYP) enzymes. *CYP2D6* gene (MIM #124030) is one of the most polymorphic pharmacogenes. Variations include point mutations, copy number variations (CNV), indels, conversions and gene rearrangements. These variants increase or decrease CYP2D6 enzyme activity resulting in four phenotypes with significant clinical implication: poor (PM), intermediate (IM), extensive (EM) and ultrarapid (UM) metabolizers. Genotyping and phenotyping test are used in clinical practice to identify variations in *CYP* allelic variants. For *CYP2D6,*more than 109 allelic variants were reported by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Database. **Objective:** Since most commercial genotyping kits are available for just few alleles, this study aimed to develop, validate and evaluate a new in-house *CYP2D6* genotyping test using Sanger sequencing and TaqMan® Copy Number Assay. **Methods:** Genomic DNA samplesfrom 10 individuals previously genotyped by Luminex xTAG® CYP2D6 Kit were used for validation. Coding sequences and flanking regions of *CYP2D6* gene were amplified by PCR. Bidirectional Sanger sequencing was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit and sequenced on ABI 3730 DNA Analyzer. Sequences were analyzed using SeqScape Software v3.0 and compared with the *CYP2D6**1reference sequence (Accession Number: AY545216.1) to identify polymorphisms. CNV was determined by TaqMan Copy Number Assay (Assay ID: Hs04083572_cn) and RNaseP assay (Assay ID: 4403326) served as the internal control. Relative quantification was performed with CopyCaller® Software v2.1 using the comparative ΔΔCT method. For each individual, the genotype that best represents the set of polymorphisms and CNV datawere defined according to The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, PharmGKB, and LOVD databases. The phenotype was predicted as PM, IM, EM or UM, based on CYP2D6 diplotypes and the activity score system recommend by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy. The results from the method developed by this study and the commercial kit were compared. **Results:** The results were 70% concordant between the two methodologies. The differences were due to the coverage of the present in-house assay, once it can detect more variants than the commercial kit, classifying haplotypes more accurately. In addition, one difference was due to a *CYP2D6* deletion that was not identified by the commercial kit. Although these differences, phenotype did not change for the patients. **Conclusions:** Strategy of CNV determination in combination with polymorphism analysis is of utmost importance to correct phenotype prediction. The new method developed combines these two assays creating a more accurate, sensitive and reliable genotyping test than the offered by most commercial genotyping kits. Although this method does not detect all polymorphism of *CYP2D6* since it does not sequence the entire gene, it can detect more than 140 variants reported in the databases and can identify undescribed mutations, contributing to new haplotypes identification.

B-194

Validation of Multiplex Ligation-Dependent Probe Amplification (MLPA) kit P081 and P082 for use in Neurofibromatosis Type 1 clinical routine diagnosis

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Neurofibromatosis Type 1 (Nf1) is one of the most common inherited neurological disorders, affecting about 1: 3.000 individuals. Mutations in the *NF1* gene [(350 kb, 60 exons located on the long arm of chromosome 17 (17q11.2)] are the cause of Nf1 disease, but there are others conditions that may confound the diagnosis. Nf1 is characterized by multiple *cafe-au-lait* spots, freckling, Lisch nodules and neurofibromas and clinical diagnosis is achieved with criteria established by Neurofibromatosis Conference at National Institute of Health, USA (NIH - 1988). Our study aimed to validate the SALSA MLPA PROBE MIX P081 and P082 kits for use in clinical practice to detect deletions of *NF1* gene in patients with clinical diagnosis of Nf1. For this purpose, 32 individuals were selected, of which 25 patients with clinical diagnosis of Nf1 and 7 non-Nf1 affected individuals (supposed healthy). All Nf1 patients were invited from the Neurofibromatosis Outpatient Reference Center at Hospital das Clínicas - Belo Horizonte / MG / Brazil. The MLPA analysis showed that 100% (7/7) of non-Nf1 individuals showed no deletion or duplication in the *NF1* gene. For the 25 patients with Nf1 clinical diagnosis, 76% (19/25) were negative for deletions / duplications in the neurofibromin gene. Microdeletions (former whole-gene deletion) were found in four (20% - 5/25) Nf1 patients with heterozygosity and one patient had only exon 47 deletion in heterozygosity. To further confirm the one exon deletion of the last patient, *NF1* gene was sequenced by massively parallel sequencing that showed, in fact, to be a point mutation in the annealing site of one of the probes from MLPA P081 kit targeting exon 47 region. In addition, one patient (4%) with positive clinical diagnosis for Nf1 remained inconclusive in MLPA analysis. Two samples were sent for analysis in another reference laboratory and the results for molecular alterations were confirmed. Importantly, the negative result does not exclude the diagnosis of Nf1. The present results suggest MLPA analysis may benefit Nf1 suspected individuals that present only one criterion or uncertain diagnosis criteria.

B-195

Improving NPM1 exon 12 sequencing using PCR amplification with a high-fidelity enzyme

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Background: Nucleophosmin (NPM1) exon 12 mutations consist in the most prevalent genetic lesion in adult acute myeloid leukemia. These mutations are small insertion/deletions in the beginning of exon 12, which code for the nucleolar localization signal of the protein. NPM1 exon 12 mutations and are associated with favorable prognosis, therefore the mutational status is crucial for treatment decision making. **Aims:** The aim of this study was to evaluate different approaches for NPM1 exon 12 sequencing and define the methodology that is most cost-effective and yields good quality sequences. **Patients and Methods:** 155 Brazilian patients diagnosed with de novo myeloid acute leukemia from February 2011 to September 2014 were included in this study. All patients were screened for NPM1 exon 12 mutations through cDNA sequencing. Twenty patients with known genotypes were chosen for DNA sequencing. Bone marrow samples were collected in PAXgene tubes (QIAGEN/BD); RNA was extracted using PAXgene Bone Marrow RNA Kit; DNA was extracted from 1ml of PAXgene solution plus blood using QIAamp DNA Blood Mini (QIAGEN). RNA was reverse transcribed into cDNA with Improm II Reverse Transcription System (Promega). Exon 12 and 3´part of exon 11 were PCR amplified from both DNA and cDNA with primers containing mutations to avoid pseudogene amplification. Amplification was carried out with Platinum Taq DNA Polymerase (Life Technologies) and with PfuUltra High-fidelity (Agilent Technologies). PCR products were column purified with the illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Science). Sequencing reactions of both strands were performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific). **Results:** Sequencing results from DNA and cDNA were completely concordant. Nevertheless, forward DNA sequencing after PCR with Taq Polymerase was hampered by the presence of extra peaks following capillary electrophoresis, probably because of the presence of a poly-T region in the 3´extremity of exon 11 that may cause DNA polymerase slippage. These peaks were not seen when DNA was amplified with the high-fidelity enzyme. **Conclusion:** In technical terms, the best method for NPM1 mutation evaluation was cDNA sequencing, although for a clinical laboratory this may not be a cost effective test due to the need of rapid sample transportation, RNA extraction and cDNA synthesis. Alternatively, DNA sequencing using PfuUltrahigh-fidelity yields good results, although in a few cases (when patients carry a rare T deletion polymorphism in intron 11) the forward DNA sequence may be misinterpreted.

B-196

Multiplex Ligation-Dependent Probe Amplification (MLPA) as a diagnostic tool for detection of copy number variations in Charcot-Marie-Tooth and HNPP diseases

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Background: Charcot-Marie-Tooth (CMT) and Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) are common inherited disorders of the sensory and peripheral nervous system that present distinct clinical manifestations and histology. Both diseases are demyelinating neuropathiesthat are mainly related to copy number variations (CNVs) of peripheral myelin protein 22 gene (*PMP22,* MIM# 601097). CMT type 1A (CMT1A) is the most commom form of CMT and is characterized by severely reduced motor nerve conduction velocities.The majority of CMT1A cases (>98%) results from duplication of region encompassing the *PMP22* gene*,* located on chromosome region 17p11.2-p12. In addition, deletions of the *PMP22* gene are the usual cause of HNPP (85%).The incidence of CMT1A is about one in 2.500 individuals and for HNPP is somewhat lower, aroundone in 6.250. Several methods for the identification of CMT1A and HNPP CNVs are reported. Multiplex Ligation-Dependent Probe Amplification (MLPA) has been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as gene duplications and deletions. **Aims:** Validate the MLPA kit P405 in CMT and HPNN patients previously evaluated by Short Tandem Repeat technique (STR).The MLPA kit is capable of discriminate CMT1A, CMT1B, X-linked CMT and HNPP diseases. **Methods:** Seven patients with CMT or HNPP were tested using the commercial MLPA kit P405 versionA1 (MRC-Holland), following manufacturer's instructions.The P405-A1 CMT probemix contains probes for copy number detection of *PMP22*, *COX10* and *TEKT3* genes in the CMT/HNPP region of chromosome 17p12 and also probes for *GJB1* (X-linked CMT gene) and *MPZ* (CMT1B gene).The analysis was performed using the Coffalyser v.140721.1958 software. Furthermore, all patients were also evaluated by STR using eight markers for CMT (D17S9A, D17S9B, D17S4A, D17S2220, D17S2224, D17S2227, D17S2228 and D17S2230) and six markers for HNPP (D17S9B, D17S9A, D17S2220, D17S4A, D17S2227 and D17S2230). **Results:**The MLPA results were concordant with STR results in all patients. Three patients did not present any variation in *PMP22*, two patients presented *PMP22* duplications and two presented *PMP22* deletions. In addition, X chromosomal variations were detected in two patients by MLPA that cannot be identified by STR markers. Although both patients presented duplications of X-linked genes, one patient presented a *PMP22* deletion. **Conclusions:** The MLPA technique appears to be more informative and high-throughput than STR test. Moreover, the former technique is robust, sensitive and sequence-specific test for detection of *PMP22* gene duplications and deletions in molecular diagnosis of CMT1A and HNPP. Therefore, MLPA could be recommended as a diagnostic tool for CMT disease.

B-197

Array-based comparative genomic hybridization identified genomic imbalance in a patient with apparently *de novo* balanced translocation

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Balanced translocations are relatively common, requiring a two-break event and involving the exchange of chromosome segments between two, usually non-homologous chromosomes. Although the great majority of cases with apparently balanced translocations are not associated with abnormal phenotypes, they can reduce the fertility and increase the risk of unbalanced gametes and abnormal progeny. In approximately 6% of balanced chromosomal rearrangements carriers, intellectual

disability, dysmorphic features and congenital anomalies can be found. The abnormal phenotype might be the result of genomic imbalance or aberrant expression caused by direct breakage of a dosage sensitive gene. Here, we report a seven-years-oldboy with intellectual disability and speech delay.Chromosomal analyses of this patient showed an apparently balanced translocation between chromosomes 2 and 9. The karyotype was designated as 46,XY,t(2;9)(q33;p22)dn. Array-based comparative genomic hybridization (array-CGH) though revealed a ~7.6 Mb interstitial deletion on chromosome 9 at 9p23-p22.2 (arr [hg18] 9p23p22.2 (10,166, 368-17,766, 067) ×1). We performed chromosomal analyses of samples obtained from his parents and the karyotypes were normal. The deletion in 9p23p22.2 region encompasses more than 15 genes, including TYRP1 (tyrosinase-related protein 1) and FREM1 (FRAS1 related extracellular matrix 1) genes. Deletions of the short arm of chromosome 9 are associated with distinct clinical phenotypes. There are reports in the literature of terminal and interstitial deletions with distinct sizes in chromosome 9p (OMIM #158170) associated with the 9p deletion syndrome. This syndrome is characterized by a variable phenotype that includes facial dysmorphism, hypotonia, intellectual disability and others. Additionally, we identified ~195 Kb duplication in 10p11.21 that could be classified as VOUS (variant of unknown significance).Studies suggested that the link between an apparently balanced translocation and the appearance of abnormal phenotype may be partly explained by the presence of cryptic and complex chromosomal rearrangements and that more breaks may lead to imbalances. Some studies were based only on FISH approaches and, therefore, small interstitial imbalances may have been missed. The development of the array-CGH overcame the limitations of cytogenetic and FISH approaches and provided the opportunity to screen the entire genome for cryptic genomic gains and losses.This study underlines the importance of a genome-wide approach in patients with an apparently balanced chromosome rearrangement and abnormal phenotype. It is crucial to examine an apparently balanced rearrangement after detection because it can be more complex than suggests.

B-198

Leukemic survival factor SALL4 contributes to defective DNA damage repair

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Background: SALL4 is aberrantly expressed in human myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). We have generated a SALL4 transgenic (SALL4B Tg) mouse model with pre-leukemic MDS-like symptoms that transform to AML over time. This makes our mouse model applicable for studying human MDS/AML diseases. In this study, by searching the common gene/pathway in leukemia initiating cells/populations (LICs), we have identified a novel mechanism that enables SALL4 contributes to leukemic development.

Methods: The molecular mechanism of SALL4 contributing to leukemogenesis was explored through gene expression profiling studies on leukemic initiating populations. Data were analyzed by dChip (http://biosun1.harvard.edu/complab/dchip/) and GSEA 2.0 algorithm (http://www.broad.mit.edu/gsea/). The regulations of a number of potentially relevant genes were further verified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Mitomycin C (MMC) clonogenic assay, chromosomal spread assay and Homologoous recombination (HR) and nonhomologous DNA end joining (NHEJ) reporter assay were performed to study the role of SALL4 in DNA damage repair and response. The gene expression data on a cohort of 542 AML patients were downloaded from publicly available Gene Expression Omnibus (GEO) dataset under series accession number GSE13159. Another cohort of 75 primary human MDS/AML samples was collected in Beijing from December 2009 to December 2015. This study was approved by the institutional review board of Peking Union Medical College Hospital and informed consent was obtained from all subjects.

Results: Characterization of the leukemic initiation population in this model leads to the discovery that Fancl (Fanconi anemia, complementation group L) is down-regulated in SALL4B Tg leukemic and pre-leukemic cells. Similar to the reported Fanconi anemia (FA) mouse model, chromosomal instability with radial changes that can be detected in pre-leukemic SALL4B Tg bone marrow (BM) cells after DNA damage challenge. Results from additional studies using DNA damage repair reporter assays support a role of SALL4 in inhibiting the homologous recombination pathway. Intriguingly, unlike the FA mouse model, after DNA damage challenge, SALL4B Tg BM cells can survive and generate hematopoietic colonies. We further elucidated that the mechanism by which SALL4 promotes cell survival is through Bcl2 activation. Parallel human study also showed that SALL4 expression of MDS/AML patients with complex aberrant karyotype was significantly higher than patients with normal or other abnormalities.

Conclusion: In summary, our studies demonstrate for the first time that SALL4 has a negative impact in DNA damage repair, and support the model of dual functional properties of SALL4 in leukemogenesis through inhibiting DNA damage repair and promoting cell survival.

B-199

Verification of the Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay

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Background: Cystic Fibrosis (CF) is the most common life-threatening autosomal recessive disorder among Caucasian populations with an incidence of 1 in 3000 and an estimated 1 in 28 carrier frequency. Caused by inheriting two defective copies of the cystic fibrosis transmembrane conductance regulator gene (CFTR), CF can affect multiple organ systems. CF typically presents with persistent pulmonary infections, sinus infections and pancreatic insufficiency. CF has a highly variable presentation, with milder forms of the disease often presenting later in life. Diagnosis is established through newborn screening programs followed by confirmation with sweat chloride testing and CFTR gene variant identification. Early identification of disease causing CF mutations is important in order to minimize the decline of lung and pancreatic function. The most common CFTR mutation (ΔF508) is responsible for ~66% of CF disease within the US population. Greater than 2000 variants have been identified and both the carrier rate and variant frequency vary between different ethnic populations. The American College of Medical Genetics (ACMG) recommends screening for a minimum of 23 common CF variants that identify 88% of Caucasian carriers but only 72% of Hispanic and 65% of African American carriers. Expanding the number of variants screened will increase the likelihood of identifying carriers in Caucasians and other ethnic groups. The objective of our study was to perform an in-house verification of the recently FDA approved Illumina MiSeqDx® Cystic Fibrosis 139 Variant IVD assay with split sample comparisons to the Luminex xTAG® 60 Variant Kit v2. This assay contains all ACMG and American College of Obstetricians and Gynecologists recommended variants.

Methods: DNA from 62 peripheral blood specimens previously submitted for CFTR testing by xTAG® Cystic Fibrosis 60 V2 Assay were de-identified and genotypes blinded. The Illumina MiSeqDx® Cystic Fibrosis 139 Variant assay was performed according to the manufacturer's protocol. The INTROL™ CF Panel III Control (MMQCI) was used to verify additional mutations not present in patient samples. Barcoded libraries were created using the CF 139-variant oligonucleotide probe pool. Amplified libraries were purified, normalized, pooled, sequenced, and analyzed using the MiSeqDx® and MiSeq Reporter 2.2.31.

Results: Prior to library preparation 14.5% (9/62) samples failed to meet established DNA quality measures and were removed from testing. Of the remaining 53 samples there was 100% intra-assay concordance. 64% (32) of samples were positive for at least one CF variant and 36% (19) contained no identified CF variants. A total of 43 variants were identified across 36 alleles and 4 manufactured controls. 99% (81/82) of CFTR amplicons had a read depth of >30X with 1 amplicon resulting in <30X in 5% of samples and an average read depth of > 1000X. A sample call rate of 99.9% was observed across all samples and variants. Cluster densities on the MiSeqDx ranged from 422-987K/mm^2 and resulted in an average of 8 million reads/run. We did not observe any additional variant pickups with the expanded panel within our highly Caucasian population.

Conclusion: Our verification of the Illumina MiSeqDX demonstrated 100% concordance with the Luminex xTAG® 60 Variant Kit v2.

B-200

Identification of three novel pathogenic mutations in NF1 gene

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Neurofibromatosis type 1 (Nf1) is a genetic autosomal dominant disease characterized by café au lait spots, Lisch nodules, neurofibromas, osseous dysplasia and optical gliomas. Nf1 is the most common genetic disease caused by a single gene [1]. The gene *NF1* is located at chromosome 17 (17q11.2) near the centromere, whose product is a protein (Neurofibromin - 2,485 amino acids) predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. The Neurofibromin is a tumor suppressor protein, that functioning as negative regulator of cellular RAS-MAPK signaling pathway. Any mutation that disrupts the function of protein could lead to uncontrolled cell growth and potentially tumorigenesis. More than 90% of these mutations are small deletions and insertions, splicing mutations, and nonsense or missense mutations. A minority of patients (4-5%) has exonic or whole-gene deletions/duplications. The *NF1* gene has the highest rate of mutation in a human gene and the causes remain unclear.In this work, we described three novel mutations in the *NF1* gene, related to typical Nf1 clinical features. The mutations were identified by Massive Parallel Sequencing complete of *NF1* gene of individuals previously diagnosed for Nf1 and with negative result for exonic or whole-gene deletions/duplications. The clinical diagnosis of Nf1 was based on the clinical diagnostic criteria outlined in the National Institutes of Health (NIH) consensus development conference in 1988. The products of PCR were loaded on Ion 316 chip and sequenced with an Ion Personal Genome Machine (PGM) System (Thermo Fisher). Entire *NF1* coding exons and their intron boundaries (25 bp) were coverage (99.44%). Screening for *NF1* deletions was performed using the SALSA P081/082 (MRC-Holland) NF1 MLPA assay following instruction's manufacturer.All the three mutations (g.248760_248763del, g.250559dup and g.69115delG) resulted in a frame shift predicted to generate a premature stop codon at amino acid positions 2268, 2305 and 102, respectively. All of them were classified as pathogenic following the Standards and Guidelines for interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG - 2015).The knowledge of new pathogenic mutations is helpful to best clinical decisions for Nf1 treatment and diagnosis.

B-201

New Molecular Assays for the Simultaneous Detection of Gastric Pathogens Using the VERSANT MiPLX Solution

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Background: Gastroenteritis accounts for more than 1.7 billion cases globally, leading to 2 million deaths annually. Children under 5 years are especially at risk, with over 700,000 deaths every year.[1] These infections can be caused by a diversity of pathogens including viruses, bacteria, and parasites. Conventional methods in the clinical laboratory can take days to identify the cause. Here we describe several new molecular assays for the simultaneous detection of gastric pathogens including Norovirus, *Clostridium difficile*, *Salmonella*, *Campylobacter,* and Giardia in stool samples using the VERSANT® kPCR Molecular System with MiPLX software.

Methods: Raw stool samples were collected from patients with Norovirus, *C. difficile*, *Salmonella*, *Campylobacter,* and Giardia infections. Ten samples of each pathogen were processed. Either 60 mg of stool was weighed, or a 10 µL loop of stool was added to pretreatment buffer. Following pretreatment, samples were loaded onto the VERSANT kPCR Molecular System for automated nucleic acid extraction and plate setup. A single extracted sample was able to be split into PCR wells for up to six different assays. Amplification and detection was performed for all pathogens using the same thermal cycling method on the QuantStudio 5 (ThermoFisher Scientific).

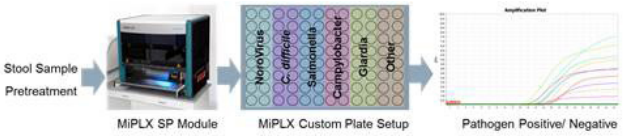

Figure 1: The VERSANT® kPCR Molecular System with MiPLX software for automated nucleic acid extraction, plate setup, and amplification/detection.

Results: The new "Company Name" gastrointestinal assays, as automated on the VERSANT kPCR Molecular System with MiPLX software, detect multiple pathogens including Norovirus, *C. difficile*, *Salmonella*, *Campylobacter,* and Giardia. Norovirus, *Salmonella*, *Campylobacter,* and Giardia were detected in both 60 mg and 10 µL loops of stool samples.

Conclusion: The VERSANT MiPLX Solution, consisting of the VERSANT kPCR Molecular System with MiPLX software, provides an automated and simplified workflow for detection of up to six gastrointestinal pathogens from one sample in a single run. The VERSANT MiPLX Solution provides a customized and flexible approach for performing high-throughput testing with a short turnaround time for the detection of desired pathogens. 1) World Health Organization (WHO). 2013.

B-202

Implementation of a molecular diagnostic method for the quantitative detection of Zika virus

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The Zika virus is a single-stranded positive sense RNA molecule belonging to the Flaviviridae family of Flaviviruses. The Flavivirus genus also includes the better known Dengue, Chikungunya, and West Nile Viruses. The World Health Organization (WHO) recently declared that the current (2016) outbreak of Zika virus in Brazil and other parts of South America poses a significant global health risk that is likely to reach pandemic status. Additionally, the United Stated Centers for Disease Control (CDC) has issued an alert level 2 (practice enhanced precautions) travel advisory for affected regions of South and Central America. Patients with an active Zika virus infection, known as Zika fever, do not present with particularly aggressive symptoms. The major cause for concern with Zika viral infection has, instead, been the possibility that Zika virus may cause microcephaly in the fetuses of infected pregnant women. Early detection of active viral infection is thus of paramount importance as it can be used to trigger more aggressive ultrasound monitoring in pregnant women with the goal of identifying the early signs of microcephaly.

Zika, and other members of the flaviviridae family, share high amounts of structural homology that render standard immunoassay-based testing methodologies of limited utility when used in viral typing. Molecular based methods, however, are well suited to identifying minor differences in the envelope and glycoprotein regions of the viral genome, and make both detection and quantification of active viral infection possible. We therefore sought to implement and validate a one-step real-time PCR-based quantitative assay (qRT-PCR) for the detection of active virus in patient plasma.

Primer sequences for real-time detection of Zika virus were provided upon request from the CDCs Arbovirus Disease Branch in Fort Collins Colorado. Primer sequences were specific for two regions of the viral genome include a viral envelope glycoprotein and one of the nonstructural protein coding regions. We performed the validation studies for our assay using a combination of commercially available viral cultures (Zeptometrix), Zika patient proficiency samples (CDC), and synthetic double-stranded DNA oligonucleotide fragments (gBlock, Integrated DNA Technologies). We determined the lower limit of quantification of our qRT-PCR assay to be $1x10^3$ copies using the GeneBlock fragments, which were also used to determine the linearity of our assay over an analytical measurement range of of $1x10^3$-$1x10^{12}$ copies. Cycle thresholds were set empirically on a per run basis and verification of the amplified PCR products was carried out by visualization of the

amplicons (75-90bp) on a QIAxcel capillary electrophoresis system. Precision studies were carried out for inter and intra-assay variability and yielded a coefficient of variation of <15% at the low end of the quantification limit. Using a combination of patient samples and synthetically designed gBlock fragments we have implemented and validated a qRT-PCR test for Zika virus, which is currently available in our institution. Having an "in-house" assay allows for faster turnaround times for our patients and clinical colleagues and enables us to provide a higher level of clinical care.

B-203

A Laboratory Developed, 106 Mutation Cystic Fibrosis Carrier Screening Test Using the Agena MassARRAY®: A Review of the First 123,000 Tests

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Background: In 2001 and 2004, ACOG and ACMG recommended population-based carrier screening for cystic fibrosis (CF) using a panel of 23 mutations. Since then, some have argued that expanded panels with additional mutations would be useful in some populations. We co-developed a 106 mutation CF screening test with Agena Biosciences (formerly Sequenom, Inc.). Analytical verification of the assay has been presented (Farkas et al. JMD 2010;12:611-9). Here we report the data obtained from the first 123,172 test preformed at Mayo Clinic, and ask whether extended carrier screening panels can increase the sensitivity of CF carrier detection. **Methods**: The MassARRAY® CF screening test was validated according to CLIA guidelines. The workflow consists of 8 multiplex PCR reactions, removal of dNTPs and primers with Agencourt® AMPure® XP resin (Beckman-Coulter), 8 multiplex single base extension (SBE) reactions, desalting with an ion exchange resin, spotting the extended SBE products onto a MassARRAY chip, and analysis by MALDI-TOF mass spectrometry. 48 samples and controls are run per batch in 384 well plates. All steps are automated, and the test requires 9 hours to complete. The method requires only general purpose laboratory reagents, thus the test is inexpensive at under $30 per test. Since validation, the MGL has tested **123,172** clinical samples. **Results**: Validation of accuracy included analysis of a set of 43 samples that contained 60 different mutations and potential interferences (such as E75Q and 711+3A>G), 28 of these samples were compound heterozygotes. In addition, 594 samples were tested in parallel (15 runs) with the current Luminex 70+5 commercial assay, 25 of these samples had one mutation detected by the Luminex assay. For both sets of samples, there were no discordant results. Of the clinical samples tested, **3,153** were for a possible or known diagnosis of CF, pancreatitis, or male infertility; the remainder had indications for testing of routine carrier screening, a family history of CF, a partner that was a known CF carrier, or no indication given. Of the samples tested, **472** had two mutations (**226** homozygotes and **246** compound heterozygotes). Of these, there were **203** deltaF508 homozygotes, and **184** cases with one deltaF508 with another mutation. There were **4,647** cases with one CF mutation identified. Of the 123,172 total cases tested, this represents a **3.8%** carrier frequency, which is concordant with literature estimates. These numbers do not include 498 carriers of one copy of the R117H variant with a 7T allele. These results are reported, but not as CF carriers - rather carriers of a mild variant not typically associated with classical CF. Of the cases with one mutation identified, **4169 (89.7%)** were ACMG panel mutations; **3,293** of which were **dF508**. We have detected **66** different mutations, including all of those on the 23 mutation ACMG panel. **Conclusions**: Of all detected mutations, **10.3%**, were non-ACMG panel mutations, indicating that a significant fraction of the population can benefit from screening with an expanded panel. The MassARRAY instrument has proven to be a robust, cost effective platform for a laboratory developed CF carrier screening test.

B-204

A rare cytogenetic finding: Ring chromosome 22

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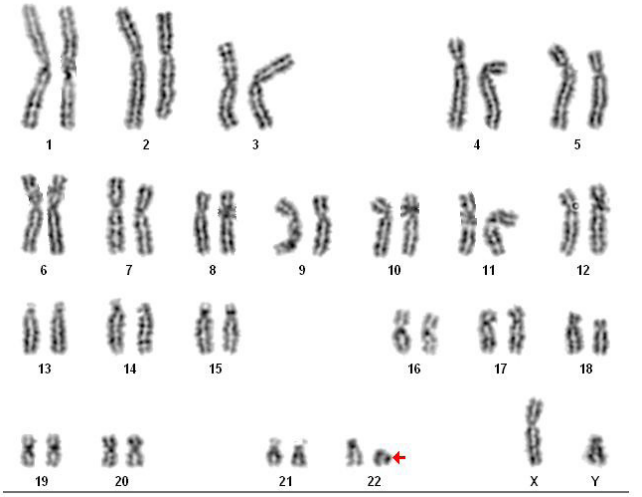
Background: Ring chromosome 22 is a rare cytogenetic finding and was first described in 1968. Since then about 60 patients have been reported. The major features of this syndrome includes intelectual disability, hypotonia and developmental delay. Here we describe a 4 years old male with intelectual disability, developmental delay and a Karyotype with ring chromosome 22.

Methods: A 400-bands Karyotype were performed on methaphase chromosomes obtained from peripheral blood lymphocyte.

Results: The patient´s karyotype showed the presence of one chromosome 22 shaped-like ring. The karyotype: 46,XY,r(22)(p13q13) (Figura1), was observed in 20 metaphases.

Conclusion: The majority of r(22) are formed de novo but there are reports of familial trasnmission, therefore the karyotype of the father and mother of the patient should be performed. The phenotypic differences of this syndrome could be the result of diferent size deletions in chromosome 22. The most frequently observed features of individuals with ring chromosome 22 overlap with the features of 22q13 deletion Syndrome, in which haploinsufficiency of SHANK3/PROSAP2 is suggested to be responsible. A few cases of ring chromosome 22 were characterized by molecular studies, and in most of them the segment containing the locus ARSA was deleted. This report underscores the variability in ring structure and clinical presentation of the patients and adds information to the limited literature on this rare disorder.



B-205

A fertile patient with an unusual karyotype finding for a mosaic of Turner Syndrome and a 4p16 translocation

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Background: Turner Syndrome is the most common sex chromosome abnormality in females, occurring in approximately one in 2500 live births. Women with Turner Syndrome are at extremely high risk for primary ovarian insufficiency (POI) and infertility. Although about 70-80% have no spontaneous pubertal development and 90% experience primary amenorrhea, the remainder may possess a small residual of ovarian follicles at birth or early childhood. The t(4;8)(p16;p23) is the second most common constitutional chromosomal translocation and is caused by an ectopic meiotic recombination between the olfactory receptor gene clusters (ORGC), located on chromosome 4p and 8p. Given that ORGCs are scattered across the genome and make-up about 0.1% of the human genome , translocations between 4p16 and other regions might be mediated by ectopic recombination between different ORGC. Purpose: To describe a fertile patient with an unusual karyotype finding for a mosaic of Turner Syndrome and a 4p16 translocation and her son with the inherited translocation and intellectual disability.

Method: A 400-band Karyotype was performed in peripheral blood lymphocyte of the patient and her son. The patient had 30 metaphases analyzed and her son 20 metaphases.

Results: The patient´s karyotype revealed 90% of metaphases with a monosomy of the sex chromosome X and the translocation between short arm of chromosome 4 and the long arm of chromosome 8: mos45,X,t(4;8)(p?16;q22)[27]/46,XX,t(4;8)(p?16;q22)[3](Figure1). The patient´s son karyotype revealed in the twenty metaphases a translocation between short arm of chromosome 4 and the long arm of chromosome 8: 46,XY,t(4;8)(p?16;q22) (Figure2).

Discussion: Primary ovarian insufficiency is a classic feature of Turner Syndrome. The pregnancy outcomes in spontaneous pregnancy are more favorable than those after oocyte donation in Turner patients but the risk of fetal chromosomal abnormalities remains high. In this case a molecular study would be important to define the breakpoints of the translocation and correlate with patient´s son phenotype.

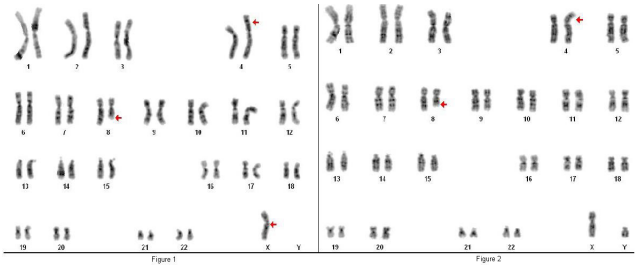

Figure 1 Figure 2

B-206

Multiplex Real-time PCR assays for Rapid Detection of group A, B streptococcus and Streptococcus pneumoniae

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Background : Streptococcus are subdivided into five main pathogenic groups commonly found in human (A, B, C, D and G). Especially group A, B streptococcus and *streptococcus pneumoniae* are risk factors cause of human disease. Streptococcus is a major infectious agent with significant morbidity and mortality, rapid and sensitive diagnosis is difficult. In this regard, real-time polymerase chain reaction (PCR) based on TaqMan technology enables the accurate detection of bacterial genome over a broad range without the necessity for post-PCR handling. In this study, we developed a multiplex real-time PCR assay (mrt-PCR) capable of simultaneously detecting group A , B streptococcus and *Streptococcus pneumoniae.*

Method: The specific primers and probes were designed based on highly conserved the capsular polysaccharide synthesis regulatory protein (cpsB) gene region of group A streptococcus, the cAMP factor (cfb) gene region of group B streptococcus and the pneumolysin (ply) gene region of *streptococcus pneumoniae*. We evaluated the analytical sensitivity, specificity, reproducibility, and reportable range of bacterial DNA load using the mrt-PCR assay. Also we established streptococcus national standards through collaborative studies with Korea Food & Drug Administation (KDFA). We have performed stability testing of certified national standards that consist of working standard (WS) and positive control (PC), since 2012.

Result: This assay showed a greatest linearity within a range from 5 x 10^2 copies/mL to 1 x 10^{10} copies/mL. The limit of detection (LOD) was 250, 350, 600 copies/mL and the percent coefficient of variation (%CV) value in the intra- and inter analysis was 0.29/0.88, 0.35/1.51, and 0.46/2.2 or group A, B streptococcus and *streptococcus pneumoniae* respectively. None of various bacteria showed a cross reactivity with three types of streptococcus. We confirmed the stability of streptococcus WS and PC, respectively.

Conclusions: The multiplex real-time PCR assay showed good analytical sensitivity, specificity and high reliability with a broad range. This assay is a powerful tool for the rapid and cost-effective diagnosis of Streptococcus.

B-207

The Shortening of Leukocyte Telomere Length in Type 2 Diabetes Mellitus with LINE1 Gene DNA Hypermethylation

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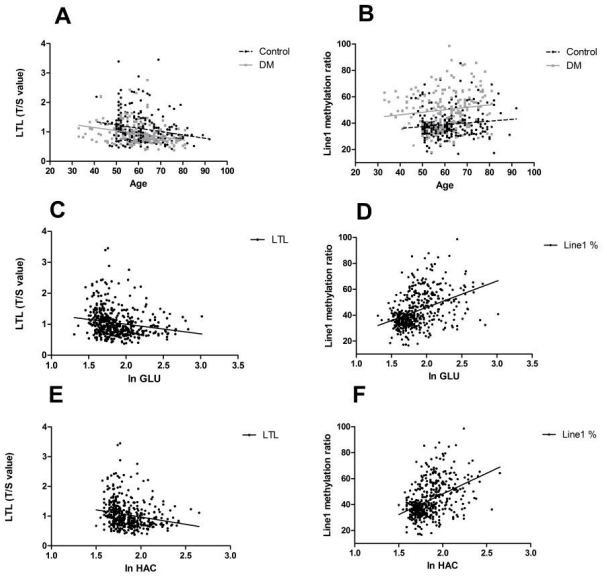
Background: Short telomeres have been linked to age-related diseases, including diabetes, but their results are inconsistent, DNA methylation has been reported with the development of type 2 diabetes mellitus (T2DM) and the shortening of telomere length, respectively. This study aims to investigate the cross-talking of leukocyte telomere length (LTL) and DNA methylation in T2DM.

Methods: We performed a hospital-based case-control study of 278 cases of T2DM patients defined as glycated hemoglobin (HbA1c) ≥6.5 %, or fasting glucose ≥126 mg/dl (7 mmol/l) and 184 of healthy normal controls visiting the hospital for a health examination, with sex and age matched, from the Peking Union Medical College Hospital, China. Circulating leukocytes were collected for LTL determination and DNA methylation of LINE1 measured by a quantitative PCR method. Biochemical variables, including serum glucose (Glu), HbA1c, homocysteine, hs-CRP, lipids-related indexs, systolic and diastolic blood pressure and body mass index (BMI) were collected. All analyses involved the use of SPSS 16.0. A two-sided P<0.05 indicated statistical significance. **Results:** LTL was significantly shortened in T2DM comparing with controls (0.93±0.38 vs. 1.11±0.48, P<0.001) and decreased steadily with age in both controls and T2DM. Reversely, we found significant increase of LINE1 DNA methylation in T2DM compared with controls (50.21±14.50 vs. 38.81±9.95, P<0.001). Spearman correlation showed LTL negatively correlated with LINE1 methylation (r=-0.280, P<0.001), ln Glu (r=-0.191, P<0.001), ln HbA1c (r=-0.196, P<0.001) adjusted for sex and age. For the multivariate linear regression analysis, we applied a principal component assay to avoiding multicollinearity of Glu and HbA1c, and found Glu, HbA1c and LINE1 methylation were stably negative related with LTL.**Conclusion:** Shortening of LTL is independently associated with the risk of incident T2DM, and closely related with the DNA methylation of LINE1. These findings provide novel insights into an epigenetic mechanism for shortened LTL in DM.



B-208

Genetic diagnosis of Familial Hypercholesterolaemia using a rapid biochip array assay for 40 common LDLR, ApoB and PCSK9 mutations.

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Background Familial Hypercholesterolaemia leads to a lifelong increase in plasma LDL levels with subsequent increase in premature vascular disease. Early diagnosis and treatment is the key to effective management of this condition. This research aims to produce a simple and cost effective genetic test which could identify the majority (71%) of mutations causing FH in the UK and Ireland.

Methods Biochip array technology was used to detect 40 point mutations in LDLR, ApoB and PCSK9 genes, over two 5X5 arrays. This technology uses multiplex allele specific PCR and biochip array hybridisation, followed by a chemiluminescence detection system and software for automated mutation calling.

Results The FH biochip array assay was validated in the Belfast Genetics Laboratory using 199 cascade screening samples previously sequenced for known FH causing family mutations, the overall sensitivity was 99%. The assay was then used for routine testing of 663 patients with possible FH, from clinics across the UK and Ireland. A total of 49(7.4%) mutation positive individuals were identified, however for the clinics in England the detection rate was 12.9%. Further analysis of 120 biochip negative patients, using DNA sequencing, did not identify any false negatives.

Conclusions The FH biochip array provides a rapid and reliable genetic test for the majority of FH causing point mutations in the UK and Ireland. A total of 32 samples can be run in 3 hours. This allows clinics to evaluate additional patients for a possible diagnosis of FH such as patients with high LDL, patients with early onset coronary disease and patients with relatives known to have FH.

B-210

Validation of a new panel for sexually transmitted infections detection at richet laboratory - Rio de Janeiro, Brazil

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Background: Sexually transmitted infections (STIs) are a public health problem, and their prevalence is rising even in developed nations. There are over 30 pathogens considered responsible for STIs; however, only eight of them are clearly related to the prevalence in the majority of clinically relevant cases. We can mention *Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum, Chlamydia trachomatis, Neisseria gonorrhoeae*, *Herpes simplex* and *Treponema pallidum*. Technological development in laboratory diagnosis has been relevant, allowing the direct detection of pathogens in clinical samples by molecular techniques [1]. Within the diagnostic technologies by amplification of genetic material, the CLART® STI's A&B test panel offers the solution to unify the analysis techniques in addition being very useful in the diagnosis of those microorganisms that are difficult to cultivate. The aim of this study was to validate to implement the STIs panel evaluating other sample types not yet validated by the manufacturer. Also to demonstrate the relevance in detecting unexpected laboratory findings.

Methods:149 samples received at the laboratory were used for this study.

41 of these samples were from urine, 25 general swabs, 5 semen, and 78 of liquid cytology in Surepath® BD (medium not recommended by the manufacturer).

The CLART® STIs A&B (CLART® Technology from Genomica S.A.U) was used for the detection and typing of bacteria, fungi and parasites causing STIs and the results were compared with those obtained previously using the techniques ABBOTT® CT/NG (Chlamydia trachomatis, Neisseria gonorrhoeae) and TIB MOLBIOL® - distributed by ROCHE® - (Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum).

Results: On liquid cytology samples, the concordance of results was 100%, 83% in urine, and 55% in swab. Despite of the swab case (55%), it is important to consider the collection area, as the recommended by the manufacturer is the urethral swab. Eleven tested samples from the liquid cytology were ordered to HPV test; therefore, they had no previous result for any of the microorganisms involved in STD's. However, after being tested, the positivity of these samples 27% for others microorganisms associated with STIs (*Neisseria gonorrhoeae*, *Mycpoplasma hominis* and *Candida albicans* were the most frequently found). In the case of semen, the results were not conclusive. It is necessary to increase the number of samples for better evaluation.

Conclusion: The panel showed to be faster than conventional methods and can get a reduction in overall time of diagnosis, more than 24 hours in many cases, allowing the physician to make adjustments in medication and / or therapies administered to each patient. The CLART® STIs kit has presented good results in liquid cytology and urine samples even not being the liquid cytology the specimen indicated by the manufacturer. For extraction

methodologies, all automated platforms from Qiagen, Abbott and Biomerieux showed satisfactory results.

B-211

Evaluation of the Invader Genotyping and TaqMan Copy Number Variation Assays for *CYP2D6*

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Background: Cytochrome P450 2D6 is involved in phase I metabolism of many different classes of commonly used drugs with four broad clinical CYP2D6 phenotypes that are inferred from genotypes: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultrarapid metabolizer (UM). Accurate CYP2D6 genotyping is challenging due to the highly polymorphic nature of the gene and the presence of the pseudogenes. The aim of this study was to validate the Invader® (Hologic, Inc., Bedford, MA, USA) CYP2D6 genotyping assay and the TaqMan™ (Life Technologies, Carlsbad, CA) copy number variation (CNV) assay.

Methods: Fifty de-identified patient samples and twenty-nine Coriell DNA were assessed. After multiplexed PCR the Invader® genotyping assay detects twenty-seven variants on three non-overlapping fragments. Genotypes were determined based on the fluorescence signal ratios of the wildtype and mutant alleles. For CYP2D6 copy number analysis, three distinct TaqMan™ real-time PCR assays targeted different regions of the gene. The copy number was determined using the CopyCaller™ Software by comparing the Crossing point (Ct) of the calibrator gene RNase P (two copies). Discrimination of the duplicate alleles was done by long-range PCR followed by the Invader® genotyping or single nucleotide extension (SNE). Invader® results were compared to INFINITI™ system (Autogenomics, Inc., Carlsbad, CA) and Sanger sequencing.

Results: Genotypes and copy number determined by the Invader® and TaqMan™ assays for the twenty-nine Coriell DNA samples were 100% concordant with previously published results except for one sample for copy number analysis. This sample was omitted from the copy number validation because of a low CopyCaller™ confidence score. The Invader® genotyping for fifty patient DNA were 100% concordant with the INFINITI™ system except for two samples, which was due to the lack of the *45 allele in the INFINITI™ panel. The presence of the allele *45 was confirmed by Sanger sequencing. Copy number analysis on the patient DNA were 100% concordant with the results obtained using the long-range PCR. Invader® inter-run and intra-run and TaqMan™ inter-run precision studies showed 100% reproducibility. Analytical sensitivity was determined to be 10 ng of DNA for the Invader® assay and 2 ng of DNA for the TaqMan™ assay, respectively. For determining the duplicate allele, seventeen DNA samples with three copies of CYP2D6 were compared to results with long-range PCR with SNE analysis. Sixteen samples showed the same results with the exception of one sample, determined to be *29/*43X2 by the Invader® test and *1/*1X2 by the comparative method, which did not test for *43. The inter-run precision of the duplicate allele discrimination assay showed 100% reproducibility.

Conclusion: Evaluation of CYP2D6 genotyping using the Invader® and TaqMan™ CNV assays shows that the results are valid, robust and highly reliable, which makes these assays particularly relevant for the implementation in the clinical practice.

B-213

Dengue Outbreak in São Paulo-Brazil (2015): Use Polymerase Chain Reaction for Detection and Typing of the Ethiological Agent

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Background: Dengue viruses (DENV) are widespread in Brazil and have been implicated with several outbreaks around the country. An important increase in the number of cases have been reported last year, particularly during summer and early autumn at São Paulo city, Brazil and an emergency unit for initial care and diagnosis was set up in the North region of São Paulo City. The discovery of Chikungunya (CHIKV) and Zika (ZIKV) viruses in Brazil reinforced the need for rapid and reliable assay to detect the presence of these infections among the patients. The objective of this study is detect and characterize DENV using real-time 3M Integrated Cycler PCR instrument in samples collected during an epidemic in São Paulo, Brazil. **Methods:** We analyzed 1,461 samples from suspected cases of DENV or other arboviruses infections attended at the the Basic Health Unit (BHU) Jardim Vista Alegre, North region of São Paulo City, Brazil during the epidemic period of 2015 were assayed at the Special Techniques Laboratory, Hospital Israelita Albert Einstein (HIAE), São Paulo, Brazil. For the detection of anti-dengue antibodies of types 1-4, immunoassay Dengue Virus IgM Capture Dx Select ™ (Focus Diagnostics Cypress, California USA) was utilized. Simplexa Dengue test using the 3M Integrated Cycler real-time PCR instrument (Focus Diagnostics Cypress, California USA) was applied for the qualitative detection and discrimination of DENV1-4 serotypes. An internal control RNA was used to monitor the efficiency of the extraction process and to detect the inhibition of RT-PCR. In addition, some of the samples with negative results for DENV were also analyzed using the Light Mix Chikungunya Kit - Virus (TIB MOLBIOL- Berlin, Germany) in Cobas z480 equipment (Roche Diagnostics, USA). **Results:** Of the 1,461 samples studied, 1.048 (71.73%) were positive and 413 (28.27%) negative for the 3M Integrated Cycler, whereas the ELISA test detected 312 (21.35%) and 207 (14.16%) samples for IgM and IgG, respectively. Indeterminate serology results appeared in 129 (8.82%) and 46 (3.15%) samples for IgM and IgG, respectively. DENV1 was the predominant strain, found in 99.7% (1.059 / 1.062) DENV positive samples, followed by 0.2% (2 / 1,062) DENV-4 and 0.1% (1 / 1,062) DENV-1/2 coinfection. DENV-3 was not detected in the study population, as well as CHIKV, that was not detected in 409 analyzed samples for this virus. **Conclusion:** As expected, the search for DENV using PCR allowed earlier detection of this infection when compared to IgG/IgM antibodies assays. The use of PCR is an alternative to NS1 test to detect the viral infection in the first days of infection. Furthermore, PCR

is the sole technique that allows distinguishing the different types of DENV. CHIKV was not found in the samples analyzed.

B-214

Prevalence of CARD15 among Crohn's disease patients and its relation to disease severity

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Crohn's disease is a chronic, episodic, inflammatory condition of the gastrointestinal tract. It causes complications outside the gastrointestinal tract. The exact cause of Crohn's disease is unknown; with genetic and environmental implications.The aim of this work was to study the prevalence of CARD15/NOD2 gene variants among Crohn's disease patients and correlate the genotypes with the extent and severity of the disease. **Subjects and Methods**: This study included100 patients and 100 age matched healthy controls. All subjects undergone immunological investigations, ASCA IgA and IgG and p ANCA by ELISA immunoassay, **The Crohn's disease endoscopy index of severity (CDEIS**) was calculated as a subscore for each segment explored, including the presence of deep ulcers, superficial ulcers, the estimated surface area of affected mucosa and ulcerated mucosa. **CDAIndex:** where Inactive (< 150), Mild activity (150-245), Moderate activity (>245) Severe (>450 Detection of CARD15/NOD2 polymorphisms was done after DNA extraction followed by PCR amplification of the targeted area then digestion of the amplified sequence (PCR-RFLP) by the appropriate restriction enzyme (**R702W, G908R and 1007fs). Results**: The CARD15 was positive among 17% of Crohn's disease patients while it was positive among 20% in control group. The distribution of CARD15variants in Crohn's disease patient group was as follows: **1007 FS** variant was positive in 6% of patients, **R702W** was positive in 7% of patients and **G908 R** was positive in 4% of patients. While in the control group; **1007 FS** variant was positive in10% of patients, **R702W** variant was positive in 5% of patients **G908R** variant was positive in 5% of patients. It was found that 19.0% of patients was inactive (<150) ,64.0% showed mild activity (150-245) 17.0% of cases showed moderate activity (>245) . The percentage of CARD15 gene was higher in ileal location of Crohn's disease and higher in inflammatory behavior, but didn't reach statistical significance. There was positive correlation between CARD15 gene, CDAI and ASCA, IgA, IgG and ANCA respectively.CARD15 status was higher with mild activity of CDAI, but didn't reach statistical significance. The present study showed the percentage of positive CARD15 gene in ASCA positive (17.6%) while in ANCA positive (0.0%). Seromarkers showed positive ASCA in 18% while negative ASCA was in 82%. Positive ANCA was found in 2% while Negative ANCA was found in 98.0%. Positive CARD15 was 17.6% with ASCA Positive, while Positive CARD15 was 82.4% with ASCA Negative. There were no statistical significant differences between CARD15 gene and ASCA and ANCA respectively **Conclusions.** The presence of CARD15 (R702W, G908R and L1007fs) mutations in unaffected persons indicates additional genes or environmental factors are necessary for CD development Thus CARD15 mutations were not sole factor implicated in susceptibility to Crohn's disease. However it could help in prioritization of genetic mechanisms that contribute to the expression of IBD. The identification of key genes serves to prioritize development of new therapeutic approaches in IBD as well as help in the development of predictive risk models

Key words: CARD15, CD, CDAI, PCR-RFLP.

B-215

Validation of a qualitative PCR for Zika virus in a private hospital in Sao Paulo, Brazil

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Background: Zika virus (ZKV) is an RNA virus from the genus *Flavivirus* which causes a tropical disease that is transmitted to humans by infected *Aedes* mosquitoes, the same vector of dengue (DENV) and Chikungunya (CHIKV) viruses. In 2015, the first autochthonous ZKV infection was confirmed in Brazil and there was a dramatic increase in reports of ZIKV infection. Although the disease is self-limiting, the Ministry of Health of Brazil recent reports an unusual increase of cases of microcephaly among newborns in the Northeast region, which indicates a possible association between ZIKV infection in pregnancy and fetal malformations. Guillain Barré syndrome cases have also been related to ZIKV infection. Clinical diagnosis is difficult due to the similar symptoms with DENV and CHIKV, so accurate diagnosis is crucial for patient management and public health interventions. Laboratory diagnosis can be accomplished by testing samples to detect viral RNA and anti- Zika IgM/IgG antibodies. However, only molecular test can identify ZKV in the first days after infection. In order to offer a high quality molecular ZKV diagnostic test in our service, we validated a laboratory developed test for this purpose.

Methods: Nucleic acids from plasma and urine samples were extracted using QIAamp Viral RNA Mini Kit (Qiagen) followed by a real time PCR assay using SuperScript® III Platinum® One-Step qRT-PCR Kit (Invitrogen™). According to CAP guidelines, we evaluated accuracy, reproducibility, analytical sensitivity and analytical specificity of the test.

Results: For accuracy, we compared the results of 30 samples and obtained 100% of correlation. Analytical sensitivity of the method was evaluated by testing dilutions of a sample with known copy number and was established in 800 copies/mL. Reproducibility was check using two negatives and two positive´s samples, tested in three different days by different persons. The results were concordant. For the specificity, we tested 10 samples positives for DENV type 1 and one sample positive for CHIKV, and all of them were negative for ZKV.

Conclusion: The results of validation demonstrated that the test is reliable and useful for detection of ZKV 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 ZKV infections in the clinical laboratory. Molecular methods are sensitive tools for the diagnosis of this viral infection in the early viremic stages of illness. Since ZKV, CHIKV and DENV infections produce similar clinical signs, it is important for physicians to differentiate them rapidly, particularly in pregnant women's.

B-216

Validation of *CFTR* complete gene sequencing, a suitable test for highly heterogeneous Brazilian population

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Background: Cystic fibrosis (CF) is a genetic disorder caused by deleterious variants in *CFTR* gene. Although p.F508del is the most common CF-causing variant, over 2000 variants have been described. The frequencies of specific variants oscillate widely across populations, and are sometimes shaped by founder effects. Generally, the first screening in *CFTR* is for p.F508del. Beyond this variant, a number of screening panels have been proposed, such as the American College of Medical Genetics (ACMG) panel composed of 23 variants. Due to the heterogeneous composition of most populations, screening panels often bypass low frequency pathogenic variants segregating in specific populations. That implies that some locally relevant deleterious variants might be missed, causing misdiagnosis, inefficient carrier screening and equivocal genetic counseling. For this reason, complete gene sequencing is the most efficient methodology for *CFTR* genetic testing, especially useful for highly heterogeneous and understudied populations. Our aim in this study was to validate the complete *CFTR* gene sequencing in a population from São Paulo, Brazil, by Next Generation Sequencing (NGS), a high-throughput, reliable and progressively accessible technology.

Methodology: DNA extracted from whole-blood samples of twenty-one patients with previously known variants for *CFTR* gene was used. Sixteen samples were positive for pathogenic variants. Of those, fourteen samples had a pathogenic or probably pathogenic variant in both alleles. Five samples were negative either for pathogenic, probably pathogenic or variants of uncertain significance (VUS). *CFTR* gene library was built using the Ion AmpliSeq *CFTR* panel, which includes exons, intron-exon boundaries and UTRs. Amplicons were fully sequenced in Ion Torrent PGM Sequencer. Pathogenic variants and regions with coverage below 50X were verified using Sanger sequencing. Validation followed CAP guidelines, evaluating accuracy, reproducibility, specificity and sensitivity of the test. To test for reproducibility, replicates were done: 2 positive samples were sequenced in triplicate, and 3 samples (2 positive and 1 negative) were sequenced in duplicate. The reproducibility tests were performed in different routines by different analysts. Read mapping and variant calling were performed in two softwares each, with the following combination: Torrent Mapping Alignment Program (TMAP) + Torrent Variant Caller (TVC) vs. 4.2 and Burrows-Wheeler Aligner (BWA) + Atlas2 vs. 1.4.3. Final variants were classified following the ACMG guidelines for interpretation of sequence variation.

Results: All samples were successfully sequenced for the entire gene. Variants were entirely concordant with previous results, resulting in 100% accuracy. In total, seventeen different pathogenic or probably pathogenic variants were detected: 7 indels (5 frameshift, 2 inframe), 10 substitutions (3 missense, 5 nonsense, and 2 splice-site), indicating 100% sensitivity and specificity. Variant p.F508del was detected in 8

patients (2 homozygotes). One VUS missense substitution was also detected. Results of replicates showed successful reproducibility.

Conclusion: We validated the complete sequencing of *CFTR* gene in a NGS platform, which resulted in a reliable genetic test for CF molecular screening. Based on our results we are confident that this genetic test can be securely offered to the highly admixtured Brazilian population.

B-218

Molecular tension probes to investigate the mechanopharmacology of smooth muscle cells

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Background: The contractile phenotype of airway smooth muscle cells is an established characteristic of asthma, a chronic lung disease characterized by airflow obstruction. Airway smooth muscle (ASM) cells generate cellular contractile forces that lead to bronchoconstriction and airflow obstruction. Bronchodilators are used to relax the airway smooth muscle cells to relieve the obstruction and increase airflow. Therefore, an assay measuring cellular forces can potentially screen bronchodilators for relative efficacy. To examine this, we used fluorescent molecular tension sensors to determine the EC50 values of albuterol in normal and asthmatic ASM cells and demonstrate that molecular tension probes are a potential diagnostic tool for personalized medicine. In this study, we measured integrin-mediated forces in ASM cells using molecular tension sensors to study the effects of chronic nicotine exposure in asthma by determining the EC50 value of albuterol in normal and asthmatic ASMs exposed to nicotine. **Methods:** Diseased and healthy human airway smooth muscle cells isolated from asthmatic or healthy donors were used in this study. Titin-based gold nanoparticle sensor surfaces were fabricated as following: I27 constructs were recombinantly expressed where the I27 domain of titin, an immunoglobulin domain found in the sarcomere, was flanked by a linear RGD peptide, a canonic motif found in fibronectin, at the N-terminal for cellular adhesion and two cysteines at the C-terminal for the attachment onto 9 nm gold nanoparticles. The gold nanoparticles were immobilized onto a glass surface through silane - NHS lipoic acid chemistry. In addition, the N-terminal of the I27 domain was decorated with a fluorescent dye that is quenched when in close proximity to the gold nanoparticle. Smooth muscle cells were added to the sensor surface where the integrin cellular receptors recognize the adhesion peptide and stretch the sensor, thus generating fluorescence signal at adhesion sites. Upon addition of the bronchodilator albuterol, we obtained a fluorescent dose response curve from which the EC50 values were determined. **Results:** The EC50 value for normal ASM cells incubated on the titin-based sensor and treated with the albuterol was 9 +/- 2 nM. The addition of nerve growth factor (NGF), a neurotrophin implicated in asthma pathobiology, shifted the EC50 to 45 +/- 11 nM. In comparison, EC50 of asthmatic smooth muscle cells without NGF was 21 +/- 4 nM and was shifted in the presence of NGF to 140 +/- 41 nM. Chronic nicotine treatment of asthmatic smooth muscle cells resulted in the EC50 value of 198 +/- 38 nM. **Conclusion:** We obtained EC50 values of albuterol for cells plated on molecular tension sensors as a readout of the contractile phenotype of asthmatic and normal airway smooth muscle cells. When comparing normal and asthmatic smooth muscle cells, the latter showed higher EC50 values both in the absence and in the presence of NGF. However, chronic nicotine treatment of asthmatic cells resulted in the highest EC50 value, which showed that nicotine changes the contractile phenotype of smooth muscle cells *in vitro*.

B-219

Expression of Matrix Metalloproteinases (MMPs) MMP-3 and MMP-8 Before and After Treatment with Etoricoxib in Patients with Chronic Periodontal Disease

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Introduction: In inflammatory periodontal pathology, presence of bacteria in subgingival level causes the host cells with the inflammatory response, resulting in the synthesis of MMPs, degradation of extracellular matrix and destruction of periodontal tissue and bone.

Aim: This study sought to highlight the expression of matrix metalloproteinases MMP - 8 and MMP - 9 in patients with chronic periodontal disease before and after treatment with etoricoxib.

Material and methods: we included in our study 23 patients with chronic periodontal disease and 11 healthy patients (control group), aged between 35 and 65 years, 16 female and 18 male, followed-up as outpatients at dental office *Dentissimo Dental Care* Timisoara, Romania.

The study was conducted during September 2014 - January 2015. Patients were administered Etoricoxib in the pharmacological dose of 90 mg, for 6 months, 7 days/month.

Biological material was collected by gingival curettage. RNA was extracted from biological material that has been amplified by RT-PCR. Gene expression of interest was highlighted by agarose gel migration.

Results: The analysis of gene expression before treatment revealed overexpression of MMP-3 in 19 (82.6%) patients and overexpression of MMP- 8 in 21 (91.3 %) of patients included in our study.

Following treatment with etoricoxib, we noted decrease of studied gene expression in two patients from the experimental group.

Conclusions: The results obtained showed the biological effects of this drug in the pathology of periodontal disease; two matrix metalloproteinases can be used in the therapeutic management of this condition.

Wednesday, August 3, 2016

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

Pediatric/Fetal Clinical Chemistry

B-220

Evaluation of GDF-15 and YKL-40 as Early Markers of Subclinical Diabetic Nephropathy and Cardiovascular Morbidity in Young Patients with Type 1 Diabetes Mellitus

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Background: Diabetic nephropathy constitutes a major long-term complication in patients with type 1 diabetes mellitus (T1DM) and its diagnosis is based on microalbuminuria. Growth Differentiation Factor-15 (GDF-15) is a protein belonging to the transforming growth factor beta superfamily that has a role in regulating inflammatory and apoptotic pathways in injured tissues and during disease processes. Chitinase-3-Like Protein 1 (YKL-40) is a protein with the ability to communicate with other signal transduction pathways to modulate various physiologic processes, such as inflammation, apoptosis, tissue remodeling, cell growth, and angiogenesis. An increasing body of evidence exists supporting the involvement of these two proteins GDF-15 and YKL-40 in cardio-renal events, therefore we aimed to investigate in an observational follow-up study their role in unravelling early diabetic nephropathy and their impact as potential risk markers for cardiovascular morbidity. **Patients and Methods**: Fifty-six patients with T1DM, aged 13.1±3.2 years and 49 healthy controls aged 12.8±6.6 years were recruited. Along with standard blood and urine chemistry, measurements of serum Neutrophil Gelatinase Associated Lipocalin (NGAL), Cystatin C, YKL-40 and GDF-15 were performed by means of immunoenzymatic and immunonephelometric techniques. eGFR values were calculated from Cystatin C based e-GFR equations[1]. The measurements were performed at enrolment and after 12-15 months. **Results**: At baseline, mean GDF-15 levels were not significantly different between children with diabetes (289.5pg/mL) and controls (278.6pg/ml). At re-evaluation, mean GDF-15 in patients increased (366.7pg/mL), ($p=0.001$) and was significantly higher than in controls ($p<0.001$). GDF-15 levels correlated negatively with eGFR values ($r=-0.27$, $p=0.04$, $n=56$) and positively with both total Cholesterol ($r=0.29$, $p=0.033$, $n=54$) and LDL-Cholesterol ($r=0.35$, $p=0.009$, $n=54$) at re-evaluation. Mean YKL-40 level in T1DM patients increased from baseline (17.4ng/mL) to re-evaluation (20.5ng/mL), ($p<0.001$), while no significant difference was observed between patients with T1DM and controls ($p>0.19$) at baseline. YKL-40 levels correlated positively with NGAL, GDF-15, total Cholesterol and Triglycerides concentrations at both time-points of evaluation ($r= 0.35$, $p=0.007$; $r=0.55$, $p<0.0001$; $r=0.29$, $p=0.04$; $r=0.46$, $p<0.001$, respectively and $r= 0.31$, $p=0.02$; $r=0.36$, $p=0.006$; $r=0.44$, $p<0.001$; $r=0.47$, $p<0.001$, respectively). A positive correlation was also found between YKL-40 levels and Systolic Arterial Pressure (SAP) values at all evaluation points ($r=0.23$, $p=0.02$). **Conclusions**: To our knowledge, this is the first study to demonstrate a predictive role for serum GDF-15 and YKL-40 as early markers of diabetic nephropathy in children and adolescents with T1DM before severe overt nephropathy occurs. In addition, the associations of these biomarkers with SAP and hyperlipidemia reflect their possible prognostic role on cardiovascular morbidity suggesting their measurement besides microalbuminuria to unravel early renal dysfunction. Defining new predictors as supplementary tests to urinary albumin excretion for the early diagnosis of diabetic nephropathy and cardiovascular morbidity would accelerate effective management and treatment approaches needed to minimize the rates of severe cardio-renal morbidity and mortality in young patients with T1DM. These data should be confirmed by further large-scale longitudinal studies before being integrated in the diabetic nephropathy risk assessment of young patients with T1DM. *[1]Clinical Chemistry, 60:974-986, 2014.*

B-221

Circulating Dickkopf-1 Protein Levels in Normal-Weight and Obese Children: Evidence of Involvement of Canonical Wnt Signaling

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Background: Dickkopf-1 protein (DKK-1) is the founding member of a multigene family of which DKK-1, DKK-2 and DKK-4 are secreted proteins that bind to the extracellular domains of the Low-Density-Lipoprotein (LDL) receptor-related proteins 5 and 6 (LRP5/LRP6) and inhibit Wnt/β-catenin signaling, making DKK-1 a drug target for multiple diseases. DKK-1 was originally identified in *Xenopus* as a molecule that can induce head structures, and this is the basis for the name. Secretion of Wnt proteins by adipose cells plays an important role in the control of adipogenesis. The Wnt antagonist, DKK-1, is secreted by human pre-adipocytes and influences adipocyte maturation and growth. DKK-1 mRNA increases six hours after onset of human adipogenesis and this is followed by an increase in DKK-1 protein. With further differentiation, mRNA and protein levels of DKK-1 progressively decline to undetectable in mature adipocytes. The transient induction of DKK-1 correlates with down-regulation of cytoplasmic and nuclear beta-catenin levels, representing a surrogate marker of canonical Wnt signaling and Wnt/beta-catenin transcriptional activity. Of note, DKK-1 protein has been implicated also in bone remodeling pathways. **Patients and Methods**: In this study we measured the circulating DKK-1 levels in 16 normal-weight and 25 obese girls using immunoenzymatic techniques and we investigated possible correlations of DKK-1 levels with parameters of anthropometric evaluation; insulin resistance; adipose tissue secretory molecules {adiponectin, leptin, retinol binding protein-4 (RBP-4) and lipocalin-2}; bone remodeling biomarkers, (osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL), osteocalcin, C-terminal cross-linking telopeptide of collagen type-I (CTX), bone alkaline phosphatase (bALP) and tartrate-resistant acid phosphatase isoform-5b (bone TRACP-5b) and a low grade inflammation marker (hs-CRP). **Results**: We found that: a) DKK-1 levels were significantly higher in normal-weight girls than obese girls 37.5±18.0 vs. 18.6±2.4 pg/mL, $p=0.009$, b) BMI and HOMA index values correlated negatively with DKK-1 levels ($r=-0.508$, $p<0.001$ and $r=-0.380$, $p<0.01$, respectively), c) logDKK-1 values correlated significantly only with adiponectin levels ($r=-0.404$, $p=0.008$), d) DKK-1 and RANKL levels correlated positively with each other, ($r=0.492$, $p<0.001$) and e) hs-CRP and DKK-1 levels correlated, negatively with each other ($r=-0.371$, $p=0.01$). **Conclusions**: These preliminary findings suggest that indices of metabolic syndrome such as obesity, insulin resistance, and low grade inflammation markers are negatively associated with circulating DKK-1 protein levels in children. Obesity is characterized by inappropriate expansion of adipose cells (hypertrophic obesity) and probably by a disrupted ability to recruit and differentiate new precursor cells (pre-adipocytes). Thus, the impairment of adipogenesis observed in obesity could be attributed to disrupted suppression of canonical Wnt signaling via DKK-1. Moreover, the lower DKK-1 levels found in obesity may also explain the connection of obesity with better bone accrual.

B-222

Serum bile acid profile in neonates with cholestasis caused by citrin deficiency

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Infants with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) are characterized by conjugated hyperbilirubinemia and markedly high levels of serum bile acids. However, the mechanisms remain unclear. This study aimed to establish a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantification of serum bile acids and compared the compositions in patients with NICCD and infants having citrullinemia but no mutations found in *SLC25A13* gene (non-NICCD). Separation of bile acids were carried out on a 2.1 mm x 150 mm Symmetry C18 column with 3.5 μm particle size using an Agilent 1200 HPLC system. Mobile phase consisted of 5 mM ammonium acetate in H_2O (87.5%), 0.1% NH_4OH (12.5%) and methanol at a flow rate of 0.2 mL/min. An API 5000 tandem mass spectrometer was used for the detection with electrospray ionization source and negative ionization mode. The established LC-MS/MS method measured 15 serum bile acids within 14 minutes and has a liner range of 0.01~1 μM for glycolithocholic acid, taurolithocholic acid and lithocholic acid, 0.1~10 μM for taurocholic acid,

glycochenodeoxycholic acid and taurochenodeoxycholic acid, and 0.05~5 μM for the others (r>0.99). The within-run and run-to-run imprecision (CV) of all bile acids was 1.2~10.9% and 3.1~10.8%, respectively, with the mean recovery of 90.5~112.6%. Compared to non-NICCD, NICCD infants had significantly elevated serum total bile acids (158.5 vs. 31.2 μM, p<0.01), glycocholic acid (13.8 vs. 0.24 μM, p<0.05), taurocholic acid (32.9 vs. 8.3 μM, p<0.001), and taurochenodeoxycholic acid (69.3 vs. 0.3 μM, p<0.01). And the resultant ratios increased in NICCD infants, including primary/secondary bile acids (516 vs. 159, p<0.05), taurine/glycine-conjugated bile acids (2.1 vs. 0.6, p<0.01), and conjugated/free bile acids (326 vs. 70, p<0.05). In summary, we established LC-MS/MS method for serum bile acid profile analysis, and found a distinct bile acid profile in NICCD patients.

B-223

Usefulness of procalcitonin to predict serious bacterial infection in febrile pediatric patients

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Background: Fever is a common symptom in pediatric patients visiting emergency department. Many of them have non-bacterial causes of fever, but some febrile infants and children would have occult serious bacterial infection (SBI) such as bacteremia, bacterial urinary tract infection(UTI), lobar pneumonia, bacterial meningitis, bacterial gastroenteritis and so on. To avoid possible complication, it is important to recognize SBI as early as possible. Various tests are are used in the laboratory evaluation of patients. However, it is still difficult to predict the presence of SBIs with complete certainty. Procalcitonin (PCT) is known as one of the acute phase reactants for identifying invasive bacterial infection. The objectives of this study is to compare the performance of serum PCT with other traditional screening tests such as C-reactive protein(CRP) and absolute neutrophil count(ANC) for detecting SBI in febrile pediatric patients.

Methods: From November 2014 to July 2015, febrile 212 infants and children younger than 7 years old, who visited emergency department, were studied for SBI. Blood, urine and/or CSF cultures were performed in most of patients. Chest radiograph were done in most of patients. Serum PCT levels were compared with CRP levels and ANC between febrile patients with SBI and without SBI.

Results: The overall prevalence of SBI was 6.1 %(13 patients) of 212 febrile infants and children. Of 13 patients with SBI, 4(30.8%) patients had positive blood culture, 7(53.8%) had positive urine culture and 2(15.4%) had pneumonia. Patients with SBI had higher PCT levels (1.3±3.2ng/ml vs 0.4±2.1 ng/ml, p<0.001), higher CRP levels (57.2±114.6 mg/L vs 27.6±92.2mg/L, p=0.005) and higher ANC ($8.22x10^9$ cells±$6.38x10^9$ cells/L vs $5.76x10^9$ cells±$9.15x10^9$ cells/L, p=0.02) than those without SBI. We also assessed the diagnostic properties of the three biomarkers (PCT, CRP and ANC) using receiver operating characteristic (ROC) curve. The area under the curve (AUC) for PCT was largest (0.76, 95% CI=71 to 0.80), followed by CRP (0.74, 95% CI=0.69 to 0.78) and ANC (0.70, 95% CI=065 to 0.75).

Conclusion: PCT had better diagnostic accuracy than traditional screening tests such as CRP and ANC for identifying febrile pediatric patients with SBI. And further study on large cohort is required to definitely determine the benefit of PCT over traditional screening tests for SBI.

B-224

A Multi-Hospital Health System's Experience with Pediatric Reference Ranges

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Establishment of age-specific and sex-specific reference intervals for pediatric population is critical for correct clinical interpretation of the lab results. Laboratories face challenges in establishing pediatric intervals since it is extremely difficult to locate and gather normal pediatric specimens. To overcome this challenge, we used our large database of patient results from our outpatient settings as a "proxy" for normal patients. We selectively looked at multiple pediatric group practices but excluded physician groups that we know would have a high probability of elevated results (e.g., endocrinology, urology, etc.). The vast majority of these outpatient pediatric subjects had routine lab work ordered with mostly normal results and few abnormal results. From this database, we pulled the data from specific sex and age intervals to verify the Canadian Laboratory Initiative for Pediatric Reference (CALIPER) Intervals for analytes such as Calcium, ALP, CO2, Creatinine, FSH, Prolactin, Albumin, Direct HDL and Total Protein measured on the Abbott ARCHITECT platform. We found that the majority of results were normal and verified many of the CALIPER intervals. In most cases, we checked one year of data using EP Evaluator Verification of Reference Interval Module with pass criteria being 10.0% and were able to verify the specific sex and age intervals for these analytes. This study shows how real world data from a five-hospital health system looks when compared to the CALIPER intervals.

We focused on this approach to verification after fielding questions from specific physician's observing what they perceived as a shift of elevated results for specific analytes. Pulling their databases indicated these elevated results. But pulling much larger group practices and excluding specific high disease probability practices showed normal results within the reference range. The elevated results for the questioning physicians were related to their small population size, practice or pre-analytical issues.

When a common reference range is used by multiple instruments at different locations it is essential that analytical quality in relation to the reference range and quality control be robust. We accomplish this using the Sunquest BDUP function (Blind Duplicate). This allows us to check one instrument against the other using established criteria to evaluate the difference in results obtained and graph them on a Levy-Jennings plot. We perform this daily at all sites with two or more instruments and monthly between all sites to help assure that each instrument is turning out similar or correlated patient results.

Other helpful tools are using inter-individual Biological Variation (CV_I) to define an Optimal (0.25 X CV_I), Desirable (0.50 X CV_I), and Minimal (0.75 X CV_I) quality control precision for these assays. In addition, we used Biological Variation and analyte precision to calculate Reference Change Value (RCV). RCV defines the critical difference that if exceeded between two sequential results indicates a significant change in patient condition. Likewise, the Index of Individuality is helpful in determining when to use the reference range (i.e., Index ≥1.0) and when to use RCV (Index ≤1.0).

B-225

Biochemical diagnosis of mitochondrial Respiratory Chain disorders in clinically suspected Egyptian children

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Background and objective: Mitochondrial Respiratory Chain (RC) disorders are a growing group of disorders with a large variety of clinical presentations ranging from well defined clinical syndromes to non specific manifestations as failure to thrive and seizures. This study aimed to describe the clinical, biochemical and histochemical spectrum of 23 Egyptian patients with confirmed mitochondrial RC disorders as the first pilot study for biochemical measurement of RC in Egypt.

Patients and Methods: Twenty three Patients clinically and radiological suspected to have mitochondrial RC disorders were referred to the Inherited Metabolic Disease Unit laboratory, Cairo University Children's hospital. Using muscle biopsy homogenate, histochemical staining of Cytochrome Oxidase and Succinate dehydrogenase and spectrophotometric assay of RC complexes were done.

Results: Eleven patients confirmed to have isolated complex I deficiency (48%), two patients had combined complex I and complex II deficiency (9%), two patients had combined (complex I, II+III, complex IV) deficiencies (9%), one patient had combined complex I & III (4%), two patients had isolated complex II deficiency (9%), one patient had isolated complex IV deficiency (4%), and four patients had normal respiratory chain enzymes activities (17%).

Conclusion: To the best of my knowledge, this is the first study reported on the Egyptian children with the clinical suspicion of mitochondrial disease. The presence of 19 positive cases out of 23 cases confirmed to have RC deficiency points to their high prevalence these disorders among Egyptian paediatric patients. With the advent of next generation sequencing technology, mitoexome and whole exome sequencing represents an appealing approach for elucidation of the molecular basis of these disorders among Egyptian patients.

B-226

Mitochondrial respiratory chain complex activities in high risk pregnancies

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Background:

Mitochondria have a central role in the energy metabolism and provide ATP in most cells. Mitochondrial oxidative phosphorylation is also the key energy source for placental functions and fetal growth. The placenta is a very important multifunctional

transient organ, essential for the healthy development of the fetus. The purpose of this study was to investigate the function of placenta by measuring respiratory chain complex (RCC) activities in high risk pregnancies, in addition to evaluate the correlation between double test risk ratio and RCC activities.

Methods:

The placenta samples were collected from 50 pregnant women following elective cesarean section; 20 normal pregnancies (controls), 6 preeclampsia (PE), 6 intrauterine growth restriction (IUGR), 6 advanced age (over age 35), 6 twins and 6 preterm deliveries were included in the study. Complex I, II-III, IV and citrate synthase (CS) enzyme activities were measured by kinetic spectrophotometric assays. Immulite 2000 and Prisca software was used for estimation of double test risk ratio.

Results:

Complex I activity was 31.43 ± 4.17 U/g protein, Complex II-III activity was 23.55 ± 5.2 U/g protein, Complex IV activity and CS activity were 144.39 ± 21.9 U/g protein and 79.9±8.81U/g protein in normal placenta, respectively. Complex I, II-III and IV activity were significantly lower in the study group than the controls ($p<0.05$). Especially, Complex I and II-III activity were significantly reduced in placenta of preterm deliveries compared to the controls ($p<0.003$). The mean activity values of Complex I in IUGR, PE, twins, and advanced age groups was also lower compared with the controls ($p>0.05$). Similarly, reduced complex II-III activity was observed in PE, twins, IUGR and preterm deliveries than the controls ($p>0.05$). Increased Complex IV activity was observed in 33% of advanced age pregnancies.

Then mitochondrial RCC complex activities were expressed per CS activity for standardization of the assay. Mean values of mitochondrial complex I/CS activity and complex II-III/CS activity was found below in all study groups than controls. Mean values of mitochondrial complex IV activity / CS activity was found below in all study groups than controls, except advance age groups.

Double test was performed for all pregnant women between 10-14th weeks of gestation. Double test risk ratio was above the cut-off limit (1:300) in 43% of the study group; Complex I and Complex II/III activity was reduced in 76% and 30% in this group, respectively.

Conclusion:

Impaired placental mitochondria RCC functions can lead to adverse pregnancy outcomes such as PE, IUGR and preterm delivery. This is the first report that documents a positive association between decreased placental mitochondrial complex activities and high double test risk ratio. Pregnant women with high risk in double test should be monitored carefully in terms of PE, IUGR and preterm delivery.

B-227

Primary Human Cytomegalovirus (HCMV) Infection in Pregnancy: Unrevealing the Metabolic Network

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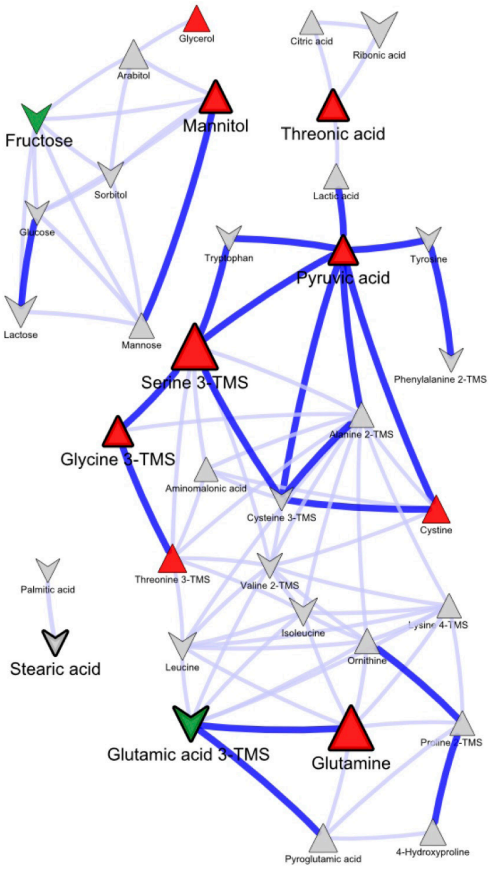
BACKGROUND: Maternal human cytomegalovirus (HCMV) infection during pregnancy represents one of the most frequent risk of birth defects and long-term sequelae. The severity of the infection depends on the clinical features: symptomatic or asymptomatic; with or without complications. **OBJECTIVE:** Our aim was to study the metabolic profile of amniotic fluid (AF) by means of metabolomics networks in HCMV-infected fetuses compared with controls. **METHODS:** we enrolled 20 pregnant women with diagnosis of primary HCMV infection developed in the neonate transplacentally (transmitters), and 23 non-infected subjects undergoing amniocentesis for cytogenetic-based diagnosis. An AF DNA level $>10^5$ copies/mL was considered a reliable predictor for symptomatic congenital infection. AF samples were analyzed by using an Agilent 5975C platform interfaced to the Gas Chromatograph (GC) 7820 equipped with a DB-5ms column. The resulting chromatograms were identified using the database NIST08 (National Institute of Standards and Technology's mass spectral database). 150 target compounds were identified; they were included into a own devoted library. Multivariate statistical analysis was done and a partial least squares discriminant analysis (PLS-DA) model was created, including transmitters and controls. Subsequently, the model was exploited for network mapping by using the software MetaMapR, in order to identify the relationship among the discriminant metabolites. **Results:** As showed in Fig.1, where red triangle means increase; green means decrease; and the size is an index of importance, PLS-DA model showed separation between the two groups (Accuracy=0.88 R2=0.75 Q2=0.58; Cross validation (CV) method: 10-fold CV, Performance measure: Q2 $p<0.01$). Eleven compounds were found responsible for the discrimination. The metabolic network analysis demonstrated that glutamine and glutamate; pyrimidine; purine; alanine-aspartate glutamate; arginine and proline; cysteine and methionine; glycine-serine threonine were the most influenced metabolic pathways in transmitters when compared with controls. **Conclusion:** Metabolomics may be considered an effective strategy in searching of useful information on pregnancy-related pathologies.

Figure 1.



B-228

Optimization of Estradiol Measurements to Improve Utility in the In Vitro Fertilization Setting

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Background: Measurement of estradiol (E2) plays an important role in clinical management for women undergoing ovarian stimulation for *in vitro* fertilization (IVF). However, inconsistent or unreliable estradiol values can pose a challenge in monitoring and management of patients. We performed a comparison study of different immunoassay platforms compared to liquid chromatography-tandem mass spectrometry in an effort to improve IVF success rate for our patient population at the Family Fertility Center.

Methods: Comparison studies were performed using estradiol assays on ADVIA Centaur CP system, Architect i1000 (Abbott) analyzer and AB Sciex 5500 LC/MS/MS system. The first part of the comparison studies were carried out on thirty-seven patient samples (ranging from 124 pg/mL to 4000 pg/mL) using ADVIA Centaur CP analyzer and Architect i1000 analyzer. The second part of the study included analysis of fifteen selected samples on the Tandem Mass Spectrometer (LC/MS/MS). Samples

were analyzed on ADVIA Centaur CP system within 1-2 hours of collection. Then they were aliquoted and stored frozen (-80 ºC) until testing on latter two instruments. Analysis of results for comparison studies was performed using the EP Evaluator Data Innovation (EE 10) program. In addition, ovarian follicle size and number were obtained via transvaginal ultrasound.

Results: Results of comparison studies between ADVIA Centaur CP analyzer and Architect i1000 analyzer showed that Centaur had a significant positive bias of 20%. Comparison studies between Tandem Mass Spectrometer (LC/MS/MS) and ADVIA Centaur CP analyzer also resulted in a significant positive bias of 20% with Centaur. The same fifteen patient samples had negative bias of 0.3% when comparison studies were carried out between Tandem Mass Spectrometer (LC/MS/MS) and Architect i1000 analyzer. Results were then analyzed (between ADVIA Centaur CP analyzer and Architect i1000 analyzer) for low concentrations of estradiol (<1000 pg/mL, n=16) and Centaur resulted in an 18% bias while the bias was much greater at high concentrations of estradiol (1000-2000 pg/mL, n=13) at 26%. In addition, using the Architect i1000 estradiol values corresponded to better prediction of ovarian stimulation based on ultrasound measurement of follicular number and size.

Conclusions: The Architect estradiol assay shows excellent precision and very little bias compared to the gold standard, LC/MS/MS at all ranges of estradiol studies. Therefore results indicate that our fertility center would benefit measurements of estradiol levels using Architect i1000 analyzer for monitoring ovulation stimulation and further clinical management for in vitro fertilization.

B-229

Efficiency of the Lecithin-Sphingomyelin Ratio and Phosphatidylglycerol in Comparison to Lamellar Body Count for Testing Fetal Lung Maturity

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Background: In fetus, immature lungs may lead to respiratory distress syndrome (RDS). Lamellar body count (LBC) is the primary laboratory test to assess the stage of lung maturity in our institution that utilizes the following ranges: <15,000 (immature fetus), 15,000-39,000 (indeterminate) and >39,000 (mature fetus). Lecithin-sphingomyelin ratio (L/S ratio) is used to test the indeterminate results of LBC testing which utilizes ranges: <2.0 (immature), and ≥2.0 (mature). Phosphatidylglycerol (PG), which is determined during the L/S ratio test, is also used to determine maturity based on whether it is present or absent. A PG positive result is indicative of mature lung. LBC is run on an automated hematology analyzer with a quick turn-around time, and is available 24 hours a day. L/S ratio and PG is a labor intensive thin layer chromatography which requires tedious sample preparation and takes ~6 hours to perform. We hypothesized that the L/S ratio and PG testing do not provide significant supplementary information when determining fetal lung maturity in comparison to the LBC testing. **Method:** Amniotic fluid was collected from 92 patients via standard clinical practice. LBC was tested immediately post sample collection. Samples with indeterminate LBC values had L/S ratio and PG performed at time of clinical care. Leftover samples with LBC >39,000 and <15,000 were stored at -70°C for L/S ratio and PG testing. Collection of leftover patient samples and clinical data for this study were approved by the Institutional Review Board. **Results:** 10 of the 92 patients were diagnosed with RDS. LBC and L/S ratio testing were compared based on their ability of predicting RDS. LBC, L/S, and PG all had sensitivity of 20%, 30%, and 40%, respectively. In terms of specificity, LBC had 60% specificity for predicting RDS, while LS Ratio had 89% and PG had 76% specificity. Positive predictive value (PPV) and negative predictive value (NPV) were also calculated. LBC had a PPV of 50% and a NPV of 94%. L/S and PG both had high NPV of 91%, but low PPV. L/S PPV was 25%, while PG PPV was 17%. **Conclusion:** L/S ratio did not improve prediction of RDS. Based on these findings and taking into account the complexity of L/S ratio testing, L/S ratio testing is not recommended in a clinical setting.

B-230

Prognostic value MR- proadrenomedullin appendicitis in pediatric population

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Background: mid-regional proadrenomedullin (MR-proADM) is a precursor of the active peptide adrenomedullin produced by the adrenal and renal tissues under stressing situations

Objective: evaluate the diagnostic and prognostic value of MR-proADM in identifying children with acute appendicitis (AA).

Methods: observational, prospective and analytical study.

170 patients were recruited from November 2013 to April 2014.Children between 3 and 16 years, 56% of males that were admitted in the emergency department with acute abdominal pain and that after initial evaluation were suspicious for AA. Patients excluded from the study (34): appendectomy or recent surgery (3 months prior), immune disease, chronic respiratory or cardiovascular disease, inflammatory bowel disease, patients who had been treated with antibiotics or steroids in the last month.

Demographic data (sex and age), clinical history and analytical data: leukocyte count (µl/mm3), neutrophil count (µl/mm3), c-reactive protein, CRP (mg/dL) were collected. The Pediatric Appendicitis Score, PAS, was also calculated for all patients. The final diagnosis of AA was determined by histologic confirmation.

MR-proADM of all samples was measured in plasma EDTA tubes. Samples were centrifuged in the first two hours after their collection at 3.000 rpm for 15 minutes, the samples were frozen at -80ºC until their analysis. Determinations were made in a BRAHMS MR-proADM KRYPTOR analyzer by means of an immunofluorescent technique using a sandwich method with polyclonal antibodies. Statistical analyses were performed with SPSS 17.0 and MedCalc 11.2.1

Results: Of the 136 children included, 44 were diagnosed with appendicitis, 74 with unspecific abdominal pain, 5 with mesenteric adenitis and 13 with others diagnostics. Mean concentration of MR-proADM for AA, nonspecific abdominal pain and mesenteric adenitis were respectively: 0.52 nmol/L (IC: 95 % 0.46-0.57), 0.37 nmol/L (IC: 95% 0.35-0.40) y 0.50 nmol/L (IC: 95% 0.17-0.85). p<0.001.

The diagnostic accuracy of the different analytical markers studied (leukocyte count, neutrophil count, CRP, MR-proADM and PAS score) was calculated.The areas under the ROC were for MR-proADM of 0.75 (95% CI 0.67-0.82), for CRP 0.72 (95% CI 0.64-0.79), for neutrophil counts 0.86 (95% CI 0.79-0.92), and for leukocyte count 0.88 (95% CI 0.81-0.93).Value for PAS score was 0.87 (95% CI 0.80- 0.92).

The cutoff point for pro-ADM was: 0.34 nmol/L (sensitivity: 93.18 % and specificity: 45.65%) and 0.3 mg/dL for PCR (sensitivity and specificity: 68.18 %, 68.48%). Patients with pro-ADM >0.34 nmol/L, no appendicitis 50 and 41 did it suffered. Patients with pro-ADM ≤0.34 nmol/L, no appendicitis 42 while 3 yes they had. The positive predictive value, PPV for pro-ADM was 45.1% and the negative predictive value, NPV was 93.3%. Considering pro-ADM and PCR together, patients with pro-ADM>0.34 and PCR>0.3 mg/dL did not have appendicitis 22 and 27 finally did it suffered. PPV= 55.1%. Patients with pro-ADM ≤0.34 nmol/L and PCR≤0.3 mg/dL did not have appendicitis 35 while 0 yes they had. VPN=100%.

Conclusion: the combination of CRP≤0.30 mg/dL and MR-proADM≤0.34nmol/L showed a negative predictive value of 100%, with 61% of specificity. This combination could be useful for excluding AA in children admitted to the emergency department.

B-232

Tanner Stage-Stratified Pediatric Reference Intervals for Dihydrotestosterone

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Background: 5α-Dihydrotestosterone (DHT) is the most potent androgen hormone. It is generated in the body through reduction of testosterone by cholestenone 5α-reductase. Clinical utilities of DHT include workup of incompletely virilized males for 5α-reductase deficiency/pseudohermaphroditism, as well as monitoring of androgen action during androgen replacement therapy. A pediatric DHT reference interval study was conducted on 1502 well-characterized subjects, using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Children from 7-17 years old (748 males and 754 females) were recruited via community advertisements. Exclusion criteria included known medical conditions, medication use, or lack of parental consent. Physical exams, including Tanner staging, were completed by a single individual per sex, to reduce subjectivity. DHT values were generated using LC-MS/MS. Non-parametric reference intervals were established for each sex separately, using StatisPro. For both male and female reference intervals, the number of subjects in Tanner Stage 5 partition was less than 120 (and showed no significant difference in DHT with Tanner Stage 4), and was therefore combined with Tanner Stage 4 to allow nonparametric analysis.

Results: Based on Tanner Stage partitioning, the following proposed reference intervals were determined. (Table below)

Conclusion: Tanner Stage-specific DHT reference intervals largely overlap between males and females at Tanner Stage 1. However, at all other stages, the upper reference limit is substantially higher in males than females. Due to the significant differences observed between sexes and Tanner Stages, this study underscores the importance

of having a large number of healthy subjects to establish nonparametric reference intervals, and the developmental differences in DHT concentration.

Tanner Stage-specific DHT reference intervals						
Sex	Tanner Stage	Age	n	95% reference interval (pg/mL)	Lower reference limit 90% CI*	Upper reference limit 90% CI*
Male	1	7 - 12	276	1.0 - 49.4	0.4 - 1.6	40.3 - 94.7
	2	8 - 14	133	3.5 - 397.9	0.9 - 5.5	220.0 - 450.0
	3	12 - 18	135	14.8 - 574.6	10.1 - 31.3	468.0 - 791.0
	4 and 5	13 - 18	204	44.9 - 511.8	4.2 - 99.8	479.0 - 693.0
Female	1	7 - 15	294	1.0 - 64.3	0.8 - 1.4	53.5 - 104.0
	2	10 - 16	121	5.5 - 95.9	5.0 - 8.1	77.1 - 110.0
	3	11 - 18	133	11.4 - 158.3	7.3 - 20.0	139.0 - 208.0
	4 and 5	12 - 18	206	18.7 - 196.8	10.6 - 23.2	173.0 - 346.0
CI* = confidence interval						

B-233

Rapid Diagnosis of Niemann-Pick Type C patients with Plasma Colestane-3β,5α,6β-triol and 7-ketocholesterol by LC-ESI-MS/MS

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Background: Niemann-Pick Type C (NP-C) is a rare autosomal recessive lysosomal storage disorder caused by impaired intracellular transport of unesterified cholesterol and glycolipids due to mutations in either *NPC1* or *NPC2* gene. NP-C is usually underdiagnosed due to a variable age of onset and heterogeneous age-dependent clinical manifestations. Moreover, definitive diagnosis is based on genetic investigations which are time consuming and not always conclusive. Development of novel therapies for NP-C in recent years emphasized the urgent need for a reliable biomarker in early laboratory diagnosis. Recently, colestane-3β,5α,6β-triol and 7-ketocholesterol that result from non-enzymatic oxidation of cholesterol have been shown to be elevated in plasma of NP-C patients. We explored the usage of plasma colestane-3β,5α,6β-triol and 7-ketocholesterol as powerful diagnostic biomarkers for rapid diagnosis of NP-C. **Methods:** Immediately separated 50 µL plasma was sufficient for the analysis. Analyses were performed on a triple quadrupole mass spectrometer (Shimadzu 8040 LC-MS/MS, Japan) equipped with an ESI source and a reversed phase column after derivatization of oxysterols with dimethylglycine esters. Eight point calibrators and 3 levels of QC were used. Statistical analysis was performed with MEDCALC. **Results:** Both colestane-3β,5α,6β-triol and 7-ketocholesterol levels in NP-C patients were significantly elevated compared to healthy individuals. Mean colestane-3β,5α,6β-triol levels was 20.9±8.4 ng/mL and 7-ketocholesterol was 31.2±14.5 ng/mL for 70 healthy individuals. Mean colestane-3β,5α,6β-triol levels was 128.3±67 ng/mL and 7-ketocholesterol was 216.9±125.4 ng/mL for 8 NP-C patients. ROC analysis yielded AUC of 0.99 and 1.00 for colestane-3β,5α,6β-triol and 7-ketocholesterol, respectively. At the cut-off of 39 ng/mL, colestane-3β,5α,6β-triol demonstrated a specificity of 98.6% and a sensitivity of 100%. Both a specificity and a sensitivity of 100% at a cutt-off of 72 ng/mL was observed for 7-ketocholesterol. NP-C patients were confirmed with genetic analyses. **Conclusion:** Our data demonstrates that plasma colestane-3β,5α,6β-triol and 7-ketocholesterol fulfills the need of rapid and reliable biomarkers for NP-C.

Oxysterol levels in NP-C patients				
Genes	**Mutation**	**Consequence of Mutation**	**Colestane-3β,5α,6β-triol (ng/mL)**	**7-ketocholesterol (ng/mL)**
NPC1	c.3160G>A	A1054T	200	243
NPC1	c.1831-1836 delGATGAA/c.3734-3735delCT		190	425
NPC1	c.3067G>T	V1023F	40	83
NPC1	c.3557G>A	R1186H	61	135
NPC1	c.1123A>C	T375P	110	86
NPC1	c.1073-1073delT	L358R	97	153
NPC1	c.3019C>G/c.3246-3247delTG	P1007A	220	259
NPC2	c.352G>T	E118Stop	108	352

B-234

Marked influence of body mass index (BMI) on biochemical markers of the metabolic syndrome in the CALIPER cohort of healthy children and adolescents

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Background: Reference intervals (RI; i.e. normative values), essential to accurately interpret laboratory tests, are severely lacking in pediatrics, potentially causing erroneous interpretation and misdiagnosis. To address this critical gap, the CALIPER project developed a comprehensive database of over 100 age- and sex-specific pediatric RIs (www.caliperproject.ca). However, body mass index (BMI) is another key covariate that may significantly affect analyte levels. The objective of this study was to determine the effect of BMI on lipid/lipoprotein, inflammatory, and nutritional markers of the metabolic syndrome (MetS) in a healthy pediatric population. If unhealthy levels manifest early in overweight/obese children/adolescents, identifying and treating these patients early may help reverse damage due to adiposity and prevent future disease.

Methods: Lipid/lipoprotein, inflammatory, and nutritional MetS biomarkers were measured in the healthy CALIPER cohort (n=998 or n=681 depending on the analyte) using the Abbott Architect chemistry assays. Exclusion criteria included history of chronic illness or use of prescription medication. Children (2-<10 years) and adolescents (10-<19 years) were analyzed separately, with each sex analyzed separately for adolescents. Variables with a skewed distribution were log transformed to achieve normality. Analyte levels were compared between normal weight (NW), overweight (OW) and obese (OB) children (based on CDC classification) using one-way ANOVA and Bonferroni's Post Hoc test. Independent Sample T-Test determined differences between NW and OW/OB combined.

Results: OW/OB adolescent males but not females had elevated ALT and ferritin, and decreased HDL-C levels compared to NW subjects. Triglycerides, apoB, and CRP were elevated in OW/OB adolescents, although more pronounced in males. Vitamin B12, C3, and C4 were elevated in OW/OB adolescents compared to NW. In children, triglycerides were higher in OW/OB (1.55mmol/L ± 0.07) compared to NW (1.24mmol/L ± 0.03) individuals, $p<0.01$, and C3 was significantly higher in OW/OB (1.31g/L ± 0.02) compared to NW (1.12g/L ± 0.01), $p<0.01$.

Conclusion: Dyslipidemia in insulin resistant states increases the risk of developing cardiovascular disease (CVD) in type 2 diabetes (T2D). Increased triglycerides and apoB (marker of atherogenic lipoproteins) and decreased HDL-C in OW/OB adolescents suggests lipid abnormalities manifest early, prior to developing insulin resistance. Inflammatory proteins, C3, C4, and CRP were elevated in OW/OB adolescents, and C3 was elevated in OW/OB children, suggesting children/adolescents with an increased BMI are in a state of chronic low-grade inflammation. Vitamin B12 levels were lower in OW/OB adolescents suggesting poor nutrition as a result of an unbalanced diet lacking in micronutrients. Critical in homocysteine metabolism, low vitamin B12 levels could result in hyperhomocysteinemia and subsequent increased CVD risk. Ferritin, an acute phase reactant and marker of iron stores, was elevated in OW/OB adolescents, possibly identifying individuals at high risk of T2D. Thus, MetS marker levels are altered in apparently healthy OW/OB pediatric subjects, suggesting T2D and CVD risk factors can be monitored early to help prevent disease development. As such, RIs should either be partitioned by BMI or OW/OB subjects need to be excluded if increased levels predict disease development.

B-235

Reference Intervals of Diagnostic Tests for Mucopolysaccharidoses in Turkish Population

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Background: Mucopolysaccharidoses (MPSs) are a group of inherited lysosomal storage disorders caused by deficiency of lysosomal hydrolases required for glycosaminoglycan (GAG) catabolism. To date, 7 main types with subtypes of MPSs have been identified. Cumulative incidence for all MPSs is 1/25000. The incidence is estimated more in in Turkish population with a high rate (21%) of consangineous marriages. Owing to the great variability of clinical manifestations in patients, clinical diagnosis is difficult and definitive diagnosis is carried out by specific enzyme activity measurements in leukocytes or genetic investigations. Currently, laboratory diagnostic tests become prominent with the approval of enzyme replacement therapies (ERT) for some types of MPSs. The aim of this study was to establish reference values for gold standard diagnostic tests of MPSs as the most common inherited metabolic disorders in Turkish population.

Methods: Reference interval study was performed according to CLSI C28 guideline from a total number of 150 healthy volunteers in both gender under 18 years, 20-30 years, 30-40 years and over 40 years in equal numbers. Measurements of leukocyte specific enzyme activities were carried out with fluorometer (Molecular Devices SpectraMax M2 Microplate Reader, USA) by using fluorogenic substrates. The normality of distribution and extreme outliners were tested by D'Agostino Pearson's and Tukey method, respectively. Outliers and extreme values were shown by box-plot analyses. Statistical analyses were carried out with SPSS v.21.0 program.

Results: Reference intervals for MPSs types were shown in table. A total number of 41 MPS patients were diagnosed with leukocyte specific enzyme activity analyses (13 MPS III, 13 MPS IVA, 13 MPS VI, 2 MPS II patients).

Conclusion: Specific enzyme activity measurements from leukocytes are gold standard for the diagnosis of MPSs. Essential reference values for diagnostic tests of MPSs were determined in Turkish population with a high rate of the disorder.

Reference Intervals for MPSs			
MPS type	**Enzyme**	**Female**	**Male**
MPS II	Iduronate-2-sulfatase (nmol/4 h/mg protein)	7.9-52.5	9.7-55.6
MPS IIIB	N-acetyl-α-D-glucosaminidase (nmol/h)	4.9-19.3	4.6-21.3
MPS IVA	Galactose 6-sulfatase (nmol/17 h/mg protein)	46.3-330.6	40.9-323.2
MPS VI	Aryl sulfatase B (nmol/h/mg protein)	9.7-81.9	11.7-92.1
MPS VII	β-glucuronidase (nmol/h/mg protein)	19.3-171.8	18.5-180.5
Reference enzyme	β –galactosidase (nmol/h/mg protein)	70.8-343.1	63.4-375.1

B-236

CALIPER Pediatric Reference Intervals for Ortho Vitros 5600 Immunoassays

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Background: Correct interpretation of laboratory test results requires accurately established reference intervals. As growth and development can profoundly influence circulating biomarker concentrations, the pediatric population requires unique reference intervals that are appropriately stratified by age and sex. The CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) program is a Canada-wide research initiative that has made considerable strides in addressing the gaps in pediatric reference intervals. This current study expands the CALIPER database by establishing covariate-stratified reference intervals for immunoassay-based tests on the Ortho Vitros 5600 System.

Methods: Healthy children and adolescents recruited from the Greater Toronto Area (GTA) and Hamilton regions as part of the CALIPER study completed a health questionnaire and provided a blood sample. Children with acute or chronic illness, or use of prescription medication within the past week were excluded. Several biomarkers were measured using the Ortho Vitros 5600 Immunoassay System, utilizing approximately 300-600 serum samples per assay. Analyte concentrations were visually inspected and statistically relevant age/sex-based partitions were determined. Outliers were removed using the Tukey or adjusted Tukey test for normally distributed and skewed data, respectively. Age- and sex-specific reference intervals with corresponding 90% confidence intervals were calculated using CSLI C28-A3 guidelines.

Results: AFP, prolactin, rubella IgG, and beta-hCG levels were fairly consistent over the pediatric age range, with no differences observed between sexes. Testosterone, progesterone, LH, FSH, ferritin, estradiol, and CEA all showed sex differences, the majority of which were seen after pubertal age. Folate and vitamin B12 both required age partitioning; however, no differences were seen between sexes.

Conclusion: Complex expression profiles were observed for several immunoassays, allowing calculation of age- and sex-specific reference intervals for the Ortho Vitros 5600 Immunoassay System. This will enable accurate diagnosis and laboratory assessment of children monitored by immunoassays run on this platform in healthcare institutions worldwide. However, we recommend further validation for the local pediatric population and specific analyzer prior to clinical implementation based on CLSI guidelines.

B-237

A Quantitative Mehtod for the Measurement of Dried Blood Spot Amino Acids using Ultra Performance Liquid Chromatography©

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Objective: Measurement of amino acids in dried blood spots has been extensively utilized for the detection of newborns with various inborn errors of amino acid metabolism including phenylketonuria (PKU) and maple syrup urine disease (MSUD). Whereas blood spot amino acid measurement has been invaluable for initial diagnosis, the relative insensitivity of blood spot measurement has found limited use in lifelong monitoring of patients with these disorders. We wanted to test if a blood spot assay was sufficiently sensitive to provide accurate monitoring of patients with amino acid disorders. The work described here outlines our evaluation of blood spot amino acids using ultra-performance liquid chromatography (UPLC).

Relevance: Most patients are currently monitored using plasma samples and measurement by ion-exchange chromatography, a process that can take up to two hours per sample. Many of the patients whom we monitor live several hours away from the hospital and find great inconvenience to travel this distance for routine monitoring purposes. We have previously found that UPLC can provide a more rapid turnaround time for analysis and routinely implement this procedure in our laboratory for plasma amino acid analysis.

Methodology: Plasma amino acids from dried bloods spots were obtained from patient samples and compared to the corresponding plasma measured using the UPLC methodology. Amino acids were extracted from dried blood spots by sonication in methanol. The eluent was dried and resuspended in 50uL of 50:50 acetonitrile:water before derivatization using 20uL of the reconstituted blood spot sample, 60uL 0.042mM norvalline in a borate buffer, and a proprietary reagent, AccQTag®. After incubation for 10 minutes at 55°C, the sample was loaded onto the UPLC with a Waters MassTrak AAA 2.1x150mm column. The derivatized samples were separated using UPLC with UV detection (260 nm) with a cycle time of 45 minutes per sample. To examine the stability of blood spots when exposed to changes in temperature, blood spots were exposed for 3 days at 4°C or 3 days at 65°C, followed by overnight storage at 4°C or room temperature.

Validation: 318 samples were collected for this study. Intra- and inter-assay imprecision (mean CVs) for alloisoleucine, leucine, isoleucine, valine, phenylalanine, and tyrosine ranged from 0.1% to 4.3% and 4.2% to 20.2%, respectively. Recoveries were lower at high levels, an observation that was not previously appreciated.

Results and Conclusions: For phenylalanine and tyrosine, dried blood spot analysis had a very slight negative bias, resulting in lower concentrations of phenylalanine and tyrosine compared to plasma amino acid analysis. For valine, leucine, isoleucine, and alloisoleucine, dried blood spot analysis had a moderate negative bias, resulting in lower concentrations of these amino acids as compared to analysis using plasma amino acids. The results of this study demonstrate that the blood spot filter papers are stable despite temperature and humidity changes, and demonstrate less bias when stored at room temperature before testing. This UPLC based method can reliably measure significant amino acids in dried blood spots and finds the method to be sufficiently sensitive for accurate long-term monitoring of patients with amino acid disorders.

B-238

Pediatric reference intervals for 1,25-dihydroxyvitamin D in the CALIPER Cohort of Healthy Children and Adolescents

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Background: 1,25 dihyroxyvitamin D ($1,25(OH)_2D$) is the most biologically active metabolite of vitamin D. $1,25(OH)_2D$ is essential to childhood growth and development and plays a role in calcium homeostasis and bone growth. Despite its importance, no $1,25(OH)_2D$ reference interval exists for the pediatric population. Traditional $1,25(OH)_2D$ assays require complex manual preparation, however Diasorin has developed a new, fully automated *in vitro* chemiluminescent immunoassay (CLIA) to measure $1,25(OH)_2D$ levels, requiring no sample pretreatment or preparation. In alignment with CALIPER [Canadian Laboratory Initiative for Pediatric Reference Intervals], we aimed to establish age- and sex-specific reference intervals.

Methods: 405 blood samples were collected from apparently healthy children and adolescents aged 0-18 y. Those aged 1-18 y were from the CALIPER cohort, while those aged 0-1 y were Mount Sinai Hospital outpatient samples. $1,25(OH)_2D$ levels were measured using Diasorin Liaison XL, an *in vitro* non-competitive three step sandwich CLIA. Statistical analysis was performed using R software, in accordance with CLSI C28-A3 guidelines. Age- and sex-specific reference intervals with corresponding 90% confidence intervals were calculated.

Results: There was a significant age-dependent decline in $1,25(OH)_2D$ levels over the first few years of life requiring data partitioning and calculation of reference values for three age groups: 0-<6m, 6m-<3y, and 3-<19y (shown in Table 1). Sub-analysis did not suggest a seasonal effect on $1,25(OH)_2D$ levels in our study group (p=0.364 based on the Mann Whitney U-Test) and sex-partitioning was not necessary.

Conclusion: This study provides, for the first time, robust pediatric reference intervals for the $1,25(OH)_2D$ Diasorin Liaison assay and will improve the accuracy of pediatric test result interpretation for this active form of vitamin D.

Age- and sex-specific pediatric reference intervals for 1,25-dihydroxyvitamin D

Age	Sample Size	Lower Limit	Upper Limit	Lower Confidence Interval	Upper Confidence Interval
0-<6 months	68	92	416	(76,111)	(385,453)
6 months - <3 years	121	104	439	(101,113)	(342,460)
3 -< 19 years	185	108	246	(104,110)	(225,355)

B-239

Comparison of Cord Blood Gas Values and Sampling Errors Pre-and Post-Universal Collection

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Background

Intrapartum asphyxia can lead to fetal metabolic acidosis which, if severe and prolonged, can result in damaging acidosis and encephalopathy. Analysis of acid-base status in umbilical cord (UC) blood can provide valuable information on the metabolic condition of neonates at birth, particularly when fetal distress is suspected during delivery. The umbilical vein and artery are located in close and intertwined proximity so analyses should be performed on both the UC artery and vein to ensure correct sampling. Oxygenated venous blood (from placenta to fetus) should have higher pH and lower pCO2 compared to the deoxygenated arterial blood (from fetus to placenta). The newborn is at higher risk of complications if the umbilical artery pH is <7.0 and base deficit ≥ 12 mmol/L. Prior to October 2014, our institution collected and performed blood gas analysis on cord blood samples only upon physician request. To improve the process for UC sample collection and avoid collection and transport errors resulting in unacceptable specimens or misleading results, a universal collection protocol was implemented in October 2014 requiring blood gas analysis on all infants at birth. We studied the impact of universal cord blood collection on the distribution of pH and base excess values, and the rates of unacceptable samples.

Methods

UC blood gas results were collected retrospectively from 9 months before (n=678) and 6 months after (n=2415) universal collection was implemented. The percentages of births involving severe metabolic acidosis (pH < 7.0 and/or base deficit ≥ 12 mmol/L) in the pre- vs. post-universal screening cohorts were compared. Data from each time period was evaluated for differences between arterial and venous cord sample pH and pCO2 results to assess accuracy of sample collection. Additionally, we calculated test cancellation rates presumably due to collection issues.

Results

Severe metabolic acidosis (pH < 7.0 and/or base deficit ≥ 12 mmol/L) was observed in 15/678 (2.2%) of samples collected pre-universal screening and 16/2415 (0.7%) post-universal screening. Four samples (0.6%) in the pre cohort and one sample (0.0004%) in the post cohort met both criteria for acidosis.

In the pre-universal collection cohort, 127/678 (18.7%) samples required cancellation, most often due to insufficient quantity or clotted sample. In the post-protocol cohort, 140/2415 (5.8%) required cancellation.

As an indication of correct source sampling, 219 of the 251 (87.3%) paired arterial/venous UC samples received pre-protocol had a difference in pH of >0.02, and 217 pairs (86.5%) had a difference of >4

mmHg pCO2 between the arterial cord- and venous cord-labeled samples. After universal collection protocol, 1027/1073 pairs (95.7%) showed a difference in pH of >0.02 and 952/1073 (88.7%) showed a difference in pCO2 >4 mmHg.

Conclusion

Collection technique (and presumably correct sample handling and transport) and correct source sampling improved after implementing universal umbilical cord blood acid-base screening as evident by a decrease in the frequency of sample cancellation and an increase in paired samples with appropriate differences in pH and pCO2. Implementation of universal cord blood screening decreased collection and sample handling errors, but did not appear to increase detection of severe metabolic acidosis in neonates.

B-240

A Technique to Enhance the Stability of a Pediatric Bilirubin Control

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Background: Bilirubin is a degradation waste product of hemoglobin, released during the breakdown of Red Blood Cells. Through a series of enzymatic steps, the heme in hemoglobin is catabolized to bilirubin which is transported to the liver where it becomes conjugated to glucuronic acid. Conjugated bilirubin is excreted from the liver in bile into the intestines where bacteria convert it into urobilinogen to be excreted by the kidneys as urobilin or in the feces as stercobilin. Bilirubin circulates in the blood stream as indirect (unconjugated) and water-soluble direct (conjugated) forms. Current clinical assays can measure total bilirubin (TBIL) and direct bilirubin (DBIL).

Elevated levels of bilirubin in the blood are clinically significant and often result in jaundice, a yellowish discoloration of the skin and eye, and may indicate various hepatic conditions, gallstones, cancer, hemolytic anemia or hepatotoxic drugs. Elevated bilirubin in newborns is a medical emergency due to the immaturity of the blood-brain barrier which may lead to irreversible brain damage. Bilirubin is very sensitive to light and oxygen and quality control materials have notoriously short open vial stability limitations, primarily due to exposure to atmospheric oxygen upon opening the vial. A technique to remove control sample without subjecting it to excessive oxygen would be advantageous to preserve the stability of the control.

Objective: To evaluate the use of a syringe and needle technique to enhance the stability of a pediatric bilirubin control.

Methods: The Quantimetrix Pediatric Bilirubin control, lots 33491 and 33501, Levels 1 and 2, were subjected to an 18 day stability protocol. In one arm, vials were uncapped and a pipette was used to remove 150µL sample before assaying TBIL and DBIL in duplicate on the Siemens Dimension® ExL. In the second arm, vials remained sealed and a 1cc syringe with 18 gauge needle was used to remove the 150µL sample through the rubber stopper. The samples were tested in this manner 9 times over an 18 day period. A linear regression was used to determine the day to failure (DTF) using ±10% cutoff.

Results: For lots 33491 and 33501 lots, the DTF for Level 1 DBIL open vial arm was extrapolated to 22.2 and 26.3 days and the syringe arm to 61.5 and 69 days respectively. The DTF for Level 2 DBIL open vial arm was extrapolated to 42.7 and 61.5 days and the syringe arm to 61 and 64 days respectively. TBIL values showed no appreciable degradation.

Conclusion: Level 1 DBIL values were most affected by oxidation from exposure to atmospheric oxygen. Using a syringe resulted in a marked increase of stability of about 24 to 65 days. This trend was less apparent in Level 2, where using the syringe resulted in an increase of about 52 to 63 days. TBIL values were virtually unaffected by this open vial protocol, actually showing a slight upward trend. Interestingly the current open vial stability claim for this Quantimetrix control is only 5 days while this data set shows excellent stability over the 18 day period across both arms.

Wednesday, August 3, 2016

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

Point-of-Care Testing

B-241

Point of Care Glucose Testing - Five-Years Performance Review with an In-house Established Quality Assessment Framework

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Introduction: In our hospital Chemical Pathology laboratory held key responsibility for the quality of point of care-glucose-testing (POC-G-T) service through the establishment of a well-designed quality management (QM) system. The QM system focused in management, technical, and quality assurance. Every three years, our laboratory participated in a well established assessment system delivered by National Association of Testing Authority (NATA) according to medical testing laboratory standard ISO 15189. Despite POCT had a quality standard ISO 22870, there was lacking a similar assessment system. Looked up PubMed as at 1 September 2015 there was insufficient publication reported assessment framework of a POCT QM system, neither was local hospital as an example

Method: In September 2015, after running the system for five years, we conducted a five-year performance review with an in-house established QM system assessment framework composing of five quantifiable quality indicators. They were categorized into two aspects, analytical and operator. In analytical aspect they were (1) RCPA QAP KPI score, (2) sigma metric , by (TEa-bias)/CVa , TEa was total error, CVa was analytical coefficient of variation (CV) of period collected low and high IQC imprecision and (3) analytical goal, by CVa/CVi, CVi was intra-individual biological variation. In operator aspect, they were (4) daily internal quality control repeat status, being interpreted as repeat frequency and reason of repeat, and (5) external quality assurance (EQA) performance, in terms of sample analysis failure rate. During the assessment process all stakeholders were involved, namely doctor, nurse, laboratory, biomedical engineering department, supplies department, laboratory information system department and vendor.

Results: Three biannual KPI scores indicated good performance. Sigma-metric ranged between 5.9 and 7.8 corresponded to error rate of 0.00050% and <0.00034% respectively. Analytical goal was achieved satisfactorily. EQA sample analysis failure rate declined from 10.0% to 0.9% in January 2015 and kept persistently low at <1.0% till July 2015. Daily IQC repeat status identified two deficiencies of sample application technique and sample verification compliance before analysis owning to lacking focus in the training program.

Conclusion: The outcome ascertained the five quantifiable quality indicators as a landmark for tangible assessment of the QM system and established reference points for another five years or shorter, such as annual, performance review.

B-242

Accuracy of i-STAT point-of-care creatinine measurements in outpatient chemotherapy patients: A direct comparison to laboratory based methods.

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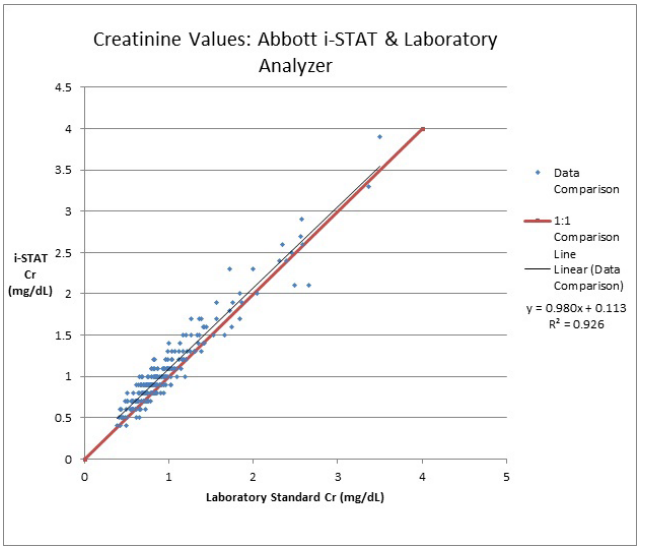
Background: Renal function is an important consideration in administration of chemotherapeutics that are either subject to renal excretion or nephrotoxic. The oncology service at our institution has transitioned to the Abbott i-STAT point-of-care (POC) device to determine kidney function in outpatients presenting for chemotherapy infusion. POC devices offer advantages in this setting by rapidly determining chemotherapy eligibility based on established cutoffs, guiding dosing, and reducing wait times associated with traditional laboratory testing. This investigation compared creatinine values obtained in the POC setting to samples submitted for standard laboratory analysis. Methods: A retrospective analysis of creatinine level was performed on POC and standard samples (N=540) collected from the same patient on the same calendar day over a 6 month period from April 2014 to September 2014. Respective samples were tested on the Abbott i-STAT CHEM8 cartridge and our core laboratory analyzer, Roche COBAS 8000. Results: The i-STAT results demonstrated a small but consistent positive bias in each of the 6 months of our investigation. When averaged over the entire six month period, i-STAT creatinine values were higher than the laboratory analyzer by 0.11 ± 0.04 mg/dL. Despite the positive bias, the two methods had an excellent linear relationship (slope = 0.980, R^2 = 0.926). When used for the calculation of glomerular filtration rate (GFR) these values result in an underestimation of 4-12%, depending on gender and absolute creatinine value. Conclusion: These findings suggest the i-STAT is an appropriate screening tool to identify patients with kidney dysfunction prior to chemotherapy infusion. However, calculations used in the dosing scheme of some chemotherapeutics rely directly on GFR and could result in sub-therapeutic dosing when POC creatinine concentrations are biased relative to laboratory results. The clinical impact of this potential dosing discrepancy may warrant further investigation.



B-243

Development of a new interface for POCT competency tracking

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OBJECTIVE: To accurately, efficiently and effectively track elements of competency for the point of care testing (POCT) department at a multi-site pediatric hospital system via electronic means.

INTRODUCTION: Per the Clinical Laboratory Improvement Act (CLIA), there are six elements of competency that must be included in assessing laboratory testing operators. Ensuring these elements are met and tracked year to year can be a daunting task. Children's of Minnesota has approximately 2200 operators trained to perform any number of the eleven different POC tests offered across over forty departments in seven physical locations. Manual tracking of competency is done by sorting operators by department and test type in TELCOR QML and then matching them up with lists of employees who have completed competency each year. These lists are two large spreadsheets generated from the e-learning software which manages the online tests of comprehension and from tracking Competency Fair attendance. Manually updating each operator takes on average 5 minutes per device type. Adding up all combinations of operators and POCT tests performed at Children's equates to thirty nine eight hour days of competency updating needed. Collaboration between Children's Human Resources Information Systems (HRIS) staff, POCT staff and POCT middleware vendor TELCOR to develop an interface providing the transfer of competency record data from HRIS to QML to track competencies would greatly simplify this process.

METHODOLGY: TELCOR QML is middleware software commonly used to transfer results from any POCT device to a hospital Lab Information System or directly to the Electronic Medical Record. Within QML, competency parameters can be set for each device type in use. Parameter options include the following: e-learning course records, patient tests, quality control tests, proficiency testing and/or linearity testing. When an operator meets all parameters set, they are automatically updated. A report created in Children's e-learning software, People Soft, is run each day looking for any staff that has successfully completed any POCT e-learning courses. This report is saved as a file in Children's network where QML knows to look for it. QML then automatically pulls that report and updates operator records within QML. In addition to course completion information, this report delivers information such as new hire additions, name changes, badge number changes, departmental changes and terminations.

RESULTS & CONCLUSION: The first production interface run resulted in the update of approximately 1500 operators. This occurred in a matter of seconds. This report run is scheduled on a daily basis and an alert occurs within QML if issues arise. The implementation of an e-learning interface drastically reduces the number of hours needed each year to update POCT competencies. It allows the POCT department to smoothly meet regulatory compliance for competency assessment tracking and it also eliminates the number of errors that occur with manual editing of competency updates and QML operator records each year. The time saved in the POCT department will be used to concentrate on other quality initiatives, instrumentation and regulatory compliance.

B-245

Operational outcomes and interchangeability of results with ABL90 Flex Plus and ABL90 Flex

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Background: One of the advantages of ABL90 Flex Plus blood gas analyzer is that up to 17 critical parameters can be determined from extremely small volume blood samples. This operational aspect is crucial for delivery rooms or neonatal intensive care units (NICU) and it should take into account for selecting a POCT device. Before using this new analyzer at any POCT setting, the comparability of results must be verified following guidelines recommendations

Methods: The frequency of "Aborted sample" and "Insufficient sample" errors were obtained by Aqure (Radiometer). 50 samples in syringes heparinized whole blood corresponding to randomly selected patients were used. After homogenization samples, blood gas samples were measured in both analyzers. Evaluation of results was performed following the EP09-A2 CLSI considering as reference method ABL90 Flex measurements. Linear regression was calculated for each parameter in order to estimate bias at the medical decision levels (www.westgard.com). The allowable bias was established according to biological variation criteria. Statistical analyses were performed using StatisPro™ (CLSI)

Results: When using ABL90 Flex Plus, the frequency of "Aborted sample" decreased from 5.9% to 3.6% and "Insufficient sample" from 3.2% to 2.2%.

Parameter (measurement interval)	Clinical decision levels	Estimated bias	Confidence interval 95%	Allowed bias	Allowed bias (%)
	7.25	0.0009	-0.0002 to 0.0019	0.0732	1.01
pH (7.053 - 7.500)	7.35	0.0025	0.0018 to 0.0031	0.0742	1.01
	7.5	0.0049	0.0033 to 0.0065	0.0758	1.01
	60.0	0.429	-1.73 to -1.19	5.82	9.7
PaO_2	80.0	0.600	-2.50 to -1.58	7.76	9.7
	90.0	0.726	-2.89 to -1.76	8.73	9.7
	100.0	0.863	-3.30 to -1.93	9.7	9.7
	35.0	0.141	-0.38 to 0.18	0.63	1.79
$PaCO_2$	45.0	0.090	-0.17 to 0.19	0.81	1.79
	55.0	0.111	-0.11 to 0.34	0.98	1.79
	4.5	0.077	-0.34 to -0.03	0.28	6.3
Hemoglobin	10.5	0.029	-0.40 to -0.28	0.66	6.3
	17	0.044	-0.60 to -0.42	1.07	6.3
	23.0	0.096	-0.86 to -0.48	1.45	6.3
	115.0	0.2129	-0.3 to 1.5	1.3	1.1
Sodium (114 - 151) mmol/L	135.0	0.45	0.2 to 0.7	1.5	1.1
	150.0	0.11	0.00 to 0.7	1.7	1.1
	3.00	0.027	-0.06 to 0.05	0.08	2.7
Potassium (2.6 - 7.7) mmol/L	5.80	0.032	-0.14 to -0.01	0.16	2.7
	7.00	0.054	-0.22 to 0.00	0.19	2.7
Chloride (87 - 118) mmo/L	90	0.17	-0.5 to 0.2	0.6	0.7
112	0.09	-0.5 to -0.1	0.8	0.7	
	0.65	0.0062	0.001 to 0.026	0.020	3.1
Ionized Calcium (0.6 - 1.3) mmol/L	1.00	0.0023	0.011 to 0.020	0.031	3.1
	1.30	0.0016	0.014 to 0.020	0.040	3.1
	45	0.59	-0.4 to 2.0	3.1	6.96
Glucose	120	0.44	4.4 to 6.2	8.4	6.96
	180	0.76	7.3 to 10.4	12.5	6.96
	2.00	0.02	-0.01 to 0.06	0.08	4
Lactate	4.00	-0.07	-0.10 to -0.04	0.16	4
	6.00	-0.16	-0.21 to -0.11	0.24	4

Conclusion: With ABL90 Flex Plus, more blood gas analysis results are obtained without sampling error. It could have a relevant clinical impact for avoiding new sample collections or reducing the timeframe to therapeutic intervention. ABL90 Flex Plus results are equivalent to ABL90 Flex. If more than one system is used to follow patients, it is also important to consider the relative bias that could be safely tolerated between methods at medical decision points.

B-246

A Preliminary, Multi-analyte Comparison Study of Whole Blood Point-of-care Testing (POCT) on the Piccolo analyzer in Our Ebola Biocontainment Unit Versus Plasma Testing on the Advia 1800 Chemistry Analyzer

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Background: Generally, the goal of bedside POCT is *to expedite diagnosis and facilitate immediate evidence-based medical decisions that can potentially improve patient outcomes. However, more recently, in high risk infectious diseases like Ebola, POCT can be more easily be isolated in a biocontainment unit and therefore testing can be performed without the risk of Ebola exposure/contamination in the core laboratory. However, the menu of FDA approved tests for POCT devices is rather limited hence, when our infectious disease physicians requested liver function testing (LFTs) on our Ebola patients, the FDA-cleared Piccolo Xpress chemistry analyzer was selected because of the availability of 1) a whole blood comprehensive metabolic chemistry panel on a single-used reagent test "disc", 2) a test disc designed for LFTs, and 3) other important analytes such as lactate dehydrogenase(LDH), magnesium, and phosphorus. We assessed the general accuracy of nineteen whole blood chemistry*

Piccolo methods by comparing plasma chemistry results obtained on our Advia 1800 chemistry analyzer. **Methods:** Lithium-heparinized whole blood samples obtained from random patients were analyzed first on the Piccolo Xpress employing test specific reagent discs/cartridges for Liver Panel Plus, Basic Metabolic Panel Plus, and other analytes. Subsequently, samples were centrifuged and analyzed on the Advia. **Results:** Piccolo Advia comparison results are shown in the Figure. Patient means were comparable across the 19 analytes with no statistically significant difference observed (r^2=0.96; p=0.86). Good-to-excellent correlation was observed for the Piccolo Liver Panel Plus when compared to the Advia (r^2=0.94-1.00) except albumin (r^2=0.79). While most analytes showed acceptable correlation and regression line slopes, weak correlation was observed for chloride, LDH, sodium, TCO2, magnesium, potassium (r^2= 0.45-0.66). Between run precision on all Piccolo analytes was generally acceptable (CV%= 0.55-7.01). **Conclusion:** The Piccolo Xpress Chemistry Analyzer appears to show generally acceptable comparison performance for testing/screening purposes in our biocontainment unit.

Comparison data for various analytes on the Piccolo versus the ADVIA 1800

Analyte	N	Slope	Y intersection	R Square	SE	Piccolo mean	Advia 1800 mean
Albumin	14	0.88	0.11	0.79	0.38	3.70	4.06
Alkaline phospatase	14	0.68	6.94	1.00	1.97	193.14	274.29
ALT	14	0.86	1.01	1.00	3.61	45.36	51.29
AST	14	0.93	1.50	1.00	6.79	59.64	62.43
Total Bilirrubin	14	0.94	0.22	1.00	0.10	2.46	2.39
GGT	13	1.01	-2.43	1.00	4.64	103.77	105.31
Total protein	14	1.25	-1.8	0.94	0.26	7.29	7.30
Phosphorus	12	1.05	0.21	0.96	0.17	2.97	2.88
LDH	13	0.70	83.66	0.67	35.56	272.23	268.85
Magnesium	13	0.92	0.15	0.89	0.13	2.07	2.08
Calcium	12	0.90	0.93	0.91	0.19	9.08	9.04
Chloride	13	0.87	11.78	0.61	2.52	104.08	106.31
Creatinine	12	0.92	0.14	0.99	0.08	1.09	1.03
Glucose	13	1.00	1.50	0.99	5.37	115.23	116.62
Sodium	12	0.86	20.12	0.59	1.83	140.08	140.50
Total CO2	12	0.54	13.50	0.45	1.20	27.75	26.33
Blood urea nitrogen	13	1.02	-0.73	1.00	1.39	27.54	27.23
Potassium	12	0.83	0.81	0.80	0.15	4.23	4.12

B-247

Determination of Analytical Performance Characteristics of RAMP® Procalcitonin

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Background: The RAMP® System is a lateral flow immunoassay platform that provides accurate and precise diagnostic information in minutes. RAMP Procalcitonin is a quantitative *in vitro* diagnostic test used with the RAMP System to measure levels of the prohormone procalcitonin (PCT) in human EDTA anticoagulated whole blood. PCT is a biomarker elevated in the blood of patients suffering from bacterial sepsis. Rapid detection of PCT aids in the early diagnosis and treatment of sepsis, leading to improved patient outcomes and reduced healthcare costs. The objective of these studies was to determine the analytical performance characteristics of RAMP Procalcitonin.

Methods: Detection limits, linearity, hook effect, repeatability, total precision, interference and cross-reactivity, and reference range (95th percentile) were determined for RAMP Procalcitonin according to methods outlined in CLSI guidelines, where applicable, using either plasma-based controls or EDTA whole blood samples.

Results: The limit of blank (LoB) and limit of detection (LoD) were determined to be 0.18 ng/mL and 0.36 ng/mL, respectively, using methods described in EP17-A. The 20% and 10% limits of quantitation (LoQ) were determined to be 1.28 ng/mL and 0.64 ng/mL, respectively, using total error estimates as described in EP17-A. Linearity analysis yielded a linear regression slope (95%CI) of 0.97 (0.93 to 1.02) and an R (95%CI) = 1.00 (0.99 to 1.00). No high dose hook effect was observed up to 2000 ng/mL.

Repeatability and within-laboratory precision were determined as per EP5-A3 by testing three levels of frozen plasma control materials in duplicate, twice per day for 20 days on three lots of RAMP Procalcitonin. Repeatability coefficients of variation (CVs) were 6.6 to 8.5% at 92.7 ng/mL, 8.0 to 9.0% at 2.72 ng/mL and 16.0 to 18.7% at 0.58 ng/mL. Within-laboratory precision CVs were 7.2 to 9.4%, 7.7 to 9.3% and 15.8 to 19.3% at the same concentrations. Simple precision was also determined for whole blood samples by testing 10 replicates of 3 blood samples in a single run on one lot of RAMP Procalcitonin tests. CVs in whole blood were 8.2% at 103.2 ng/mL, 6.8 % at 3.00 ng/mL and 14.1% at 0.75 ng/mL.

As per EP7-A2, no interference was observed for RAMP Procalcitonin as the result of hemoglobin (500 mg/dL), bilirubin (conjugated 40 mg/dL, unconjugated 40 mg/dL), triglycerides (3260 mg/dL), human serum albumin (2.4 g/dL) or 11 common pharmaceutical compounds. No statistically significant cross-reactivity was observed with calcitonin (5 ng/mL), katacalcin (10 ng/mL), α-CGRP (30 ng/mL) or β-CGRP (30 ng/mL).

The RAMP Procalcitonin reference range study was conducted at one site and included 125 apparently healthy subjects. The 95th percentile from these results was determined to be 0.36 ng/mL, using nonparametric analysis methods described in C28-A3C.

Conclusion: RAMP Procalcitonin demonstrated robust analytical performance for the quantification of procalcitonin, based on methods outlined in applicable CLSI guidelines.

B-248

Multivariable Statistical Techniques for the Acceptance of Several Glucose Meters. A Practical Example with AccuChek Inform II ®.

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Background: In Hospitals, several glucose meters are used for assessing the patient glycemia to direct insulin therapy at the bedside. To maintain a seamless operation, the values as obtained with a glucose meter should be interchangeable with those as determined with other glucose meters and the laboratory method. We describe the application of multivariable statistical techniques for acceptance of Accuchek Inform II ® glucose meters upon receipt from the manufacturer. Methods: Upon receipt, three AccuChek Inform II® (Roche) meters were compared to two meters in use. QC material (Level 1,2 lot # 50100531, Roche). Reagent strips (lot #474135, (Roche). Linearity material (lot #5010720, Roche). Five assays with level 1 and 2 control material were performed for five days. Three assays, for each value of the linearity material, were performed for each meter. Twelve patient specimens for each meter, with values between 40 and 550 mg/dL, were obtained in green top tubes (Becton-Dickinson) and assayed in parallel and within twenty minutes with AccuChek Inform II and the laboratory method (Cobas c501®, Roche). The data were transferred to Minitab® (Version 17, Minitab, Inc.) statistical software and analyzed with multivariable statistical techniques for equality of means variances, linearity and equality of regression lines and their graphic representations. Results: Precision study: The multivariable parallel box plots showed equality of means and variability (height of the boxes and whiskers) by day and meter. This was corroborated by the multiple comparisons of the means by day and instruments obtained with the GLM and Tuckey's multiple comparisons (Level 1: day F=0.7, P=0.6, meter F=0.4, P=0.8; Level 2: day F=0.8, P=0.5, meter F=0.3, P=0.9). Bonett's and Levene's statistical tests showed homogeneity of variance by day and meter for both levels of control (level 1: P=03, P=0.7; level 2: P=0.05, P=0.8). Linearity study: The weighted polynomial regression (y=-0.6+0.97x, Sy/x=0.35,) showed that the relationship was linear (Pure error test: F=1.1, P=0.3) and the meters regression lines were not statistically significantly different from each other (F=0.54, P=0.71). This was also visualized by the lowess regression model. Comparison with the laboratory method: The regression line obtained with the orthogonal model (y=2.4+0.96x) was similar to that obtained with the weighted least squares model (y=2.9+0.96x). Furthermore, the weighted polynomial model showed equality of regression lines for the meters (beta for meter not statistically significantly different from zero: F=0.76, P=0.56). The plot of the absolute and relative differences by the values, as obtained with the laboratory method, showed that all the differences were within the CLIA's criterion (target value ± 6 mg/dL, 10% greater). Conclusions: This practical example showed that multivariable statistical techniques offered straightforward numerical results and compelling, multidimensional graphics to evaluate the performance of new glucose meters for acceptance prior use for patient testing. Multivariable methods are more powerful and parsimonious than univariate methods for comparing the performance of several variables. However, they require intense numerical analysis. Consequently, the availability of statistical software, such as Minitab, was of critical importance for numerical and graphic analyses and the storage of their results.

B-249

Evaluation of four simplified protocols to verify the interchangeability of POCT blood gas patient results

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Background:

When new equipment is introduced into a laboratory, verification of patients´ results interchangeability with the previous method is mandatory.This is especially difficult in complex POCT blood gas networks due to short stability of samples and multiple magnitudes and devices. Existing protocols (CLSI EP09, C54) are complex when facing these scenarios.

Aim:

To evaluate the performance of four simplified evaluation protocols to verify the interchangeability of blood gas patient results.

Methods:

CLSI EP09-A3 protocol results were considered as reference results. Comparison through bias estimation between results of ABL-90 and ABL-90 plus (Radiometer®) was performed (40 blood gas syringes randomly selected). pH, pO_2, pCO_2, sodium, potassium, chloride, glucose, lactate and hemoglobin were measured.

We designed four simplified protocols:

P1 (n:10): 1 day, 5 samples, two replicates/sample.

P2 (n:10):5 days, 1 sample, two replicates/sample.

P3 (n:18): 3 days, 3 samples/day, two replicates/sample.

P4 (n:9): 3 days, 3 samples/day, one replicate/sample.

Results of bias estimation (BE) were compared with those obtained from CLSI EP09-A3.Bias desirable biological variation specification was selected as criteria to determine the interchangeability of patient results at clinical decision levels

Results:

Table shows the bias estimation of CLSI EP09-A3 and the simplified protocols.

Magnitude (ITs %)	Clinical Decision levels	Allowable Bias	EP09-A3 BE(95%IC)	P1BE(95%IC)	P2BE(95%IC)	P3BE(95%IC)	P4BE(95%IC)
pH (<1.01%)	7.25	0.073	0.0009 (-0.0002to0.0019)	-0.0004 (-0.0042to0.0033)	0.0032 (-0.0048to0.0112)	0.0033 (0.0014to0.0051)	0.0053 (0.0022to0.0085)
	7.35	0.074	0.0025 (0.0018to0.0031)	0.0014 (-0.0014to0.0041)	0.0027 (-0.0013to0.0068)	0.0035 (0.0026to0.0045)	0.0068 (0.0053to0.0083)
	7.50	0.076	0.0049 (0.0033to0.0065)	0.0040 (-0.0021to0.0102)	0.0020 (-0.0029to0.0069)	0.0039 (0.0018to0.0061)	0.0089 (0.0057to0.0122)
pO_2 (<9.7%)	60	5.82	-1.46 (-1.73to-1.19)	-2.20 (-4.78to0.38)	-1.12 (-2.18to-0.07)	-0.86 (-1.31to-0.42)	1.13 (-0.83to3.09)
	80	7.76	-2.04 (-2.50to-1.58)	-3.08 (-6.57to0.40)	-1.63 (-3.36to0.10)	-0.88 (-1.64to-0.13)	2.17 (-1.68to6.02)
	90	8.73	-2.33 (-2.89to-1.76)	-3.52 (-7.72to0.67)	-1.89 (-4.10to0.33)	-0.90 (-1.84to0.05)	2.69 (-2.24to7.62)
	100	9.70	-2.61 (-3.30to-1.93)	-3.96 (-8.95to1.02)	-2.14 (-4.88to0.59)	-0.91 (-2.05to0.23)	3.21 (-2.83to9.24)
pCO_2 (<5.75%)	35.0	0.63	-0.10 (-0.38to0.18)	-0.07 (-1.07to0.92)	0.44 (-0.85to1.74)	-0.39 (-0.83to0.05)	-0.86 (-1.65to-0.07)
	45.0	0.81	0.01 (-0.17to0.19)	0.39 (-0.20to0.98)	0.54 (-0.89to1.96)	-0.40 (-0.83to0.03)	-1.12 (-1.74to-0.50)
	55.0	0.98	0.12 (-0.11to0.34)	0.85 (-0.04to1.74)	0.63 (-1.79to3.06)	-0.41 (-1.18to0.37)	-1.38 (-2.15to-0.60)
Hemoglobin (<6.3%)	4.5	0.28	-0.18 (-0.34to-0.03)	**-0.32*** (-1.31to0.67)	0.07 (-0.69to0.82)	-0.01 (-0.39to0.38)	**1.50*** (0.03to2.97)
	10.5	0.66	-0.34 (-0.40to-0.28)	-0.38 (-0.75to-0.02)	-0.25 (-0.59to0.08)	-0.30 (-0.47to-0.14)	0.29 (-0.34to0.91)
	17.0	1.07	-0.51 (-0.60to-0.42)	-0.45 (-1.07to0.18)	-0.60 (-0.93to-0.27)	-0.63 (-0.84to-0.42)	-1.03 (-1.39to-0.66)
	23.0	1.45	-0.67 (-0.86to-0.48)	-0.51 (-1.81to0.80)	-0.92 (-1.67to-0.17)	-0.93 (-1.37to-0.48)	**-2.24*** (-3.43to-1.04)
Sodium (<1.1%)	115	1.3	0.6 (-0.3to1.5)	**-6.1*** (-17.2to5.0)	**-4.0*** (-9.8to1.7)	**-3.8*** (-7.4to-0.1)	**3.7*** (-3.8to11.2)
	135	1.5	0.5 (0.2to0.7)	-1.1 (-3.7to1.5)	-0.6 (-2.1to0.8)	-0.6 (-1.6to0.5)	**1.7*** (-0.5to3.9)
	150	1.7	0.3 (0.0to0.7)	**2.7*** (-1.2to6.5)	**1.9*** (0.1to3.7)	**1.8*** (0.8to2.9)	0.2 (-1.7to2.1)
Potasium (<2.7%)	3.0	0.08	0.00 (-0.06to0.05)	-0.03 (-0.12to0.06)	0.07 (-1.01to1.14)	0.00 (-0.18to0.18)	**0.11*** (-0.12to0.35)
	5.8	0.16	-0.08 (-0.14to-0.01)	0.08 (-0.12to0.28)	**-0.24*** (-1.27to0.79)	-0.08 (-0.35to0.20)	**0.24*** (-0.20to0.68)
	7.0	0.19	-0.11 (-0.22to0.00)	0.13 (-0.19to0.45)	**-0.37*** (-2.26to1.52)	-0.11 (-0.56to0.34)	**0.29*** (-0.42to1.01)
Chloride (<0.7%)	90	0.6	-0.1 (-0.5to0.2)	**-0.8*** (-3.1to1.5)	-0.5 (-3.5to2.6)	-0.2 (-1.2to0.8)	0.2 (-0.7to1.2)
	112	0.8	-0.3 (-0.5to-0.1)	0.3 (-0.7to1.2)	-0.1 (-1.1to0.9)	-0.2 (-0.7to0.3)	-0.3 (-0.9to0.3)
Glucose (<6.96%)	45	3.1	0.8 (-0.4to2.0)	0.5 (-3.6to4.7)	0.7 (-1.5to2.9)	-0.5 (-2.1to1.1)	-0.5 (-2.3to1.2)
	120	8.4	5.3 (4.4to6.2)	4.7 (-1.4to10.8)	3.2 (1.6to4.8)	3.4 (2.3to4.4)	3.5 (2.3to4.6)
	180	12.5	8.9 (7.3to10.4)	8.1 (-4.4to20.6)	5.2 (3.3to7.2)	6.4 (5.0to7.9)	6.6 (5.2to8.1)
Lactate (<4.0%)	2.0	0.08	0.02 (-0.01to0.06)	-0.04 (-0.12to0.05)	-0.04 (-0.21to0.14)	0.00 (-0.07to0.07)	0.00 (-0.11to0.12)
	4.0	0.16	-0.07 (-0.10to-0.04)	-0.10 (-0.21to0.01)	-0.08 (-0.27to0.10)	-0.08 (-0.13to-0.03)	-0.03 (-0.10to0.04)
	6.0	0.24	-0.16 (-0.21to-0.11)	-0.17 (-0.38to0.04)	-0.13 (-0.53to0.27)	-0.16 (-0.26to-0.06)	-0.06 (-0.17to0.04)
False positive rate			Reference	3/9	2/9	1/9	3/9

(*BE higher than allowable bias)

False positive: Non interchangeable result of the simplified protocol when EP09-A3 result is interchangeable. False positive rate: non interchangeable magnitudes / number of magnitudes.

No false negative results were found. Protocol 3 showed the best agreement

Conclusion:

After the laboratory performs the whole verification protocol with the first analyzer, when facing a change in POCT blood gas network involving multiples devices, these simplified verification protocols could be used as a screening tool for the rest of devices.

When applying these protocols, if the expected bias is higher than allowable bias, it can constitute a trigger to initiate a more complete validation protocol

B-250

Comparison of Siemens DCA Vantage and Alere Afinion Point of Care Analyzers for Hemoglobin A1c Determination

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Background: Point of Care (POC) A1c testing may improve compliance with diabetes monitoring guidelines and promote better glycemic control in diabetic patients. We compared assay performance characteristics and ease of use of two CLIA-Waived POC analyzers for hemoglobin A1c testing: the Siemens DCA Vantage (Siemens Healthcare, Erglangen, Germany) which utilizes a monoclonal antibody agglutination reaction and the Afinion S100 (Alere Inc., Waltham, Massachusetts, USA) which is a boronate affinity assay. The two CLIA-Waived analyzers were compared to our internal central laboratory reference method, BioRad Variant II Turbo Hemoglobin A1c assay (BioRad Laboratories, Hercules, California). **Methods:** Precision was determined by performing 4 replicates each of 2 levels (low, high) of commercially available quality control material for 5 days. To test accuracy we compared capillary whole blood A1c on both POC devices to reference A1c in a venous sample in 32 diabetic patients and in 21 non-diabetic laboratory volunteers. Usability features such as footprint, required maintenance and turnaround time were also compared for the two point of care methods. Statistical significance of differences in mean bias between devices was determined using unpaired t test.

Results: Precision studies yielded coefficients of variation (CVs) on the Afinion of 1.8% and 1.3% and 2.8% and 2.5% for DCA at low and high levels. . Among the 53 comparison samples spanning a reference A1c range of 4.6-10.0%, mean bias on the Afinion was -0.06 ± 0.17 while mean bias on the DCA was -0.17 ± 0.16 (p=0.0007). Compared to the DCA, the Afinion also had a smaller footprint, no required maintenance, and a faster turnaround time of 3 minutes on the Afinion compared to 6 minutes on DCA.

Conclusion: Both devices demonstrated acceptable precision, and all capillary samples on both DCA and Afinion were within 0.5% of the paired venous A1C reference value. The Afinion showed less systematic bias and better usability features compared to the DCA.

B-252

Clinical Evaluation of the Next Generation I-STAT® Point-of-Care Instrument using a Sodium Test* *Submission pending FDA review

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Introduction: The next generation i-STAT is an *in-vitro* diagnostic instrument developed for Point-of-Care use with singleuse i-STAT cartridges to perform simultaneous quantitation of specific analytes in whole blood. A study was performed to evaluate the performance of the next generation i-STAT using the i-STAT sodium test. The study evaluated next generation i-STAT precision using the i-STAT sodium test and compared performance of the i-STAT sodium test on the next generation i-STAT to the i-STAT 1 wireless in a Point-of-Care setting. This was done using i-STAT E3+ cartridges. **Methods:** The clinical evaluation included multi-day precision, whole blood precision and method comparison using one lot of i-STAT E3+ cartridges. Multi-day and whole blood precision were completed at 3 sites. Multi-day precision was performed using aqueous solutions (i-STAT TriControls Calibration Verification Set) containing 5 levels of sodium. Each site tested the panel once daily for 5 days on 5 next generation i-STAT instruments. Whole blood precision testing was performed using venous whole blood samples representing low, normal and high sodium levels. At each site, each of 3 samples was tested 3 times on each of 7 next generation iSTAT instruments (for a total of 21 results per sample). Method comparison was performed at 4 sites. Testing was performed using whole blood samples prospectively collected

or leftover from routine patient care. Duplicate testing and analysis of 174 samples was performed on both the next generation i-STAT and i-STAT 1 wireless. Two next generation i-STAT instruments and two iSTAT 1 wireless analyzers were used for this testing. **Results:** Multi-day imprecision (Total SD) for Sites 1, 2, and 3 ranged from 0.29 mmol/L to 0.65 mmol/L, 0.41mmol/L to 0.62 mmol/L, and 0.37 mmol/L to 0.53 mmol/L, respectively. Whole blood imprecision (Total SD) across all levels for Sites 1, 2, and 3 ranged from 0.00 mmol/L to 0.50 mmol/L, 0.36 mmol/L to 0.45 mmol/L, and 0.30 mmol/L to 0.51 mmol/L, respectively. Method Comparison analysis was performed for the measuring interval of 100-180 mmol/L. For all four sites combined, the slope for Passing-Bablok regression results was 1.0; correlation coefficients for the first replicate result of the next generation i-STAT vs. first replicate result of i-STAT 1 wireless, first replicate result of the next generation i-STAT vs. mean result of i-STAT 1 wireless, and the mean replicate result of the next generation i-STAT vs. mean result of i-STAT 1 wireless are 0.998, 0.999, and 0.999, respectively. The Estimated Bias and Estimated Percent Bias for Passing-Bablok regression at all 3 medical decision points (115 mmol/L, 135 mmol/L, and 150 mmol/L) for all sites combined are 0.00. **Conclusion:** Multi-day and whole blood precision results demonstrate acceptable precision on the next generation i-STAT. Method comparison results demonstrate acceptable correlation between the next generation i-STAT and the i-STAT 1 wireless for whole blood specimens. Overall the clinical evaluation of the next generation i-STAT using the sodium assay demonstrated equivalent performance between the next generation i-STAT and the iSTAT 1 wireless in a Point-of-Care (POC) setting. The study was funded by Abbott Laboratories.

B-253

Analytical and Clinical Evaluation of the NowDx Whole Blood Qualitative hCG Device

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Background: Point-of-Care (POC) urine qualitative hCG devices are frequently used to rapidly assess pregnancy status but these devices are susceptible to false negative results caused by elevated concentrations of hCG beta core fragment (hCGβcf) that are frequently encountered during normal pregnancy. In this study, we evaluate the analytical and clinical performance of the NowDx qualitative hCG device approved for use with capillary fingerstick whole blood, a specimen that does not contain hCGβcf.

Methods: hCG-negative heparinized whole blood was spiked with purified hCG to generate samples with known hCG concentrations and the resulting samples were used to evaluate device sensitivity, low-end reproducibility, high-dose hook effect, linearity, acceptable specimen volume, acceptable hematocrit range and lot-to-lot variation. Device results were interpreted by ten laboratory technologists with a range of experience with qualitative hCG testing. To confirm the hCG concentrations of the spiked whole blood specimens, quantitative hCG measurement was performed in plasma isolated from each specimen using the Roche Cobas e602. Device performance was also prospectively evaluated in 40 pregnant and 40 non-pregnant women aged 18-44 in a hospital-based clinic or an academic hospital emergency department. Capillary fingerstick whole blood hCG results were compared to urine qualitative (Beckman Coulter Icon 20) and plasma quantitative (Roche Cobas e602) hCG test results generated during the same clinic or hospital visit. IRB approval was obtained for this study.

Results: 100/100 device observations were positive when used to test a whole blood specimen containing a plasma hCG concentration of 18 IU/L and 18/20 were positive at 17 IU/L. 20/20 device observations were positive at 2.2 x10^6 IU/L, although test line intensity began to decrease

above 6.0 x 10^5 IU/L. 100% of device observations were positive over a range of 18 IU/L to 1.2 x 10^3 IU/L and from 2.5 x 10^4 IU/L to 2.2 x10^6 IU/L but three invalid results were observed in the intermediate range (2/20 invalid at 5.7 x 10^3 IU/L and 1/20 invalid at 1.2 x 10^4 IU/L) due to decreased control line intensity. 60/60 observations were positive at a specimen volume ≥ 30 µL but 1/60 was positive at a specimen volume ≤ 25 µL. 20/20 observations were positive at a hematocrit of 46.2% and 16/20 were positive at 50.3%. The non-positive observations were invalid due to the absence of a control line. In 40 pregnant and 40 non-pregnant prospectively recruited women aged 18-44, the NowDx device generated 100% agreement with urine qualitative and plasma quantitative test results.

Conclusions: The NowDx qualitative whole blood hCG device demonstrates acceptable performance for the determination of pregnancy status using capillary fingerstick specimens.

B-254

Moving a Point of Care Program from Vision to Action

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A Point of Care (POC) program has the unique opportunity to bridge the gaps that exist between the laboratory and nursing in a healthcare institution. There are many challenges that need to be overcome in order to have a quality POC program. Our objective was to demonstrate the usefulness of a visual management board (VMB) in overcoming barriers, empowering a POC program to address challenges collaboratively within an organization. We implemented a VMB that allowed us to prioritize POC specific challenges within the organizational goals. The VMB was implemented in August of 2014 in the POC division as part of an organizational wide Lean management initiative. Goals were selected that aligned with the organizational scorecard. The priorities are categorized by focus areas such as: quality, patient experience, and employee engagement. The top 3 priorities are then displayed on the VMB in separate columns. It provides a visual display of the top level metrics, the problem analysis, and the quality metrics after improvements have been put in place. The organizational leadership rounds to the VMB to offer support and recognize accomplishments. We have found that the VMB has positively impacted patient safety, staff engagement, and leadership engagement. It allowed the problem solving process to be transparent and teachable within the department. First, POC was able to improve the utilization of scanning technology when identifying a patient for POC testing in the outpatient setting. A Pareto was used to analyze patient identification errors revealing that a majority of errors were caused by manual entry. Baseline metrics revealed that staff were using the scanning function only 8% of the time when identifying patients for POC glucose testing. An A3 problem solving tool revealed there was a lack of availability of a scannable barcode in the outpatient setting. The countermeasure required redesigning the patient visit label. Changes could not be implemented without understanding the unique needs of multiple departments. After implementation, scanning improved to 85.2% compliance. Second, we focused on improving critical glucose documentation by operators in the inpatient setting. This project is on its third A3 and there have been multiple countermeasures put in place. The baseline compliance was 12.1% in January 2015. The current compliance is 67.1%. A third A3 was completed and trending increased towards our scorecard goal of >90% compliance. Third, the POC team has worked together with nursing leadership and the electronic health record (EHR) team to develop countermeasures that will help staff be successful. Nursing leadership also developed an operator compliance policy in partnership with POC to standardize coaching and discipline. In conclusion, the implementation of a VMB in POC has fostered a collaborative environment and created more opportunities to build relationships across the organization. Each project has involved working with various disciplines and levels within the organization. It created a dynamic process improvement system that allowed the POC division to rapidly improve the overall quality of the POC testing program. The VMB gives an ongoing sense of purpose and drives the POC division's vision to action.

B-255

Development and Evaluation of The Transthyretin Assay for Point-of-Care Testing

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Background: The nutritional management for the elderly is getting more important than ever. The malnutrition results in the impairement of the immunity and the decreased muscle amount; as a result, it leads to the various problems such as the high morbidity from infectious diseases and the decline of the ADL. Therefore, the early detection of the malnutrition and the necessary medical intervention are important. Transthyretin (TTR), a rapid turnover protein (RTP) with a half-life of 2 days, is considered to be a sensitive indicator of the nutritional status. We have newly developed a rapid assay of TTR by immunochromatography using whole blood samples for the intended use at home or in clinic. In the prototype assay, the whole blood sample needed the two-step dilution which made the assay difficult to handle. To overcome this problem, we have made the device collecting a small amount (1uL) of blood followed by the one-step dilution and dispensing. In this paper, we report a performance of the sample collecting device and the TTR assay.

Methods: We first evaluated a quantity of whole blood by the sample collecting device. To characterize the analytical performance of the TTR assay, we evaluated the limit of Quantitation (LOQ), linearity, within-run precision, an effect of interfering substances, correlation with turbidimetry and nephelometry and comparison of blood collection sites. The fundamental assay performance was evaluated using control sera. For testing correlation, whole blood samples were measured by IC and the results were compared with measured levels of plasma samples by turbidimetry and those of serum samples by nephelometry. For the comparison of blood collection sites, we measured whole blood samples collected from antecubital vein, fingertip and earlobe in 10 healthy individuals.

Results: The precision of a quantity of blood collected by the device when measured 5 times was CV3.9% and average value was 1.26uL. The device shortened the sample preparing time to one third (the device: 24sec, the prototype method: 83sec). The LOQ of the TTR assay was 8.3mg/dL (CV≤10%). The linear range was 8.7mg/dL - 35.4 mg/dL, when the error of measured value is within +/-10%. The within-run precision of 10-time measurement of two sera with different concentration were CV6.1% and 8.6%, respectively, with an average value of 15.7mg/dL and 26.5mg/dL. Interference substances tested did not influence the measurement. The correlation between IC(y) and turbidimetry(x) was determined to be y=0.968x+0.883(R=0.958, n=43). The correlation between IC(y) and nephelometry(x) was determined to be y = 0.927x + 2.62 (r=0.935, n=64). There was no significant difference of measured values in each of the two groups by paired t-test (venous-earlobe p=0.108, venous -fingertip p=0.206, earlobe-fingertip p=0.781).

Conclusion: The sample collecting device made it possible to easily prepare the diluted sample. The TTR assay using the sample collecting device will be useful to evaluate the nutritional status of the elderly at home and in clinic and to monitor the effect of nutrition intervention.

B-257

Development of an assay for measuring biochemical parameters in 65-µL fingertip blood samples collected at home

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BACKGROUND

The prevention of lifestyle-related diseases is important for improving health and reducing medical costs in aged societies like Japan. Thus, we newly produced a test kit for performing health checkups based on 65-µL fingertip blood samples. The examinees collect the blood samples according to written instructions and isolate diluted plasma themselves. The samples are then mailed to a laboratory.

METHODS

1. The **DEMECAL kit**

The **DEMECAL kit** (Fujifilm, Japan) is composed of a tube, dilution buffer solution, a lancet, a blood-aspiration sponge, a cylinder with a blood cell separation filter, a cap, a swab, and a sticking plaster. The dilution buffer solution is composed of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and ethylenediaminetetraacetic acid dipotassium salt dihydrate (EDTA-2K).

2. Calculation of biochemical concentrations in diluted plasma

The plasma sample dilution ratio was measured by assessing the sodium level of each diluted sample using an enzymatic assay. The concentrations of each molecule in diluted plasma were then multiplied by the dilution ratio.

3. Measurement of sodium dilution

The dilution of plasma sodium was determined using an enzymatic rate assay involving β-galactosidase and *o*-nitrophenyl-*β*-galactopyranoside.

4. Instrument and measurement conditions

A JCA-BM6050 automated analyzer (JOEL Ltd., Japan) was used to obtain the biochemical measurements with commercially available reagents. The sample volume was greater than that of the original sample.

RESULTS

The sodium analysis exhibited good linearity from 0-25 mmol/L. The proportionality of the dilution rate of sodium was twenty fold admitted. The measurements were performed 20 times, and the mean co-efficient of variation (CV) at a dilution rate of 9.8-fold was 2.2%. To examine the within-run variation of the **DEMECAL kit**, 20 EDTA whole blood samples were analyzed as venous blood samples. The CV (%) of the examined parameters were as follows: aspartate aminotransferase (AST): 3.8, alanine aminotransferase (ALT): 2.6, gamma-glutamyl transferase (GGT): 2.3, total cholesterol: 2.3, high-density lipoprotein (HDL)-cholesterol: 2.4, low-density lipoprotein (LDL)-cholesterol: 2.3, triglycerides: 1.6, creatinine: 3.4, urea-nitrogen: 2.2, uric acid: 2.3, and glucose: 2.7. The correlations between the results obtained with the **DEMECAL kit** and venous plasma analysis were as follows: AST: 0.990, ALT: 0.998, GGT: 0.998, total cholesterol: 0.973, HDL-cholesterol: 0.987, LDL-cholesterol: 0.990, triglycerides: 0.999, urea-nitrogen: 0.993, creatinine: 0.966, uric acid: 0.994, and glucose: 0.994. The diluted plasma remained stable in three different temperature conditions (4°C, room temperature, and 37°C).

CONCLUSIONS

This measurement system can provide reliable clinical data about various molecules in diluted plasma derived from 65-µL samples of fingertip blood collected at home. The **DEMECAL kit** can be used to collect blood at distant locations, providing a suitable transport system is available, and hence, can contribute to nationwide healthcare provision. The **DEMECAL kit** can also be employed for other biochemical and immunity tests.

B-258

Basic Evaluation of a novel Glycohemoglobin Analyzer RC20 for POCT

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Background: An HbA1c (glycohemoglobin) test can check the long-term control of blood glucose levels, and is essential to diagnose prediabetes or diabetes. Therefore, HbA1c can be hopefully checked in clinics and small-sized hospitals as well as large hospitals. Glycohemoglobin Analyzer RC20 (RapidColumn A1c) is a compact autoanalyzer based on HPLC and can be used for point of care testing (POCT). Here we evaluated analytical performance of the glycohemoglobin autonalyzer.

Methods: Rapid Column A1c and its dedicated reagents (Sekisui Medical Co., Ltd.) were compared with HLC-723 G9 (Tosoh Co.).

Results: The within-run precision (CV) examined by using patients' specimens was 0.4%, and between-run precision using HbA1c control (Sekisui, JCCRM411-3, Sysmex Co.) for 8 days (n=2) was 0.6 to 1.0%. The trueness examined by using JCCRM411-3 level 1 to 5 for triple assay was below 0.12% for the bias and -1.21 to 1.00% for the relative error. The relation between the two analyzers examined by using patients' specimens was 0.995 for the correlation efficient and y=0.971x + 0.10 for the regression line.

Conclusion: The basic performances of Glycohemoglobin Analyzer RC20 were satisfactory. We assessed the device useful for POCT. In addition, the device has some excellent properties, for example easy handling and maintenance, user friendliness, small sample volume (3 microL), short measuring time (3 min), comfortable reporting and search function. The excellent performance could lead to improved patient care in POCT.

B-259

Activated Clotting Time (ACT): Comparison of the Hemochron Signature Elite and the Abbott i-STAT

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Background: ACT is commonly used for heparin anticoagulation monitoring during procedures including cardiopulmonary bypass surgery, coronary angioplasty, and interventional radiology. To prevent thrombosis, moderate to high levels of heparin anticoagulation are required. The Hemochron Signature Elite (HSE, Accriva Diagnostics, formerly ITC, San Diego, CA) was implemented at TJUH as a replacement for the older model Hemochron Response (Accriva/ITC Model HRS.110, San Diego, CA). Operating room (OR) perfusionists reported irreproducible high results using HSE that could not be explained clinically. In consideration of use of i-STAT analyzers (Abbott Point of Care, Princeton, NJ) as an alternative to HSE, we performed a comparison of ACT results as analyzed by HSE and i-STAT (Abbott Point of Care, Princeton, NJ) analyzers.

Methods: A comparison of inter-device reproducibility of results for each method was performed using the same samples for both the HSE and i-STAT (22 OR patient specimens, sampled across all analyzers within a 10 sec interval), measured in duplicate across two separate analyzers for each method. Measuring ranges were: HSE-68-1005 sec; i-STAT-50-1000 sec. Linearities for HSE (Kaolin ACT+ cartridges) and i-STAT (ACT-K cartridges) analyzers were assessed by heparin dilution curves. Precision for each method was measured by repeat testing of controls. In the duplicates experiment, 10 additional specimens were excluded because HSE results exceeded the HSE measuring range.

Results: Precision for i-STAT controls (n=40) were 3.6% CV and 3.8% CV (91-169 sec and 406-754 sec, respectively) across devices. These were comparable to precision for HSE controls (n=120), which were 5.5% CV and 2.3% CV (110-196 sec and 284-504 sec, respectively). A heparin curve demonstrated acceptable linearity for the i-STAT ACT therapeutic range (units heparin/mL = x, heparin response (sec) = y, R^2 = 0.9613, y = 99.607x + 174.61, range = 107-748 sec) and also for the HSE ACT therapeutic range. A comparison of inter-analyzer reproducibility between HSE and i-STAT showed significant differences, however. Among 22 specimens, the average % difference from the mean for HSE results across two analyzers (4.6%; median ACT = 477 sec, range 91-757 sec) was significantly different ($p < 0.002$) from that for i-STAT results (1.8%; median ACT = 374 sec, range = 106-951 sec). Correlation of average results was r^2=0.05881, 0.946x + 35.5.

Conclusion: Both i-STAT (ACT-K cartridge) and Hemochron Signature Elite (Kaolin ACT+ cartridge) analyzers showed acceptable linearity, and there was reasonable correlation between ACT results from each. However, we found that the iSTAT ACT-K cartridge performed with better precision and cross-device reproducibility than the Hemochron Signature Elite (Kaolin ACT+ cartridges) for patient samples. The i-STAT was subsequently chosen for measurement of ACT in the OR.

B-260

Performance of Creatinine and Chloride on the epoc Analyzer

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Background: The epoc Blood Analysis System (Alere, Orlando, FL) performs blood gases, electrolytes, and metabolites using a Blood Gas Electrolyte and Metabolite (BGEM) Test Card panel on 92 uL of whole blood. The BGEM test card uses potentiometric sensors to measure sodium, potassium, ionized calcium, pH, pCO2; amperometric sensors to measure pO2, glucose, and lactate; and a conductometric sensor to measure hematocrit. Results are available in 3-10 minutes, depending upon the time between calibration and patient testing. TJUH implemented the epoc in its ICUs in 2012 to provide Point of Care (POC) results. Alere recently added creatinine and chloride sensors to its BGEM cartridge. At the request of our Emergency Department, we evaluated creatinine and chloride on the epoc.

Methods: Precision and method comparisons were performed at our Center City (CC) and Methodist Hospital (MH) sites. Whole blood was collected from 40 CC patients and 24 MH patients. Inter- and intra-precision was performed on 3 levels of controls (Eurotrol, Burlington, MA). Comparison studies were performed on the RapidLab 800 (Radiometer, Brea, CA) at the CC site. Comparison studies were performed at the MH site on the GEM 3500 blood gas analyzer (Instrumentation Laboratories, Bedford, MA) and the Cobas 6000 (Roche, Indianapolis, IN) using plasma from concurrently drawn specimens.

Results: Intra-precision for creatinine on the epoc (n=20, 4 devices) was 6.6%CV, 2.1%CV, and 3.9%CV for Levels 1, 2, and 3, respectively, and 0.7%CV, 0.6%CV, and 0.5%CV, respectively for chloride. Inter-precision for creatinine (n=20, 4 devices over 5 days) was 3.9%CV, 2.0%CV, and 2.7%CV for Levels 1, 2, and 3, respectively and 1.1%CV, 0.5%CV and 0.5%CV, respectively for chloride. Method comparison for CC for creatinine (n=40) was: mean=1.636, median=1.135, r^2= 0.979, y=1.0343x+0.0317, and for chloride (n=40) mean=107.8, median=107.25, r^2=0.888, y=0.839x+17.634. Method comparison for MH for creatinine (n=24) was: mean=1.105, median=0.730, r^2=0.8944, y=1.1125x-0.1095, and for chloride (n=24) mean=103.667, median=104.000, r^2=0.8172, y=1.0275x+1.7272.

Conclusion: The epoc creatinine and chloride studies performed showed comparable results when compared to laboratory results and considered acceptable for implementation at those sites where epoc is currently in use.

B-261

The Stability of Color and Clarity in Urinalysis Quality Control Products

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Background: Color and clarity are important parameters in urinalysis. Changes in urine color can be caused by urine concentration, presence of blood, drugs, or ingestion of certain foods. The clarity of the urine is an important indicator of infection, but can also be affected by many other factors. This study was designed to determine whether clarity and color would remain stable in human urine preparations designed to act as quality control products for urinalysis.

Methods: : Human urine from multiple donors was combined and preserved using antibiotics to prevent microbial growth during the duration of the study. The combined urine was then split into two pools. The first pool was held without further modification and designated the normal pool. The second pool was further modified by the additions of reagents to produce a positive reaction on all the pads found on Siemens Multistix® 10SG reagent strips. This was designated the abnormal pool. The urine was then dispensed into either 15 mL glass tubes (dipper format) or 25 mL plastic bottles with a dropper tip (dropper format). The filled tubes and bottles were held at either 2-8 or 25°C. The Dipper tubes were dipped into 20 times using the Multistix 10SG. Color and clarity were determined using a Siemens Clinitek 500 analyzer. This study was repeated three times.

Results: Color and clarity were determined to be yellow and clear in both the normal and abnormal urine pools at the beginning of the study for both the dipper and dropper format. The pools remained yellow and clear for up to 18 months when stored at 2-8°C. They also remained unchanged for up to 48 days when stored at 25°C. It was found that 20 dips of the Multistix 10SG urinalysis strips into the dipper tubes did not change the color or clarity of either the normal or the abnormal pool, when using the dipsticks as per the manufacturer's instructions. The addition of the reagents to the abnormal pool including; protein, bilirubin and urobilinogen reactive compounds, and hemoglobin did not lead to detectable changes to the color or clarity of the material either initially or over time at either of the tested temperatures.

Conclusion: The results of this study demonstrate that color and clarity are stable in both the normal and abnormal pools of preserved human urine for up to 18 months when stored at refrigerated temperatures and up to 48 days when stored at room temperature. In addition, the color and clarity of the pooled urine placed in Dipper tubes is not affected by up to 20 dips using Multistix 10SG reagent strips. These results demonstrate that the color and clarity parameters can be incorporated into the quality plan of laboratories performing urinalysis using the Siemens Multistix 10SG strips and the Clinitek 500 reader.

B-262

Development of a Novel Quantum Dot-based Immunoassay for Point-of-Care Testing

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Background: There is a need for point-of-care testing (POCT) of common lateral flow immunoassays that can do all common diagnostic tests reliably, high sensitive, and low in cost (device and consumable cost). Here we have developed a novel quantum dot-based immunoassay for POCT. The tunable optical and physical properties of semiconductor quantum dots (QDs) based on their size and shape have attracted considerable interest for biological and medical applications. Due to QD's highly photoluminescent quantum yields and stability, it is potentially a good candidate to replace organic dye as new generation fluorescence labels and can be widely used in POCT.

Methods: Starting hydrophobic CdSe/ZnS core-shell QDs were prepared according to low-cost, green, phosphine-free method. Then, an amphiphilic oligomer was used to prepare water-soluble CdSe/ZnS QDs. Certain amount of oligomer and QDs was dispersed in chloroform and stirred for 1 h (room temperature, molar ratio of QDs/oligomer was 1:2) in a flask. After stirring, chloroform was gradually evaporated, and a clear solution of water-soluble QDs was obtained after the pH value of solution was kept between8-10. After the conjugation with selected antibodies by an EDC/NHS-mediated course, such QD-based photoluminescent probes was stored at 4degree before use. To prepare QD based-lateral flow immunoassays strips, selected antibody was dispensed onto a nitrocellulose membrane as a test line and the QD probes and selected antigen were dispensed onto a sample pad by using the XYZ Dispensing System (BioDotInc, Irvine, CA). The sample pad was treated with 10 mM sodium phosphate (pH 7.4) buffer, BSA (0.5%, w/v) and Triton-X100 (2%, w/v) and dried before the dispensation. The membrane and sample pad were then dried overnight at room temperature under 10% relative humidity condition. Then the membrane and sample pad were assembled and cut into strips with a width of 3 mm/strip by using the CM4000 Guillotine Cutter (BioDotInc, Irvine, CA). To perform the detection, different test solutions were added to the end of the sample pad. The QD fluorescence signal on the test line was observed by home-made photoluminescent detection device.

Results: Data analysis indicates that the QD-based lateral flow immunoassays have a CV <15%.The linearity fell in the range of 0-200 mg/L of C-reactive protein (CRP), and the analytical detection limit was 0.048 mg/l within 5 min. The mean recovery of the control was 102.63% in a working range. Through cross-reactivity, it can be out of interference of bilirubin, hemoglobin, lipoidaemia, rheumatoid factor. The QD-based lateral flow immunoassays correlated well with Tina-quant CRP (Latex) for quantification of CRP concentration (r = 0.956, N = 213).

Conclusion:The developed technology platform for QD-based lateral flow immunoassays is reliable with high sensitivity. It meets all the performance specifications of POCT. Hence, we believe it can be easily applied for low cost, sensitive, quantitative, and rapid detection of common diagnostics tests for infection diseases (such as CRP, Procalcitonin, HIV, Syphilis, Hepatitis, *etc*), tumor, and chronic cardiovascular/cerebrovascular diseases (like Myo, cTn, CKMB, AST, LDH, *etc*).

B-263

A Quantum Dot-based Fluorescence-linked Immunosorbent Assay for Rapid Disease Detection

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Background: The conventional and widely used enzyme-linked immunosorbent assays (ELISA), due to fast and high-sensitive, were suitable *in vitro* diagnosis and a large number of samples screening. In this study, we have developed a novel quantum dots-labeled immunosorbent assay for rapid disease detection. Semiconductor quantum dots (QDs) have been successfully used in biological and medical research, with high luminescence and high resistance to photobleaching. Furthermore, the developed immunoassay has immense potential for the development of rapid and cost-effective *in vitro* diagnostic kits.

Method: The aqueous CdSe/ZnS QDs was conjugated with antibody to produce QDs-Ab probes using EDC and NHS as coupling reagents. Then, the QDs-Ab was purified by centrifugation and the product was stored at 4degree before use. The monoclonal antibody (mAb) was immobilized on a standard 96-well microplate by the following protocol: Primary antibody was diluted in 50mM carbonate-bicarbonate buffer (pH 9.6) and incubating 24h at 4degree. Then excess binding sites were blocked with BSA (0.5%, w/v) in 10mM PBS (pH 7.4) incubating overnight at 4 degree after removing excess coating antibody by washing three times with washing buffer(10mMPBS containing 0.05% Tween-20, PBST). Then different concentrations of antigen (Ag) were captured by the mAb immobilized on the microplate. At last the QDs labeled detection antibody is introduced to form a mAb-Ag-mAb sandwich complex. Within a certain range, the fluorescence intensity which can be read out using the SpectraMax i3 Multi-Mode microplate reader was enhanced gradually with the increase of antigen concentration.

Results: Through the optimization of reaction conditions, we established a new detection method *in vitro* with the reaction time regulated within 50min,which is shorter than the commercialization ELISA. The novel assay provides a linear analytical range, such as C-reactive protein (CRP) assay range of 0-400 ng/mL with a detection limit of 1.61 ng/mL and procalcitonin (PCT) assay range of 0-100 ng/mL with a detection limit of 0.09 ng/mL. The precision of the assay has been confirmed for low coefficient of variation (CV), less than 10% (intra-assay) and less than 15% (inter-assay), and together with recoveries of 85-105%. Through interferences and cross-reactivity, it can be out of interference of bilirubin, hemoglobin, lipoidaemia, and rheumatoid factor.

Conclusion: This developed analytical method meets all the needs of the rapid, sensitive, and high-throughput determination of inflammation factors (CRP and PCT). It has not only shortened the reaction time, but also simplified the operation steps, and more important, the detection sensitivity was greatly improved. This result indicates that the developed method can be applied to rapid disease detection effectively.

B-264

Clinical Equivalence of the VerifyNow PRUTest and P2Y12 - Target Values for Patients Treated with Thienopyridine Inhibitors

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VerifyNow (VN) uses light transmission technology to measure platelet function in whole blood and the effectiveness of specific platelet inhibitors. The thienopyridine class of inhibitors including clopidogrel and prasugrel are used in acute coronary syndrome (ACS) to block the ADP platelet P2Y12 receptor, thus reducing platelet induced vascular obstruction. The native VN P2Y12 test has been used in large randomized trials, including GRAVITAS, which defined the P2Y12 value of 208 as an effective platelet inhibition for reducing thrombosis and ischemia. PRUTest (P2Y12 Reaction Units) is the next generation assay. This study was undertaken to demonstrate the equivalence of the two assays to ensure transference of the ACS trial information to the current PRUTest platform. Following an initial direct method comparison study, in which normal donors were evaluated showing equivalence of the PRU lower limit reference range (x = 183; 2.5 centile; 90%CI= 169-198, N=147) and P2Y12 (x = 180; 2.5 centile 90%CI=164-197, N=84), a survey of PRU values in an ACS platelet inhibited population was conducted. The table below demonstrates PRU mean values, SD, central 90% CI for untreated (N=231) and treated (N=101) patients with drug dosage shown for clopidogrel, prasugrel and aspirin, usually given in a dual antiplatelet regimen.

Parameter	No treatment	Clopidogrel	Prasugrel
N	231	71	30
Mean	297	160	98
SD	56	73	71
2.5th centile (90% CI)	186 (175-196)	12 (-11-37)	3.5*
97.5th centile (90% CI)	408 (398-419)	307 (283-330)	268*
Mean aspirin dose (SD)	NA	167 (117)	171 (120)
Mean P2Y12-RI dose (SD)	NA	79 (28)	13 (13)
*Calculated by non parametric percentile method (CLSI C28-A3). All others by "robust" method			

Summary: The P2Y12 receptor inhibitors clopidogrel and prasugrel inhibit platelet aggregation measureable as a statistically lower PRU range compared to that of the untreated ACS population. In the study population 19.0% of clopidogrel treated patients did not achieve a PRU value <200 PRU; which coincides with medical literature which cites 20-25% patients retain high residual platelet reactivity (HRPR) on clopidogrel ("clopidogrel resistance"). These patients are at a much higher risk of cardiovascular events, e.g., myocardial infarction and stroke and require more aggressive antiplatelet drug treatment. The VerifyNow PRUTest provides the means to identify and properly manage clopidogrel effectiveness.

B-265

Evaluation of the LABGEO PT10 point-of-care testing: Comparison between capillary whole blood and lithium heparin whole blood

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Background: Point-of-care (POC) testing device has been widely used because of its rapid availability of results making diagnosis and management as early as possible. Collection of venous blood samples through venipuncture requires technical expertise of sample. However, capillary blood can dramatically reduce the difficulty of obtaining samples and allows the prompt testing results facilitating patient management even faster. In this study, by comparing the measurements in Samsung LABGEO PT10 using capillary blood with those using lithium heparin whole blood and in central laboratory using serum, we evaluated the usefulness of capillary blood in Samsung LABGEO PT10.

Method: A total of 51 patients and 18 healthy adults aged between 20 and 65 were enrolled. Capillary and venous blood samples were collected after informed consents were obtained. Venous blood samples were split into lithium heparin tube and serum separating tube. Measurements using capillary blood and lithium heparin whole blood were performed in LABGEO PT10. Venous samples in serum-separating tube were centrifuged and serum was used for measurement by Toshiba 2000FR NEO in central laboratory duplicate. Comparison of measurement results were as follows; 1) Measurements in LABGEO PT10 using capillary blood and lithium heparin whole blood, 2) Measurements in LABGEO PT10 using lithium heparin whole blood and in central laboratory using serum, and 3) Measurements in LABGEO PT10 using capillary blood and in central laboratory using serum.

Results: In comparison between measurements in LABGEO PT10 using capillary blood and lithium heparin whole blood, the slope ranged between 0.9289 and 1.0795, R^2 was over 0.95 except albumin, high density lipoprotein and total protein. Comparison between measurements in LABGEO PT10 using lithium heparin whole blood and in central laboratory using serum revealed that the slope ranged between 0.6255 and 1.1602 except alkaline

phosphatase, R^2 was over 0.95 for most of anlaytes. And in comparison between measurements in LABGEO PT10 using capillary blood and in central laboratory using serum, the slope ranged between 0.6433 and 1.1364 except alkaline phosphates, R^2 was over 0.95 for most of analytes. In Bland-Altman analysis, all measurements were within 95% limit of agreement.

Conclusion: Measurements in LABGEO PT10 using capillary blood was well correlated with those in LABGEO PT10 using lithium heparin whole blood and also with in central clinical laboratory using serum. In conclusion, capillary blood provides reliable measurements across a clinically relevant range and can be trustfully used in LABGEO PT10.

B-266

Use of the Piccolo XPress (Abaxis) as a point-of care analyser in an ebola setting

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Introduction The Piccolo Xpress (Abaxis) is a CLIA-waived portable point-of-care analyser, able to perform multiple assays simultaneously on a single-use reagent disc, after the addition of 100 µL Li-heparin whole blood, plasma or serum. This device was placed in the laboratory of the Ghent University Hospital for testing of blood from suspected ebola patients. The aim of this study was to carry out a performance characterization of the AmLyte 13 reagent disc (albumin, ALT, amylase, ALST, total bilirubin, calcium, CK, creatinine, CRP, glucose, K, Na and blood urea nitrogen) on the Piccolo Xpress.

Methods Accuracy (n=10) was tested on 2 levels Abaxis Chemistry quality control material. Within-run and between-run imprecision were assessed with both quality control material (n=10) and 2 patient samples (n=6). Method comparison was performed on 70 whole blood Li-heparin samples (50 ICU patients, 20 healthy volunteers) against the routinely used core laboratory Cobas 8000 chemistry analyser (Roche Diagnosics) and the RP450 blood gas analyser (Siemens) for glucose, K and Na. Check of reference values was done on 20 healthy volunteers and lot-to-lot comparability was verified on 11 samples.

Results Imprecision and accuracy did not all fulfil Westgard acceptance criteria, with i.e. AST, ALT and creatinine suffering from high variability on patient samples (11%, 11% and 9% respectively) However, this variability was considered to be acceptable for the purpose of this device. Method comparison showed a good correlation to both Cobas 8000 and RP450, with exception of sodium (correlation coefficient 0.80 compared to Cobas and 0.75 to blood gas analyser), which showed a high scattering of results on the Bland-Altman plot. A significant bias was observed (Piccolo vs Cobas) for both albumin (-17%; to be explained by differences in methodology) and amylase (-22%). Check of reference values was within criteria for all parameters, however very wide reference intervals were observed for both albumin (33 - 55 g/L) and sodium (128 - 145 g/L). Wilcoxon test for paired samples was performed for lot-to-lot comparability of the 13 different parameters and although statistically significant differences were observed for some assays, none were found to be clinically significant.

Conclusions The point-of-care Piccolo Xpress analyser of Abaxis is easy to use and demonstrates acceptable performance for the tested AmLyte 13 reagent disc. Significant differences were found for albumin and amylase compared to the core lab Cobas 8000 chemistry analyser, and hence, these results are not 'as such' interchangeable. Sodium results on the Piccolo show a high variability and are not reliable for routine practice. We conclude that the Piccolo Xpress is suitable for analysis of critical sera from suspected ebola patients in an urgent setting.

B-267

Development and Performance Evaluation of urinary soluble CD14 immunoassay on FREND system

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Background: FREND System is a portable FREND cartridge reader which is based on immunoassay technology capable of quantifying single or multiple analytes by measuring laser-induced fluorescence in a single-use disposable reagent cartridge. CD14 is a component of innate immune system which recognizes pathogen-associated molecular patterns such as LPS. Soluble CD14 (sCD14) is soluble form of CD14 and it either appears after shedding of membrane anchored CD14 (mCD14) or is directly secreted from intracellular vesicles. Recently, it has been reported that urinary sCD14 had a high predictive value for rheumatoid arthritis (RA) disease activity when combined with a conventional serum biomarker. We developed a fluorescence immunoassay (FREND uCD14) to measure the level of urinary sCD14 on the FREND System, which may serve as a valuable monitoring tool for RA patients. **Objective:** The objective of this study is to evaluate the analytical performance of FREND™ uCD14 assay. **Methods:** The imprecision, linearity, method comparison and detection limit of FREND uCD14 were evaluated according to CLSI guidelines EP05-A3, EP 06-A, EP 09-A3 and EP 17-A2. The FREND uCD14 assay is traceable to the calibrators prepared with the recombinant soluble CD14 (R&D systems 383-CD-050). For the method comparison, aliquots of urine samples over the measuring ranges were measured with FREND uCD14 assay on NanoEnTek FREND™ system. The comparative assay was Human CD14 DuoSet ELISA (DY383, R&D systems) on TECAN Infinite M200 Plate reader. For the clinical performance evaluation, urine samples were collected from the patients with written consent who visited St. Mary Hospital. Urine samples from RA patients were tethered to the disease activity score 28-joint assessment (DAS28) which is measurement values of the disease activity from RA patients. Additionally, 100 apparently healthy subjects were enrolled for establishing the reference interval. **Results:** The imprecision for urinary sCD14 assay produced coefficient of variation (CV) of <10% (range 5.8-9.9%) at concentrations of 51.23, 182.90 and 398.16 ng/mL. The AMR of the assays were 10 ~ 500 ng/mL with ordinary least squares regression fit of y=1.0255x - 2.1767 (r^2=0.9898). LoD was determined to be 6.077 ng/mL. In the method comparison studies with R&D Systems Human CD14 DuoSet ELISA, the correlation coefficient(r) was 0.9956 (95% CI= 0.9930 to 0.9972), and the slopes /intercepts were 1.015 (95% CI= 0.9868 to 1.0441)/-1.3493(95% CI= -4.5261 to 1.9733) by Passing-Bablock regression fit. **Conclusion:** Data indicates that the newly developed FREND uCD14 assay exhibits reliable analytical performance and can be useful as an easy-to-use urinary RA monitoring kit. Clinical studies for Receiver-Operating Characteristic (ROC) curve analysis and reference interval establishment will be conducted shortly. **Acknowledgements:** This work was supported by grants from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs (No. HI14C3417).

B-268

A new one-step direct-sampling HIV antibody rapid test.

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Background: The human immunodeficiency viruses 1 & 2 are the retroviruses that cause acquired immunodeficiency syndrome (AIDS). There are a number of tests that are used to determine whether a person is infected with HIV. They include the HIV antibody test, P24 antigen test and PCR test for HIV RNA or DNA. HIV antibody tests are the most appropriate test for routine diagnoses of HIV infections. Most people develop detectable HIV antibodies within 6 to 12 weeks of infection. There are many commercially available HIV antibody tests intended for use either in the laboratory or rapid tests for use at the point-of-care. Most rapid tests are multi-step in their operation requiring, for example, the addition of a chasing buffer or a pre-run dilution in order to complete the test. Each step possibly contributes to operator error and additional labour costs. However, a rapid test that is single-step in operation and requires only a small volume of whole blood and without the need of a sample transfer device, is ideal for point-of-care as well as self-testing for HIV infection. The objective of this study is to demonstrate the clinical utility of a new one-step HIV antibody test.

Principle: The ADEXUS-Dx HIV-1/2 Antibody Test was developed using a direct sampling immunoassay technology for whole blood, plasma or serum. HIV -1/2 recombinant antigens were employed for the detection of both HIV-1 and HIV-2 antibodies. A small sample volume (35µL) is required to run the test and no extra buffer is needed. Capillary blood from a finger tip can be directly applied to the test without any transfer device. When the sample is sufficient to fill a built-in receiving channel, it flows into a dry porous test strip composed of a membrane array with gold conjugated HIV antigens. The appearance of one or two visible purplish-red band(s) at the test region indicates the sample contains a detectable level of HIV antibodies for HIV-1 and/or HIV-2.

Performance: The ADEXUS-Dx HIV-1/2 Antibody Test requires less than 40µL of sample and was completed in 15 minutes without any additional step. Testing with the WHO HIV Antibody Reference Panel confirmed that the test recognized HIV-1 subtype A, B, C, E, Group O antibodies as well as HIV-2 antibodies. Clinical studies of the ADEXUS-Dx HIV-1/2 Antibody Test were conducted in three different countries: Brazil, Cameroon and Senegal. A total of 937 clinical samples (442 positive and 495 negative) were tested. There was 100% agreement for HIV antibody positive samples and 99.8% agreement for HIV antibody negative samples. Testing of a characterized 15 member low titer panel showed 14 positive samples.

Conclusion: The ADEXUS-Dx HIV-1/2 Antibody Test is a true one-step rapid test with excellent sensitivity and specificity. It is suitable for use in the detection of HIV infection at the point-of-care and for self-testing.

B-269

Design and evaluation of a mobile nucleic acid amplification testing system in a hospital emergency setting

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Nucleic acid amplification tests (NAAT) possess remarkable sensitivity and speed capable of producing actionable results in an hour or less. However, today's clinical environment based on batch processing of samples in centralized laboratories introduces delays on the order of days or more. We previously reported the development of a low-cost mobile nucleic acid analysis platform for streamlined analysis of vaginal swabs for chlamydia screening. Here, we report the development of a comprehensive mobile phone user interface and evaluation of our platform in hospital emergency rooms by naïve operators. The platform consists of three components: a disposable cartridge, a phone charger-powered handheld instrument and a mobile phone user interface. The cartridge is designed based on the principle of droplet magnetofluidics which enables integration of sample preparation with amplification without sophisticated fluidic manipulation. Each cartridge costs less than $2 to manufacture, which is an order of magnitude cheaper than currently available molecular POC tests. Fluorescent LAMP assay is implemented on the cartridge, yielding analytical sensitivity of 10^2~10^3 copies of gene targets and excellent specificity against a panel of vaginal flora and human genomic DNA. The phone charger-powered instrument coordinates thermal incubation and magnetic particle manipulation via microcontroller-driven thermoelectric module and a servomotor. Initial validation using a panel of 20 blinded vaginal swabs from clinical sample archives was evaluated against the standard of care NAAT assay, yielding full agreement. In order to facilitate easy access to the POC platform, we developed a comprehensive user interface around a mobile phone app. The interface consists of three features including a tutorial module, data archive and a 1-click test routine capable of acquiring POC data in a digital format. The tutorial provides concise video instructions outlining platform design and operation for naïve operators. The entire platform workflow takes up to 72 minutes from start to finish for a first-time user, including 6 minutes of user tutorial, 60 minutes of incubation and minimal hands-on time. Samples tested to this date amplified within the first 40 minutes, suggesting a workflow that is capable of delivering results within an hour of collection. Patients visiting the emergency department at Johns Hopkins Hospital were recruited in a chlamydia POC study, where two sets of swabs were collected during pelvic examination. One set was analyzed using the gold standard Gen-Probe AC2 CT assay. The second set was aliquoted and evaluated using the POC NAAT platform deployed in the emergency room. The two results were in agreement for 30 out of 30 samples, demonstrating that the POC assay performance is comparable to the gold standard for the samples tested. Subsequently, the remaining aliquot of tested samples was evaluated by a research staff member at the emergency department, whose sole exposure to the POC platform was via training module embedded in the mobile phone app. A total of 13 samples were evaluated, with results in full agreement with the standard of care assay. This study illustrates the potential utility of nucleic acid tests that are both mobile and user-friendly in a clinical environment.

B-270

The Variance between Point-of-Care (POC) and Clinical Chemistry Laboratory Glucose Testing Results in Critically Ill Patients

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Background: It is desirable that the results of POC glucose testing are comparable to those measured by clinical chemistry laboratory method in all patients. FDA recently proposed guidelines states that the current glucose meters may not be used for critically ill patients as the accuracy of the results in this patuent population is unknown. The objective of this study is to determine if the currently used glucose meters meet the FDA new criteria in critically ill patients.

Methods: Glucose was measured by POC glucose meters (FreeStyle Precision Pro, Abbott) and clinical chemistry analyzers (ARCHTECT c16000, Abbott) with a difference in blood sample collection of ≤ 5 minutes. POC glucose tests were performed using finger stick or arterial/venous blood. Electronic medical records of these patients were accessed to determine if they fall under any one or more of the following categories: hypotension (BP < 90/60 mm Hg) and receiving vasopressors. The variances between POC test results versus clinical chemistry analyzers were calculated. Fisher's exact test was used for statistical analysis for the percentages of patients with the variance greater than 10% between testing and control groups as well as between finger stick and arterial/venous subgroup. $P < 0.05$ was considered statistically significant.

Results:

Control		**Vasopressor**				**Hypotension**			
Finger Stick (N=60)		Arterial/Venous (N=49)		**Finger Stick (N=54)**		Arterial/Venous (N=68)		Finger Stick (N=80)	
≤ 10%	> 10%	≤10%	> 10%	≤10%	> 10%	≤ 10%	> 10%	≤ 10%	> 10%
35 (58%)	25 (42%)	39 (80%)	10 (20%)	25 (46%)	29 (54%)	47 (69%)	21 (31%)	47 (59%)	33 (41%)

For finger stick, the p values for hypotension vs control and vasopressor vs control were 0.543 and 0.086, respectively. In the vasopressor and hypotension groups, the p values between arterial/venous and finger stick subgroups were 0.0006 and 0.2313, respectively.

Conclusion: The results of this preliminary study suggest that the current glucose meters do not meet the accuracy criteria proposed by the new FDA guidelines for both general and critically ill patients.

B-271

A Rapid Dilute and Shoot-Flow Injection Tandem Mass Spectrometric Method for Quantification of Phenobarbital in Urine

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Background: In recent years, UDT (urine drug testing) is the mainstays for drug compliance monitoring/ abuse in pain management. Although, LC-MS/MS is considered as a gold standard for UDT, these methods are highly time consuming and require 2 separate LC-MS screening procedures, since most of the pain drugs are usually split into positive mode and negative mode panels, where majority of the drugs are positively ionized and only barbiturates and EtG (ethanol metabolite) ionize better in negative mode. Due to this, completely separate and time consuming LC-MS/MS runs were applied just to analyze two drugs, where approximately 50 positively ionizing drugs can be analyzed in the same time frame, utilizing different buffers, mobile phases and chromatography columns. In order to address this, we have developed a fast and robust tandem mass spectrometric method to analyze and quantify phenobarbital in urine in 2 minutes with a simple sample preparation: 10 times dilution with deionized water, with no chromatographic separation.

Methods: The samples were rapidly prepared by simple one step dilution of blank urine (mixture of 6 lots) spiked with phenobarbital followed by flow injection of sample to mass spectrometer using 5mM Ammonium acetate/70% Acetonitrile as a carrier solvent, without HPLC. Quantification and detection of phenobarbital was achieved via mass spectrometry analysis by electro-spray ionization triple-quadrupole mass spectrometry in multiple reaction monitoring mode employing a stable isotope-labeled internal standard (phenobarbital-d5). **Results:** The validated method was linear at the dynamic range of 5-200ng/ml with correlation coefficient > 0.9998. The coefficients of variation and relative errors for intra and inter assay at four QC levels (i.e., 5, 12.5, 45 and 160 ng/ml) were > 3.0% and > 5.0% respectively. The major advantages of our method are: (1) Simple dilution, as sample preparation, (2) FI-MS/MS analysis (no HPLC) of phenobarbital in urine with 2 minutes run time enabling much higher throughput.

Conclusion: The acquired results proved that this novel method is simple and robust, has the capacity to process 720 samples/day, and can lead to the transfer of existing methodologies to the newer robust platforms; application of this method can permit rapid screening of multiple pain drugs in urine with short sample preparation recommended for clinical UDT studies. Nevertheless, no change in the signal intensity or sensitivity was found even after 1200 injections proving that this method was robust.

B-272

Validation of the Hemochron MCS 7000; A new Point of Care Coagulation Instrument for Heparin Anticoagulation Management

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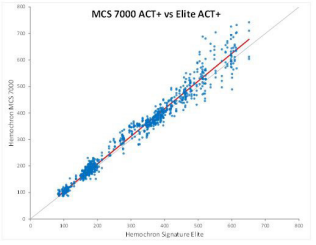
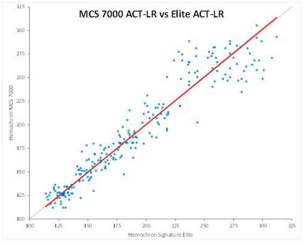
The Hemochron MCS 7000 (MCS) is the next generation Hemochron® point-of-care microcoagulation instrument capable of assessing heparin anticoagulation using either the ACT+ (high range therapy) or the ACT-LR (low range therapy). The clot detection method uses a high optical resolution camera for end point identification yielding improved precision and accuracy. The MCS system also uses unique bar coded ACT+ and ACT-LR test cuvettes results to ensure assay type identity with each test and cuvette expiration dating. Critical to the transition to the new system

is ensuring comparable results with those obtained with the predicate Hemochron® Signature Elite (Elite) instrument. In a laboratory based validation protocol, donor blood samples were spiked with heparin and tested using ACT+ cuvettes (n = 794) and ACT-LR cuvettes (n = 227) on both the MCS and the Elite. Reference Range was identical in the two systems. Across the reportable range, the weighted Deming fit showed a correlation of $R^2 = 0.97$ and a slope 1.04 for ACT+ and a correlation of $R^2 = 0.91$ and a slope 1.01 for ACT-LR, demonstrating the clinical equivalence of results.

Summary: This validation demonstrated the equivalence of ACT results with the MCS and Elite ensuring consistency of clinical use and heparin management. The MCS expands the user capabilities of the Elite system, offering state-of-the-art graphical user interface (GUI) with a capacitive color touch screen, wireless communication (Wi-Fi & Bluetooth), POCT1A compliance to support network connectivity, a 2D bar code scanner, user replaceable battery, expanded database for test record storage, and QC lockout menu. An additional feature is a browser based configuration manager allowing broadcast system configuration to multiple instruments.





B-273

"Inconclusive qualitative pregnancy test, followed by quantitative pregnancy test of hcg with discrepancies between the values and the real clinical pregnancy status"

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Objectives: The aim for this study is to assess a false negative in pregnancy identification by the qualitative point of care (POC) devices, when compare to the human chorionic gonadotropin (hCG) quantitative assays and to the chart review. The POC device cut off for HCG concentration above which POC shows positive results is 10IU/dL. **Relevance:** Females at reproductive age are tested for a possible pregnancy before diagnostic or therapeutic procedures which might be contraindicated in the case of positive results. An accurate diagnosis of pregnancy should be pursued to avoid possible teratogenic effect or life treating complication causing by a missing ectopic pregnancy. **Methodology:** The POC *using Sure-Vue® Serum/Urine hCG-STAT* pregnancy test, is a rapid chromatographic immunoassay for qualitative detection of hCG utilizes mouse monoclonal anti alpha subunit-hCG and goat monoclonal anti beta subunit- hCG, using Sure-Vue® antibodies. The manufacture's instruction advises to evaluate the results of the test after 5-6 minutes. Because large number of ambiguous results had been detected, a pilot study was performed which showed discrepancy of the results between readings after 5 and 10 minutes of incubation. The sample with such discrepancy was resulted as an "inconclusive". Subsequently, 124 patients form the University Hospital at San Antonio with inconclusive results of a serum qualitative pregnancy test were reflexed to the HCG quantitative testing and for the chart review. **Validation:** The charts review confirmed viable pregnancies, complete or incomplete abortions in 18 of 124 females with inconclusive qualitative pregnancy test (viable pregnancies= 7; miscarriage=11). In addition, qualitative test showed low accuracy (86.2%) when compared with the gold standard (quantitative tests) since the reading at 5 minutes missed 17 patients who presented higher levels to the cut off (≥ 10 IU/dl). Due to the retrospective character of our study we couldn't assess an accurate relation between those 17 patients and their pregnancy status. **Conclusions:** Qualitative pregnancy test (read at 5 minutes) yield 124 negative results that switch to positive when read at 10 minutes, among which 18 females from this population were found to be pregnant (NPV=85.4%). 17 out of the 124 patients with inconclusive results have a positive quantitative test (hCG ≥10-31 mIU/dl). A common cause for false negatives results by qualitative test is the low accuracy of used devices when compared to the chemistry instrumentation. To improve a detection of the pregnancy in the female patients in the hospital settings, serum HCG quantitative testing should be offered, or detailed validation of qualitative POC device should be performed before the assay is offered to the patients. The cut off for positivity of such devices should be set at the level, which guarantees a maximal negative predictive value performance.

B-275

A rapid H-FABP test for qualitative measurement of H-FABP in serum, plasma and whole blood.

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Background: Human cardiac FABP ("**H-FABP**") is one of the most abundant proteins found in cardiomyocytes. H-FABP is mainly responsible for the transport of fatty acids. In the event of an acute myocardial infarction ("**AMI**"), the concentration of H-FABP in blood rises within 1-3 hours and returns to normal after 24 hours. Therefore, H-FABP is clinically useful as an early biomarker of AMI. According to industry literature, 99% of normal populations have an H-FABP level below 6 ng/mL. An increased level of FABP in circulation correlates with an increased risk of cardiac muscle damage. Early diagnosis is important because timely treatment of an AMI significantly improves the prognosis of a patient. An easy-to-use rapid test for the detection of H-FABP can facilitate an early diagnosis of an AMI. The objective of this study is to evaluate the performance of a new H-FABP rapid test.

Principle: The ADEXUS-Dx H-FABP Rapid Test ("**H-FABP Test**") is a solid phase immunochromatographic assay. The H-FABP Test uses a sandwich format to detect the presence of H-FABP above an established reference concentration in blood, plasma, and serum samples. The appearance of a purplish-red band in the test window indicates that the sample contains H-FABP above normal levels. The H-FABP Test has a unique feature of finger-stick, capillary whole blood sampling needing only 35 µl blood.

Performance: The H-FABP Test was negative to other forms of FABP, including liver-FABP, intestine-FABP, adipocyte-FABP, epidermal-FABP, ileal-FABP, brain-FABP. There is no hook effect at the highest H-FABP concentration present in patient serum (700 ng/ml). Sera containing human anti-mouse antibodies (HAMA) up to 327 ng/mL tested negative suggesting minimal interference by HAMA in a normal population based on the reference range for HAMA (0-188 ng/mL). The same test results were obtained for serum and plasma samples, the later of which were unaffected by anti-coagulants. A method comparison study between the H-FABP Test and the Randox quantitative assay showed the H-FABP Test cut-off was 6 ng/ml relative to Randox assay. The agreement between the two assays [for samples with H-FABP concentrations] is: 100% [below 1.5ng/mL]; 80% [1.5-3ng/mL]; 52% [3-12 ng/mL]; 73% [12-18 ng/mL]; and, 100% [above 18 ng/mL]. The discrepancies are likely due to the different antibodies used in the H-FABP Rapid Test and the Randox assay.

Conclusion: The H-FABP Test is a one-step rapid test with demonstrated specificity to cardiac FABP. It has a cut-off at 6 ng/ml based on comparison with the Randox assay, a generally acceptable clinical reference value for H-FABP. Therefore the H-FABP Rapid Test is a useful test for the early detection of AMI.

B-276

Correlation of HbA1c Measurements by Three Different Methodologies: Ion Exchange HPLC, Boronic Acid Affinity Reflectometry, and Monoclonal Antibody Agglutination

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Background: Hemoglobin A1c (HbA1c) concentration is important in diagnosing diabetes mellitus and in monitoring glycemic control in these patients. HbA1c measurements are performed both in our outpatient clinics as point-of-care tests (POCT) as well as in our hospital's main laboratory. The aim of this study was to determine the HbA1c correlation among two POCT methods and the main laboratory. The outpatient clinics assessed the Alere Afinion AS100 (AF) POCT instrument (boronic acid affinity reflectometry) due to its faster turn-around-time (≈3 minutes) and on-board quality control checks. Capillary HbA1c concentrations from AF were correlated to the current Siemens DCA Vantage POCT (DCA) instrument (monoclonal antibody agglutination). In collaboration with physicians, an additional correlation study between the AF and the main laboratory's BioRad Variant II Turbo (BioRad) (ion exchange HPLC) was conducted on venous samples. **Methods:** Capillary blood from thirty-two patients was collected as part of routine care and assayed on both the AF and DCA according to manufacturer's recommendations. Venous blood was collected in EDTA tubes on nineteen of the thirty-two patients after consent was obtained and assayed on the AF and BioRad instruments according to the manufacturer's recommendations. The data were analyzed on EP Evaluator. **Results:** HbA1c measurements ranged from 4.5 – 14.6%. DCA and AF results of capillary

blood samples were well correlated (y=1.043x-0.17, R^2=0.9858, n=32). BioRad and AF results also showed good correlation with the capillary (y=0.995x-0.01, R^2=0.9960, n=20) and venous blood (y=0.991x+0.14, R^2=0.9968, n=19). In comparison, the results of the BioRad venous blood and DCA capillary blood were not as highly correlated (y=0.929x+0.30, R^2=0.9950, n=20) because the 95% confidence interval of the slope (0.883-0.975) did not include 1.00. Additionally, results of AF capillary and venous samples were well correlated (y=1.012x-0.13, R^2=0.9985, n=20). **Conclusions:** Overall, the three methods for assaying HbA1c demonstrated good correlation with each other. Afinion AS100 is our chosen POCT instrument because its on-board quality control checks, faster turn-around-times, and acceptable correlation to the BioRad methodology of the main laboratory.

B-277

Eliminating i-STAT Invalid Patient and Operator Identification Errors: How Collaborating with Anesthesia Improved Point-of-Care Compliance

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Background: Point-of-care tests (POCTs) must meet specific accreditation standards, including patient identification (ID) requiring patient results to be in medical records, and operator ID requiring medical records to indicate the operator. A principal challenge with POCTs is enabling non-laboratory trained clinical staff to maintain compliance with accreditation standards. This was the case at our institution where i-STATs are widely used. We noticed high invalid patient and operator ID rates from the same location in the hospital, the operating room (OR): patient ID that did not match the electronic health records were being entered, hence patient results could not cross in to the medical records. Incorrect operator IDs were being entered in to the system, and consequently medical records were lacking the name of the operator. We hypothesize that these high invalid patient and operator ID error rates may be due to errors in manual entry. These are both operator-related errors so we decided to engage the operators. A review of our records showed that i-STAT operators in the OR included anesthesiologists, anesthesia techs and nurses.

Method: Quality improvement collaboration was established between point-of-care and anesthesia and tasked with: identifying the root cause of the high invalid patient and operator ID error rates and, implementing practical strategies that will eliminate these errors and bring the OR into compliance with accreditation standards. A previously unidentified group of operators was identified: residents. Every month, a new set of residents rotate in the OR. These residents perform the bulk of i-STAT testing and although they were trained by anesthesiologists this was not recorded. Also they were not assigned operators so were not recognized by the system when their IDs were scanned. Furthermore, we found that operators sometimes scanned a patient label that was not associated with the patient account number resulting in an invalid patient ID. To address these issues: (1) i-STAT training added to on-boarding process for OR residents where they are assigned operator IDs, (2) clinical educator ensures competency for all OR operators and, (3) education provided on the correct patient label to scan. Invalid patient and operator ID error rates (Number of invalid IDs per month/Total number of tests per month x 100) were determined over a 17 month period including 9 months before and 8 months after initiating the collaboration.

Results: Previous efforts by POC to decrease invalid patient ID error rate in the OR were partially successful, however it showed a slow increase from 14% to 62% over 8 months. After the POC team collaborated with anesthesia, invalid patient ID error rate decreased to 0% in 7 months. Similarly, previous efforts to decrease invalid operator ID error rate in the OR reduced it from 99% to 68% over 7 months. After collaboration with anesthesia, the operator ID error rate decreased to 0% in 7 months.

Conclusion: Engaging in quality improvement projects with clinical teams to improve communication, identify underlying problems, and collaborate to provide solutions, is an effective way of overcoming some of the challenges that plague point-of-care testing.

B-278

Clinical Significance of Accurate Total Hemoglobin Measurements in the Perioperative Setting

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Background: Conductance-based techniques are often employed by point-of-care (POC) devices to determine total hemoglobin (tHb) in the perioperative settings. These techniques are low cost and convenient, however, only calculate tHb through the measurement of hematocrit and may be susceptible to hemodilution effects encountered in surgical settings. Inaccurate determination of tHb may result in inappropriate blood transfusions. *The objective of this study is to evaluate the clinical significance of POC calculated tHb versus measured tHb for intraoperative blood transfusion practices.*

Methods: We compared the clinical performance of a handheld POC analyzer to a central laboratory hematology analyzer (LH 750, Beckman Coulter, Brea, CA) serving as a reference method. The POC device (epoc, Alere, San Diego, CA) utilize conductance methods. Paired remnant whole blood specimens were obtained from adult (age ≥18 years) requiring cardiac surgery at risk for hemodilution, and tested on each analyzer. Patient chart review and physician arbitration was conducted to determine the potential clinical impact of each tHb result intraoperative transfusion decisions. Units of administered packed red blood cells (PRBC) were recorded. The paired t-test was used to compare the two devices.**Results:** Sixty unique patient samples were collected and used for paired comparisons. Mean (SD) tHb for the cohort was 9.5 (2.4) g/dL. Mean bias for the POC device versus the hematology analyzer was -1.4 (1.1) g/dL, P=0.011 (**Figure 1**). Ten patients received 12 potentially unnecessary units of PRBC based on POC results and case arbitration—resulting in $7,584 of additional costs ($632/PRBC).**Conclusion:** The POC analyzer exhibited a negative mean bias compared to reference methods. Conductance-based determination of tHb may have resulted in unnecessary blood transfusions and leading to excess cost. Further studies are needed to determine when conductance based measurements become unreliable such as in cases of hemodilution.

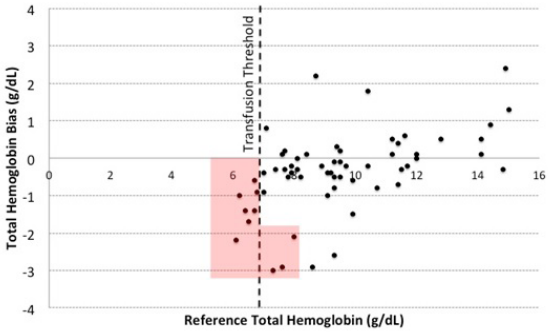

Figure 1. Bland-Altman Plot Comparing POC versus Lab Total Hemoglobin. The figure compares bias (POC – Lab) on the y-axis against reference total hemoglobin measurements. The vertical broken black line indicates the institutional transfusion threshold for total hemoglobin of 7 g/dL. Red zones indicate data points, which may have prompted unnecessary transfusions.

B-279

An Enzymatic Creatine Assay Based on a Single Reagent

O. Beeks. *Instrumentation Laboratories, Bedford, MA*

Objective

To develop a reliable method for the determination of creatine in aqueous and clinical samples.

Relevance

Creatine (Cr) is an essential metabolite synthesized naturally in the body by two amino acids, glycine and arginine. This chemical provides a base for ATP re-synthesis, allowing for continued muscle energy. To improve the performance, oral ingestion of creatine monohydrate is widely used by athletes. Although the majority of the ingested Cr is removed by the kidneys, the chronical effect on renal function with long-term Cr supplementation is of interest in the field of sports medicine.

An accurate and reproducible method is essential for measuring Cr in aqueous and clinical samples. Unlike Creatinine (Crea), there is no NIST traceable standard available for Cr. Furthering this challenge is the fact that there are only few Cr testing

reagents available on the market. All of the commercialized reagents are either for research purposes only, or unfit for high throughput testing. Therefore, our primary goal is to develop both the standard and the reagent which can provide accurate Cr determination, which is also important for Crea assay development which contains both Cr and Crea.

Methodology

The proposed method is based on a single-part reagent via enzymatic conversion of Cr with colorimetric end-point detection at 547 nm. In contrast to commercialized microtiter plate assay kits, this method can be fully automated and easily adapted to any clinical chemistry analyzers with programmable parameter settings. Cr in clinical samples can be analyzed simultaneously with other commercially available assays, such as Crea, etc. To develop a robust and accurate method a total error of < 5% must be met. Listed below are the reagent, standard and instrument used in the development process.

Gravimetric Cr standard: freshly prepared, by dissolving creatine monohydrate in 1 mMole sodium hydroxide

Reagent: A mixture of creatinase, sarcosine oxidase, 4-aminoantipyrine and horseradish peroxidase

Instrument: Roche Cobas c311, via one available open channel (programmable parameters)

Sample volume: 30 uL

Reagent volume: 150 uL

Incubation time: 10 min

Results

We have shown performances as the following:

Linearity: 0.2−15mg/dL (R^2 >= 0.999)

Imprecision: 0.2% with-in run (n=12), 0.8% run-to-run (n=3 runs, n=36 samples total)

Inaccuracy: less than 2%

Recovery: ranging from 97-102% versus gravimetric

Interference: None from Crea, glucose, lactate and urea

Total error: 4% maximum

Recovery in serum samples: Three levels of serum creatine show correlation versus IDMS determined Cr

Cr assay= 1.2509*IDMS - 0.3079, R^2>0.999. CV% is 7.9% at the low level (0.5mg/dL) and 1.7% between levels 2-5mg/dL.

Conclusions

We have demonstrated that the Cr assay meets our specifications in aqueous and clinical samples.

References

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B-280

Comparative analysis of lactate quantitation via the Radiometer ABL800 and point-of-care i-STAT and GEM4000 systems

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Background: Early identification of sepsis with rapid medical intervention improves patient outcomes and decreases sepsis-related mortality. The Surviving Sepsis Campaign (SSC) recommends lactate measurement within the first 3 h of sepsis suspicion and fluid resuscitation for patients exhibiting hypotension or lactate ≥ 4 mmol/L. Additional lactate testing within 6 h is recommended if the initial lactate is elevated to facilitate monitoring patient response to treatment when targeting normalization of lactate concentration. The Center for Medicare and Medicaid Services Severe Sepsis/Septic Shock Early Management Bundle (SEP-1) requires lactate testing within 6 h if the initial lactate is above a 2 mmol/L threshold. Point-of-care (POC) lactate testing is rapid and may aid in early diagnosis and management of sepsis patients. The aim of this study was to assess the analytical performance and overall concordance of lactate measurements using POC devices, i-STAT and GEM4000, as compared to lactate measurement using the Radiometer ABL800.

Methods: Lithium-heparin whole blood samples submitted to UNC hospitals during January 2016 for routine lactate measurement were quantified via the Radiometer ABL800 Series and assigned the reference method (Radiometer A/S, Bronshoj, Denmark). Residual samples (n=42) spanning threshold limits of 2 mmol/L and 4 mmol/L were selected for subsequent analysis using the i-STAT (CG4 cartridge, Abbott Diagnostics, Irving, TX) and GEM Premier 4000 (Instrumentation Laboratory, Bedford, MA). Linear regression, Bland-Altman, and concordance analyses were performed. Overall concordance was calculated as the percent of lactate values falling into the correct lactate category (i.e. below or above 2 mmol/L or 4 mmol/L) using each POC method compared to the Radiometer ABL800.

Results: Comparative analysis of lactate results yielded the regression equations: [i-STAT]= 0.98[ABL800]- 0.12, (r^2 = 0.99) and [GEM4000]= 0.95[ABL800]+ 0.11, (r^2 = 0.99). The mean (± standard deviation) bias in measured lactate concentration < 4 mmol/L using the i-STAT and GEM4000 compared to the Radiometer ABL800 was -0.15 ± 0.12 mmol/L and 0.03 ± 0.12 mmol/L, respectively. In analysis of high-range lactate (> 4 mmol/L), the i-STAT and GEM4000 demonstrated a mean bias of -0.28 ± 0.51 mmol/L and -0.29 ± 0.54 mmol/L compared to the Radiometer ABL800, respectively. The overall concordance of i-STAT and GEM4000 lactate measurements compared to the Radiometer ABL800 at a 2 mmol/L threshold was 90.5% and 92.9%, respectively; at a 4 mmol/L threshold both POC devices demonstrated 100% concordance.

Conclusion: The i-STAT and GEM4000 devices demonstrated excellent correlation with lactate measured by the Radiometer ABL800. Both POC methods exhibited minimal bias in lactate measurements < 4 mmol/L compared to the Radiometer ABL800 suggesting an acceptable alternative testing approach for initial patient assessment in clinical settings where lab testing is unavailable or has prolonged turnaround time. Discordance in lactate measurements between testing methodologies at a 2 mmol/L threshold will impact additional lactate testing required based on SEP-1 criteria. Both POC methods demonstrated a systematic negative proportional bias compared to the Radiometer ABL800 which may confound interpretation of high lactate concentrations (≥ 4 mmol/L) if serial measurements are performed using multiple methods.

B-281

Comparative study of Protein C-reactive Ultra sensitive assay in Point of Care

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Background: The C Reactive Protein (CRP) is an acute protein phase produced by the liver in response to factors released by macrophages and adipocytes during infections, tissue injuries, and acute inflammation. The CRP high concentration indicates an increased risk of myocardial infarction, cardiovascular acute syndromes prognosis, and it is strongly associated with cardiovascular disease adding predictive value for others cardiovascular disease and peripheral vascular markers. Previously, the CRP evaluation was reported as positive or negative, but currently, the circulate CRP is already quantified which support the clinical and therapeutic protocols. The AHA/CDC guideline referee that CRP measurement from intermediate values could alert the physician for the monitoring of the coronary disease. According with this risk stratification guideline, the CRP value above 10 mg/L, detected into 6 to 24 hours, after onset IAM symptoms; it is an indicative of recurrent cardiac events in a short time (30 days to 1 year). This study compared the results from CardioPhase® hsCRP assay in Point of Care Stratus® CS 200 Acute Care (Siemens Healthcare Diagnostics) and for RCRP assay in Dimension® RXL MAX (Siemens Healthcare Diagnostics) at Central Laboratory

Methods: We evaluated forty (40) plasma samples with Lithium Heparin. We used the CardioPhase hsCRP in POC - Stratus® CS 200 with Solid Phase Radial Partition Immunoassay Technology and Extended Range CRP assay with Dimension® RXL MAX system by Turbidimetric Immunoassay Technique. According to literature, for healthy individuals, expected values are under 3 mg/L (0.3 mg/dL) for both methodologies. Results were 100% concordant between the methods (n=40 samples - 75% of pathological samples (n=30) and 25% normal (n=10)). The Kappa coefficient was 1.00 and r = 0.989.

Results & Conclusion: The data showed a high concordance between Stratus® CS 200 (POC) and Dimension® RXL MAX (Central Laboratory), which suggest that Stratus® CS 200 is a point of care technology for quantitative determination of high sensitivity CRP simplifying the pre analytical process without compromise the quality of the patient care.

B-282

Analytical Evaluation of the Roche CARDIAC Troponin T Assay Performed on the POC Cobas® h232 Instrument

M. P. Rozmanc[1], D. C. C. Lin[2], E. Proctor[1], A. Wong[1], Z. McIntyre[1], M. Cheung[3], T. O'Brien[4], L. Fu[3], D. E. C. Cole[3]. *[1]Sunnybrook Health Sciences Centre, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Sunnybrook Health Sciences Centre and University of Toronto, Toronto, ON, Canada, [4]Women's College Hospital and University of Toronto, Toronto, ON, Canada*

Background: Measurement of cardiac troponins is of central importance for the diagnosis of acute myocardial infarction (AMI). Point-of-care testing (POCT) devices for troponin measurement can produce rapid results and facilitate timely evaluation of patients with suspected AMI. The aim of this study was to evaluate the Roche CARDIAC T Quantitative assay (POC TropT) performed on the POC Cobas® h232 instrument.

Methods: Method comparison between the CARDIAC Troponin T assay on the POC Cobas® h232 and the Troponin T hs (high sensitivity) (cTnT-hs) assay on core facility Roche MODULAR® ANALYTICS E170 system was first evaluated by assaying 119 heparinized whole blood samples from patients with a wide range (6-3161 ng/L) of cTnT-hs values. Precision was assessed by measurement of two levels of liquid quality control materials (Roche CARDIAC) Control Troponin T) and patient samples at two different concentrations. After implementation, the assay was further validated with parallel testing of patient samples using the cTnT-hs assay.

Results: Comparison between the POC TropT and cTnT-hs methods in the quantitative range (100 to 2000 ng/L) yielded a linear correlation with a slope of 0.96, y-intercept of -10.7 ng/L and R^2-value of 0.96 (n=62). A mean negative bias of -29.6 ng/L (-6.6%) was observed. For POCT purposes, qualitative intervals of <50 ng/L are reported, yielding a false negative rate of 10%. For POCT samples reported in a qualitative range of 50-100 ng/L, a false positive rate of 12% and a false negative rate of 12% were observed. For imprecision, a CV of 8.6 - 10.8% was determined using quality control material across two different reagent lots and two different POCT instruments. Using two patient samples with mean concentrations of 147 ng/L and 271 ng/L, CV's of 15.1% and 13.4%, respectively, were observed. After implementation, 32 patient samples were tested by both POC TropT and core facility cTnT-hs. The resultant POCT values (<50 ng/L, n=29; and 50-100 ng/L, n=3) were all consistent with the cTnT-hs values.

Conclusions: The semi-quantitative CARDIAC Troponin T assay on the POC Cobas h232 instrument demonstrates acceptable imprecision in our hands and correlates well with the central laboratory cTnT-hs assay. Elevated results (>100 ng/L) identify high risk patients; however, negative results (<50 ng/L) cannot be used reliably to "rule out" cardiac ischemia. In addition, this assay cannot detect/monitor change in troponin levels when the concentration of troponin is <100 ng/L. In settings with no immediate access to central laboratory testing, this POC TropT assay might be used as an aid in the diagnosis of patients with suspected AMI, in conjunction with clinical pre-test probability assessments and electrocardiogram (EKG) findings.

Wednesday, August 3, 2016

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

Proteins/Enzymes

B-283

Validation of a Serum S-100B ELISA for Measuring S-100B in Cerebrospinal Fluid

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Background: S-100B is a calcium binding protein with a molecular weight of approximately 22 kD. The protein has a homodimeric ß,ß-chain structure and is found in astroglial and Schwann cells. S-100B is highly specific for tissue of the nervous system and also for malignant melanoma cells. As with other S-100 proteins, it is suggested that S-100B serves as a calcium sensor regulating the function and subcellular dissemination of specific target proteins. Various studies have reported cerebrospinal fluid (CSF) S-100B concentrations to be valuable prognostic markers for patients suffering traumatic brain injury and may have diagnostic potential for Creutzfeldt-Jakob disease. The purpose of this study was to assess the performance characteristics and validate the CanAg® S100 EIA (Fujirebio Diagnostics, Inc., Gothenburg, Sweden), an ELISA intended for and previously validated for serum, for use in quantifying S-100B in CSF.

Methods: Residual CSF specimens sent to ARUP Laboratories were used for this study. S-100B was measured according to the test kit manufacturer's protocol. The performance characteristics evaluated were analytical sensitivity, linearity, recovery, precision and S-100B stability. CSF specimens used for the reference limit study were selected based on results that were within the reference limits for all the following tests: albumin, IgG, IgG albumin ratio, IgG index, albumin index, CSF IgG synthesis rate, and oligoclonal bands. The University of Utah's Institutional Review Board approved this study.

Results: The parametric limit of blank was 11 ng/L (zero calibrator, 20 replicates). Dilution of an elevated S-100B CSF specimen with the zero calibrator to 11 samples with different concentrations, and each measured in triplicate, produced linear regression results of $y = 0.999x + 1.34$, $r^2 = 0.996$. Adding aliquots of a serum specimen with a high S-100B concentration to five different CSF specimens (maximum ratio 1:10) generated a mean recovery of 92% (84 - 103%). Precision was determined from two CSF pools tested over 20 days in triplicate. Repeatability and within-laboratory CVs were 3.5 and 4.0% at 140 ng/L, and 2.3 and 3.1% at 1177 ng/L, respectively. S-100B was stable in CSF for 4 hours at room temperature, and minimums of 7 days and 4 weeks at 4 - 8 °C and -20 °C, respectively. The analyte was also stable over a minimum of three freeze/thaw cycles. S-100B measured from 141 CSF specimens (69 males, 72 females, ages 3 - 77 years) generated an upper reference limit of 692 ng/L (nonparametric analysis, 95th percentile). A slight increase was observed with age but was considered clinically insignificant because of the large confidence interval of the slope (slope:1.963 ± 0.957; 95% CI, 0.08744 - 3.838). No significant difference between genders was observed ($p = 0.542$).

Conclusions: The CanAg S100 EIA demonstrates acceptable performance for quantifying S-100B in CSF. A CSF reference interval has been established for the ELISA which potentially may be of value in assessing and/or monitoring traumatic brain conditions.

B-284

Evaluation of Performance of the New VITROS® LDHI (IFCC) Slide Assay (In development)

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VITROS Chemistry Products LDHI Slides quantitatively measure lactate dehydrogenase (LDH) activity in serum and plasma using VITROS 250/350/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System. Lactate dehydrogenase is an enzyme with a widespread tissue distribution and increased levels reflect tissue damage. Causes of elevated LDH include neoplastic states (i.e. leukemia, lymphoma, solid tumors), anemia (i.e. megaloblastic, hemolytic), inflammatory and infectious states, disorders of the lung and muscle, renal and myocardial infarctions, hepatic disease, trauma and shock[1-4]. The VITROS LDHI Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Lactate dehydrogenase catalyzes the conversion of pyruvate and NADH to lactate and NAD+. The oxidation of NADH, which is monitored by reflectance spectrophotometry, is used to measure lactate dehydrogenase activity. LDHI measurements using the slide pyruvate to lactate reaction are traceable to results from the *IFCC primary reference procedure for LDH* via commutable calibrators. We evaluated the accuracy of 90 patient serum samples (90 - 851 U/L) on the VITROS 350 System compared to the IFCC comparative method. The VITROS LDHI Slides assay showed excellent correlation with the IFCC method. VITROS 350 System = 1.01 * IFCC - 7.5; (r) = 0.997. A 10-day precision study conducted on four VITROS Systems showed excellent precision. Mean LDHI concentrations of 195 U/L and 627 U/L resulted in within-laboratory percent coefficient of variation (%CV) of 3.5% and 1.3% respectively on the VITROS 350 Chemistry System, 3.0% and 1.5% on the VITROS 5,1 FS Chemistry System, 3.1% and 1.5% on the VITROS 4600 Chemistry System, and 3.2% and 2.0% on the VITROS 5600 Integrated System. The Limit of Blank (LoB) is 27.6 U/L based on 72 determinations with 4 blank samples. The VITROS LDHI Slides assay has exhibited good correlation with serum samples across a broad measuring range compared to the IFCC comparative method. In addition excellent precision and good low end sensitivity has been observed on the VITROS 350, VITROS 5,1 FS, VITROS 4600, and VITROS 5600 Systems. 1. Tietz Fundamentals of Clinical Chemistry, Seventh Edition, Saunders, 2015 2. Quist J, Hill AR. Serum lactate dehydrogenase (LDH) in *Pneumocystis carinii* pneumonia, tuberculosis, and bacterial pneumonia. Chest. 1995;108:415-418. 3. Winzelberg GG, Hull JD, Agar JW, Rose BD, Pletka PG. Elevation of serum lactate dehydrogenase levels in renal infarction. JAMA 1979; 242: 268-269. 4. Zein JG et al., Prognostic Significance of Elevated Serum Lactate Dehydrogenase (LDH) in Patients with Severe Sepsis; *Chest.* 2004;126(4_MeetingAbstracts):873S

B-285

An Acute Phase Response Score from Serum Protein Capillary Electrophoresis Analysis

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Many types of tissue injury, from many sources, provoke an acute phase response (APR), also called a systemic inflammatory response. Common APR cellular and molecular markers are the white blood cell count, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). All APR markers reflect an injury, but their responses may differ greatly from each other in intensity and time course, and may also vary according to the source of injury. Due to marker variability, more than one is often used to test for injury. Marker results may also be combined into a score for a more reliable diagnosis or prognosis. Most of the proteins that form the peaks of a serum electrophoresis (SELP) have been used as APR markers. Increases in alpha-1-antitrypsin (AAT), in the alpha-2 proteins, haptoglobin and alpha-2-macroglobulin, and in C3 and C4 complement, which form the beta-2 peak; and decreases in prealbumin (PA) and in transferrin (TRF), the beta-1 peak, have served as APR markers. OBJECTIVES: (1) To determine whether the total protein of an SELP zone, or the peak protein, is a better APR measure; and (2) to create a score from the peak or zone protein values that is a superior APR measure. METHOD: CRP and ESR are common measures of the APR. A CRP or ESR test, or both, were ordered on 200 recent SELP tests. For each SELP the protein in the PA, alpha-1, alpha-2, beta-1, and beta-2 zones, and in the peaks of these zones, was calculated from the total protein and the SELP curve data from a CapillaryS(tm) capillary electrophoresis analyzer. Subtraction of the peak protein from its zone protein yielded the zone's non-peak protein. These three protein measures from each zone were correlated with the corresponding ESRs and CRPs. The peak and zone proteins from each SELP were also combined in various ways to create a score which was correlated with the CRP and ESR and analyzed by multilinear regression for the contribution of its components. SELP curve analysis was by our Python programs and statistical analysis was via "R". RESULTS: In every case the peak proteins produced far better correlation coefficients than the non-peak zone proteins. Only the alpha-1 zone non-peak protein produced a good, but smaller, correlation coefficient. The alpha-1 zone is known to contain other APR proteins. Several linear combinations of the five peak proteins were formed into APR scores. The simple sum of the peak z-values (the number of standard deviations of a peak from the peak mean), with negation of PA and TRF z-values, was one of the best scores, correlating much better with the ESR and somewhat better with the CRP than the individual peak values. For correlation with CRP, the alpha-1 peak protein made a

dominant contribution. For correlation with ESR, the alpha-2 and beta-1 peaks made major contributions and alpha-1, beta-2, and PA peaks made smaller contributions. Combination SELP scores can be excellent APR metrics.

B-286

Clinical Validation and Identification of Non-Protein Solute Interferences for Serum Total Protein Measurement by Digital Refractometry

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OBJECTIVES:

Clinical refractometers are exempt from FDA premarket notification as Class 1 devices. As such, there are few comprehensive clinical validations of refractometers in the literature or available from manufacturers. A subset of laboratories (based on proficiency testing self-reporting) still use refractometry as their method for serum total protein (TP) measurement in conjunction with protein electrophoresis. The primary objective of this study was to conduct a clinical validation of a refractometer for serum TP measurement. A digital refractometer was chosen to eliminate subjectivity involved in manual refractometry methods. A secondary objective was to identify and characterize potential interference of non-protein dissolved solutes on TP measurement.

METHODS:

Experiments were designed in adherence to Clinical and Laboratory Standards Institute® (CLSI)-based protocols. A preliminary evaluation of the Palm Abbe™ model PA202X (MISCO) refractometer was conducted through device familiarization and carryover studies. Precision was measured over 20 days with 2 runs daily at 2 concentrations of quality control (QC) materials (Bio-Rad Liquid Unassayed Multiqual®). Accuracy was assessed by testing TP in 50 clinical serum specimens over 5 days on the Palm Abbe digital refractometer, a manual refractometer, and the cobas c502 chemistry analyzer (Total Protein Gen. 2, Roche Diagnostics). Linearity was evaluated using dilutions of high TP sample pools with either low TP sample pools or ddH_2O. An interference screen was conducted using low concentration (TP=6 g/dL) and high concentration (TP=8 g/dL) analyte pools, spiked with hemoglobin, bilirubin, glucose, sodium chloride, or Intralipid®. Characterization of interference was then evaluated through serial dilutions when required. Reference interval (RI) verification studies were conducted using 20 male and 20 female serum samples from healthy donors.

RESULTS:

Carryover of 8.5% was lowered to acceptable levels (<2%) when a ddH_2O wash step was included between specimen measurements. Precision studies demonstrated overall repeatability of 0.8% CV (low QC, TP=2.4 g/dL) and 0.3% CV (high QC, TP=10.6 g/dL). Accuracy studies demonstrated a correlation to both manual refractometry (Deming regression, y=1.1148x-1.039, r^2=0.97) and the Roche TP assay (Deming regression, y=1.008x+0.356, r^2=0.93), although a negative bias was noted at lower concentrations of TP (versus manual refractometry) and an overall positive bias (+5.8%) was observed versus the Roche chemistry assay. Linearity was verified using a) low and high concentration patient pools (TP range tested, 4.4-10.8 g/dL; slope 1.009), and b) ddH_2O and high concentration patient pools (TP range tested, 1.7-13.9 g/dL; slope 0.950). A trend toward decreased recovery was observed when using ddH_2O as a diluent at lower TP concentrations. Interference (>3.63%) was observed at high concentrations of glucose (>267 mg/dL) and triglycerides (>580 mg/dL). Our current laboratory adult male and female RIs for TP (6.3-8.2 g/dL) were verified on the Palm Abbe refractometer.

CONCLUSIONS:

The performance characteristics of the Palm Abbe digital refractometer using a human TP scale were validated in a clinical laboratory setting. TP results were in general comparable to those obtained using manual refractometry and an automated chemistry analyzer. Refractometer results for TP measurement, however, may be falsely elevated in the presence of high concentrations of non-protein solutes such as glucose and triglycerides.

B-287

Study of reference materials suitable for the IFCC method of ALP measurement

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Background: Reference procedures for the measurement of catalytic activity concentrations of alkaline phosphatase (ALP) were recommended in 2011 by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The reaction principle of the IFCC procedure is based on the use of 2-amino-2-methyl-1-propanol (AMP) and 4-nitrophenyl phosphate (4-NPP) . This procedure will be used as a reference measurement system for the certification of primary reference materials. However, there is no current certified reference material for ALP by this method.

In the IFCC procedure for ALP presented in 1983, AMP concentration in the reaction mixture was 350 mmol/L. In the current primary reference method, the AMP concentration increased to 750 mmol/L for pH stability and improvement. Currently, three types of commercial kits of AMP are available; these are at final concentrations of 350, 750, and 900 mmol/L.

Objective: Primary reference materials must be essential for traceable data for the primary reference IFCC methods. Therefore, we examined raw materials suitable for this purpose.

Methods: Patient serum, liver, bone, small intestine, and placenta-derived ALP isozyme (Joko Ltd. Japan) were used as samples. Freeze-dried control material was prepared using human liver type recombinant ALP (Asahi Kasei Pharma Ltd. Japan). Reagents were prepared using the IFCC procedure and various AMP concentrations (125-1250 mmol/L). Three types of commercial kits with different AMP concentrations were also prepared. Using these reagents, ALP activity of above-mentioned samples were measured, and each isozyme sample reactivity was compared with that of the patient serum.

Results: For placenta-derived ALP, a significant inhibition was confirmed at AMP concentration >250 mmol/L. The liver type ALP demonstrated similar reactivity to human serum and to recombinant ALP. The reactivity of the placental type differed from patient serum in the measurement using the commercial kits.

Conclusion: Previously, BCR371 (porcine kidney origin), from the Institute for Reference Materials and Methods (IRMM), and SRM 909b, from the National Institute of Standards and Technology (NIST), were used as reference materials of ALP. However, these are no longer available. For the past several years, new reference materials have been investigated by the IRMM and IFCC. The placental type ALP is not suitable as a reference material because it causes inhibition by AMP. The IFCC has recommended that the focus is on the measurement of liver type ALP, which is abundantly present in human serum.

The control material of recombinant ALP had similar properties to the human liver type ALP and would be precisely applied to a reference material for the primary reference method of ALP activity measurement.

B-289

Prediction of Pressure Ulcer Based on the Protein Fraction Waveform Data

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Background: The development of pressure ulcer is influenced by the patient's nutrition status and nursing care. However, the Braden scale, generally used for NST (Nutrition Support Team) activities, is limited by its lack of objectivity.

Objective: The aim of this study was to establish the indices for early detection and intervention of bedsore development by using protein fraction waveform data.

Method: Raw electrophoresis waveforms obtained by Sebia CAPILLARYS2 PROTEIN(E)6 were normalized as previously described. Positive group consisted of 64 patients with bedsore development and a negative group consisted with 7642 patients without pressure ulcer during hospitalization. Waveform data one week before the development of pressure ulcer were used in the positive group and wave form data at admission was used for the negative group. Raw waveform data were normalized without eliminating the information between the ALB and α1 fractions.

Mobility data was corrected by positioning the peak at 75, and DMF at 300 and β1 peak position at 203. ROC analysis was conducted at each 300 point of the normalized mobility. Logistic regression analysis was performed using these data as explanatory variables and a prediction formula was obtained to detect bedsore risks.

Results: The tail position of the albumin in the β1 fraction (mobility 200) dropped, which was strongly correlated with Cu and inflammatory proteins (AUC = 0.814). The tail position of the prealbumin (mobility 69) strongly correlated with C4 (AUC = 0.789). The tail position of on the α2 albumin (mobility 164) negatively correlated with Zn (AUC = 0.753). The logistic regression formula was found to be -4.471+0.0251 * age+0.0526 * P114+0.00244 * P163-0.0177 * P199+0.00233 * P216 (AUC = 0.876).

Conclusion: A high diagnostic characteristic to detect bedsore by the protein fraction pattern existed at the mobility 200, 69 and 164. By combining these results with that of logistic regression analysis, had a high performance to predict the development of pressure ulcer.

B-290

Evaluation of the Kappa Freelite and Lambda Freelite assays on The Binding Site Optilite turbidimetric analyser using urine samples

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Elevated levels of free light chains in urine maybe indicative of kidney disease or a malignant lymphoproliferative condition such as malignant melanoma, lymphocytic neoplasms, Waldrenströms macroglobulinemia, AL amyloidosis or light chain deposition disease. In a healthy kidney, the tubular cells selectively absorb free light chains so very low levels are found in urine of a healthy individual. There are differing glomerular filtration rates for free kappa (κ-FLC) and free lambda (λ-FLC) light chains and hence differing measuring ranges and reference intervals. Samples of unknown antigen concentration are assayed on the Optilite and the results are read from a calibration curve. The κ-FLC assay has a measuring range of 2.9 - 127mg/L at the standard 1/10 sample dilution (reference interval 1.35 -24.19mg/L). The λ-FLC assay has a measuring range of 5.2-139mg/L at the standard 1/8 sample dilution (reference interval 0.24 - 6.66mg/L). High κ-FLC samples are automatically re-measured with an upper measuring limit of 127000mg/L and high λ-FLC samples are automatically re-measured with an upper measuring limit of 139000mg/L. Low samples are measured at 1/2, with sensitivities of 0.58mg/L and 1.30mg/L respectively. Correlation to the Binding Site Freelite assays for the Siemens BN™II was performed for κ-FLC using 228 urine samples including 20 from normal subjects (Range <0.31 - 207000mg/L). Using a medical decision point of the top end of the normal range, this gave 2x2 table agreement of 100% positive, 87.91% negative and 95.22% overall. For lambda, the comparison comprised 146 urine samples including 23 from normal subjects (Range 1.25 - 47600mg/L), this gave 2x2 table agreement of 100% positive, 96.7% negative and 97.95% overall. Precision was assessed using a method based on CLSI (EP05-A2), measuring samples at two concentrations for κ-FLC and λ-FLC on a single analyser over 5 days. Precision results produced total precision CVs of 8.6% (8.2mg/L κ-FLC), 6.5% (54.0mg/L κ-FLC), 9.8% (8.9mg/L λ-FLC) and 5.7% (43.1mg/L λ-FLC). Interference was tested by running interferents; ascorbic acid (342umol/L) and urobilirubin (45mg/L) and negative controls in urine samples. Urobilirubin interference was 4.13% at 9.08mg/L κ-FLC, 2.61% at 100.0mg/L κ-FLC, 3.85% at 10.6mg/L λ-FLC and 4.95% at 134.5mg/L λ-FLC. Ascorbic acid interference was -2.82% at 4.38mg/L κ-FLC, -5.78% at 85.2mg/L κ-FLC, -4.49% at 10.3mg/L λ-FLC and -3.1% at 102.7mg/L λ-FLC. We conclude that the Kappa Freelite and Lambda Freelite assays for the Optilite provide a reliable, accurate and precise method for quantifying free light chains in urine and would be useful in identifying patients with kidney disease or a malignant lymphoproliferative condition.

B-291

Development of a Multi-Analyte Biochip Array for the Early Diagnosis of Ischaemic Stroke

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Background: Stroke is the second leading cause of death globally. Following acute ischaemic stroke, thrombolytic therapy can be administered and early administration (within 3 hours of symptoms onset) can help limit stroke damage and disability. This therapy is not applicable to patients with haemorrhagic stroke. There is an unmet clinical need for the development of rapid assays to complement existing medical examination for the early diagnosis of ischaemic stroke and to discriminate between ischaemic and haemorrhagic stroke. Studies reported Glutathione S-Transferase-Pi (GST-Pi), Nucleoside Diphosphate Kinase A (NDKA), Parkinson Protein 7 (PARK7) as plasma markers for early diagnosis of ischaemic stroke; Glial Fibrillary Acidic Protein (GFAP) was found to be a biomarker enabling differentiation between ischemic and haemorrhagic stroke.

This investigative study aimed to develop a biochip array for the simultaneous determination of these four biomarkers from a single sample to facilitate the identification of ischaemic stroke patients that qualify for thrombolytic treatment within the first hours post-symptoms onset.

Methods: Simultaneous chemiluminescent sandwich immunoassays were developed on the Evidence Investigator analyser, the capture antibodies being immobilised on the biochip surface at discrete test sites. Serum samples from stroke patients on admission (within 6 hours of onset of neurological symptoms) and day 4 (ischaemic n=26, haemorrhagic n=6) and healthy control samples (n=9) were assessed. The Mann-Whitney t-Test was applied to determine statistical significance ($p<0.05$) of the results.

Results: All assays presented clinically relevant assay ranges with sensitivities <1.0 ng/ml and within-run precision CV(%)<10. Results from ischaemic stroke patients revealed that median concentration levels on admission were significantly increased for GST-Pi (12.93 ng/ml; p=0.0005), NDKA (102.79 ng/ml; p<0.0001), PARK7 (10.75 ng/ml; p=0.0004) and GFAP (0.16 ng/ml; p<0.0001) when compared to control levels [GST-Pi: 1.17 ng/ml, NDKA: 13.77 ng/ml, PARK7: 1.9 ng/ml and GFAP: 0.00 ng/ml]. Four days after admission, levels decreased for GST-Pi (5.58 ng/ml; p=0.0293), NDKA (75.06 ng/ml; p=ns) and PARK7 (5.80 ng/ml; p=ns), whereas GFAP levels continued to increase (0.65 ng/ml; p=ns). Results from haemorrhagic stroke samples revealed that on admission, GFAP levels were significantly elevated (4.06 ng/ml; p=0.0008) when compared to controls, the GST-Pi levels were also elevated (3.94 ng/ml; p=ns), however the difference from control values was not significant. Levels of GFAP (14.31 ng/ml; p=ns) and GST-Pi (20.9 ng/ml; p=ns) were increased at day 4 when compared to levels on admission but the difference was not significant. As expected, on admission serum levels of GFAP were significantly higher in haemorrhagic (4.06 ng/ml; p=0.0004) than in ischaemic stroke patients (0.16 ng/ml).

Conclusion: The developed biochip array allowed simultaneous determination of GST-Pi, NDKA, PARK7 and GFAP from a single serum sample. These biomarkers were significantly elevated in serum samples from ischaemic stroke patients when compared to controls. Furthermore, serum levels of GFAP on admission were significantly higher in haemorrhagic than in ischaemic stroke patients and controls. The results suggest that this array will complement existing imaging techniques in the identification of ischaemic stroke patients that qualify for thrombolytic treatment within the first vital hours post symptoms onset.

B-292

Evaluation of the IgA CSF assay for use on the Binding Site Optilite turbidimetric analyser

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The measurement of IgA in cerebrospinal fluid (CSF) and paired CSF and serum samples aids the assessment of the body's ability to resist infectious disease in conjunction with other clinical and laboratory findings. An increased CSF protein level can be indicative of barrier dysfunction and/or intrathecal synthesis of immunoglobulin (Ig) within the central nervous system (CNS). More specifically, an increase in intrathecal IgA in CSF can be indicative of bacterial infections such as tuberculous meningitis. Calculation of CSF/serum ratios and comparison of the Ig ratios to the albumin CSF/serum value can differentiate between the serum-derived Ig and intrathecal Ig synthesis. Here we describe the evaluation of the IgA CSF assay for use on The Binding Site Optilite® analyser. The measuring range of the assay for CSF are 1.65-40 mg/L at the 1/2 (standard) analyser dilution and 0.91-20mg/L at the 1/1 analyser dilution. For serum, the assay range is 330-8000mg/L at the 1/400 analyser dilution. Correlation to the Binding Site IgA CSF assay for the SPAPLUS® was performed using 98 CSF samples (range 0.959-36.079mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=1.10x + 0.12. Correlation to the Binding Site IgA serum assay for the SPAPLUS® was performed using 85 serum samples (range 620-4750mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.92x + 79.02. The precision study was based on CLSI EP5-A2. The study was performed over 5 working days for CSF samples and 21 days for serum samples, with 2 runs per day, where each sample was run in duplicate within each run. 3 CSF levels (3.53, 4.59 & 28.9mg/L) and 7 serum levels (557, 687, 1142, 2995, 3797, 6204, 7463mg/L) were

assessed on 3 different analysers using 3 different reagent lots for serum and two reagent lots for CSF. All levels gave total precision values of <9% CV. A linearity study was performed following the CLSI EP6-A. The linearity has been confirmed using a serially diluted CSF sample over the range of 1.446-44.745mg/L and a serially diluted serum sample over the range of 258-8930mg/L with deviation from linearity <10%. Interference testing followed CLSI EP7-A2. Serum and CSF samples close to the medical decision points were tested. No significant assay interference effects were observed in serum when tested with triglyceride (500mg/dL), Intralipid (2000mg/dL), bilirubin (100mg/L) or haemoglobin (5g/L). No significant assay interference effects were observed in CSF when tested with haemoglobin (2.5g/L), bilirubin (200mg/L), acetaminophen (1324µmol/L) or acetylsalicylic acid (3.63mmol/L). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and gave a limit of 0.91mg/L. In conclusion, the IgA CSF assay for the Optilite® analyser provides a reliable, accurate and precise method for quantifying IgA in CSF and if abnormal levels of IgA are detected it can be useful in identifying patients who have a variety of CNS disorders.

B-293

Evaluation of the serum IgG Hevylite Kappa and IgG Hevylite Lambda immunoassay on the Optilite® turbidimetric analyser

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Elevated serum concentrations of monoclonal protein are indicative of an underlying abnormality such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma and other lymphoproliferative disorders. International guidelines recommend serum protein electrophoresis (SPE) densitometry is performed to quantify monoclonal proteins. Turbidimetry can also be used in these instances to measure total IgG but this will include non-tumour immunoglobulin, and measurement of either IgGκ or IgGλ may give a more accurate representation of tumour production. Here we describe a polyclonal antibody immunoassay for IgG heavy/light chains on the Binding Site Optilite® analyser. The measuring ranges are 2.3 - 30g/L and 1.5 - 17.5g/L at the 1/20 standard analyser dilution for the IgG Kappa assay & IgG Lambda assays respectively. Correlation to the Binding Site IgG heavy/light chain assays for the Siemens BN™II analyser was carried out using 117 samples for IgG Kappa (range 0.810 - 26.5g/L) and Lambda (range 0.186 - 14.300g/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; kappa y= 1.01x - 0.19, lambda y=0.93x - 0.03. Precision studies were run based on the CLSI approved guideline EP5-A2. The precision was tested using 7 targeted levels covering the medical decision point, pathological concentrations, the reference interval and the minimum dilution, on 3 kit lots and 3 analysers over 21 days. All levels gave a total precision result of <10%. Interference testing was carried out using bilirubin (200mg/L), haemoglobin (5g/L), intralipid (2000mg/dL), triglyceride (1000mg/dL) and 15 other common drug and metabolite interferents. The interferents were spiked into serum base pools targeted at 6 levels for both specificities. The difference between the interferent spiked base pools and the negative control showed no significant interference at any level with <10% interference seen at any level. The linear range was established for both the IgG Kappa and IgG Lambda assay by analysing a serially diluted spiked sample covering the measuring range at the standard 1/20 analyser dilution. Both assays passed with an acceptance criteria of <10% for the recovery at each dilution upon expected versus observed calculated results. We conclude that the Hevylite IgG Kappa and IgG Lambda assays for the Optilite turbidimetric analyser provide a reliable, accurate and precise method for quantifying intact IgG immunoglobulins in human serum and show good agreement with existing assays.

B-294

Performance of IgM CSF assay for use on the Binding Site Optilite® turbidimetric analyser

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The measurement of IgM in cerebrospinal fluid (CSF) serum samples aids the assessment of the body's ability to resist infectious disease. An increase in CSF levels can be indicative of barrier dysfunction and/or intrathecal synthesis of immunoglobulin (Ig) within the central nervous system (CNS). Here we describe the evaluation of an IgM CSF assay for use on the Binding Site's Optilite® analyser. The measuring range of the assay for CSF samples is 0.11-40.0mg/L. For serum, the range is 60-3200mg/L. Correlation to the Binding Site IgM CSF and serum assays for the SPAPLUS was performed using 97 CSF samples (range 0.29 - 4.418mg/L) and 50 serum samples (range 402 - 4152mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.97x + 0.03 for CSF and y=0.99x - 0.01 for serum. The precision study was based on CLSI approved guideline EP5-A2 and ran over 5 working days for CSF samples and 21 days for serum samples, with 2 runs of duplicate testing per day. 3 pooled CSF samples and 5 pooled serum samples were assessed on 3 analysers using 3 reagent lots. Results are shown in table 1. A linearity study was performed following the CLSI approved guideline EP6-A. and demonstrated linearity over the range 0.109 - 5.227mg/L for CSF and 50.175 - 2639.351mg/L for serum. Interference was tested by spiking base pools at one CSF level (1.30mg/L) with 200mg/L bilirubin and 2.5g/L hemoglobin and at two serum levels (310.01 & 2492.43mg /L) with 200mg/L bilirubin, 5g/L hemoglobin, 2000mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control. Interference at all analyte concentrations of <7% was detected. We conclude that the IgM CSF assay for the Optilite® analyser provides a reliable, accurate and precise method for quantifying IgM in CSF and shows good agreement with existing assays.

Table 1: Precision of IgM CSF Optilite assay

Analyte concentration	Total precision (%CV) (Acceptance <10%)	Within run precision (%CV) (Acceptance <5%)	Between run precision (%CV) (Acceptance <8%)	Between day precision (%CV) (Acceptance <8%)
0.4382mg/L CSF	5.90%	2.80%	2.50%	4.60%
1.201mg/L CSF	3.60%	2.20%	2.60%	1.30%
3.0237mg/L CSF	4.20%	2.30%	2.90%	2.00%
242.51mg/L serum	6.40%	1.70%	1.90%	5.90%
394.93mg/L serum	4.40%	1.80%	1.70%	3.70%
1492.86mg/L serum	5.80%	2.00%	3.70%	4.00%
1882.43mg/L serum	5.00%	2.30%	2.20%	3.90%
2576.82mg/L serum	4.90%	2.70%	2.40%	3.30%

B-295

Performance of a low level IgG assay for use on the Binding Site Optilite® turbidimetric analyser.

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Serum is the predominant source for proteins present in CSF, the levels of which are regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can indicate barrier dysfunction and/or local (intrathecal) synthesis of immunoglobulin (Ig) within the central nervous system (CNS). Albumin in CSF originates exclusively from blood, therefore the albumin CSF/serum ratio provides a measurement of barrier function. The assessment of barrier function, intrathecal synthesis and other variable CSF analytes can be useful in the diagnosis of a variety of CNS disorders. Here we describe the performance of a low level IgG assay for measurement of Serum, CSF and Urine samples on the Binding Site's Optilite analyser. Precision was verified using a protocol based on CLSI (EP05-A2). Samples were spiked with purified IgG to give serum levels of 13 mg/L, 25.5 mg/L, 42.5 mg/L, 80 mg/L, 114.25 mg/L and 1000mg/L; CSF levels of 37.24 mg/L, 116.75 mg/L and 1114.44 mg/L and urine levels of 13 mg/L, 70mg/L and 110 mg/L. To validate the serum precision, samples were tested in duplicate, twice per day on 1 kit lot over 3 analysers for 21 days. The CSF and Urine studies were carried out testing samples in duplicate twice per day, on one batch for 5 days. CSF was tested across 3 analysers and urine on one. All matrices and analyte concentrations gave total precision CVs of <10 %. Linearity was verified by assaying serially-diluted serum and CSF samples across an extension of the reportable measuring range and comparing expected versus observed results. Linearity was validated for CSF over the range of 3.67-154.50mg/L and 486.59-31310.42mg/L for serum. This provides a measuring range of 4.2-135mg/L at neat for CSF and urine and of 75-1350mg/L for serum, with an upper limit of 1350mg/L utilizing auto-dilutions. Interference was validated by spiking serum pools between 2300- 22950 mg/L with 200 mg/L Bilirubin, 5 g/L Haemoglobin, 1000 mg/dL Intralipid and 500 mg/dL Triglyceride; Urine pools of 12.559 and 108.064 mg/L with 45mg/L urobilinogen and 1000mg/L Ascorbic Acid; and CSF pools between 9.65-827.56mg/L with 50mg/L Bilirubin, 0.625 g/L Haemoglobin,

200mg/L Acetominophen and 600mg/L Acetylsalicylic Acid. All data was compared to a negative control. Interference at all analyte concentrations was ≤ 9.24%, for all substances tested. Correlation to the Binding Site SPAPLUS IgG assay for the serum comparison study was performed using 70 normal serum samples (Total range 5398 - 22249 mg/L). Analysis by Passing-Bablok regression (y=1.01x + 255.01) demonstrated acceptable agreement. Comparison to the Binding Site SPAPLUS IgG CSF assay using 66 clinical CSF samples (Total range 7.78 - 761.22 mg/L) established acceptable agreement when analyzed by Passing-Bablok regression; y=0.96x + 4.48. In conclusion, the low level IgG assay for the Binding Site Optilite analyser is reliable, accurate, precise and shows good agreement with existing assays.

B-296

Performance of Rheumatoid Factor assay for use on the Binding Site Optilite® protein analyser

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Measurement of Rheumatoid Factor (RF) in serum has been shown to be of use in the detection and monitoring of Rheumatoid Arthritis (RA).Rheumatoid factors are antibodies directed against the Fc portion of IgG. Most factors are IgM antibodies, but may also be IgG or IgA. Rheumatic conditions and chronic inflammatory processes give rise to Rheumatoid Factors, which are produced by plasma cells present at sites of tissue injury. Between 60 and 80% of patients with active RA possess the RF protein in their blood or joint fluid, and therefore its detection is of great value in the diagnosis and monitoring of the disease. Turbidimetry is used in this assay to determine the protein concentrations. The measuring range at the standard 1/1 analyser sample dilution is 7 - 100 IU/mL. The lowest medical decision point for this assay is 12.5 IU/mL. The performance characteristics of the RF assay for use on The Binding Site Optilite® analyser assessed in the following manner. Comparison to the Randox RF assay on the Randox Imola analyser was performed using 20 patient samples (range = 8.18 - 402.01 IU/mL). Good agreement was seen with a Passing-Bablok regression slope of y = 0.95x + 2.97. Precision was assessed according to CLSI (EP05-A2), measuring samples at 5 concentrations, on 2 kit lots and 3 analysers over 21 days. Precision acceptance was ≤5% within-run CV, ≤8% between-day and between-run CV and ≤10% total CV. All %CV for the precision study came within the acceptance criteria (total precision results: 9.894 IU/mL - 9.30%, 16.587 IU/mL - 5.70%, 37.113 IU/mL - 3.90%, 76.501 IU/mL - 3.50%, 144.453 IU/mL - 7.00%). Linearity was assessed by assaying a serially-diluted sample pool across the width of the measuring range and comparing expected versus observed results. The acceptance criteria was nonlinearity ≤10% of the low medical decision point up to and including the low medical decision point. Above this point, acceptance criteria was nonlinearity within ± 10% for each dilution. CV acceptance criteria was <8.0% at each point in the series. Linearity passed these criteria across the range 6.23 - 111.627 IU/ml. Interference was tested by running triglyceride (10g/L), bilirubin (0.2g/L), haemoglobin (5.0g/L) and 13 other potential interferents at 3 levels, acceptance being ≤10% difference to a negative control. No significant interference was observed at any level with the interferents studied. In conclusion, the RF Optilite assay for the Optilite analyser provides a reliable, accurate and precise method for quantifying RF in serum and correlates well with existing methods.

B-297

Alanine Aminotransferase activity in plasma as a marker of severity of liver disease

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BACKGROUND: Alanine aminotransferase (ALT) activity is an important screening, diagnostic, and monitoring test for liver disease. High levels of ALT can be a marker of liver disease with variable aetiologies and prognosis.

Several studies showed that the majority of cases with ALT concentrations >1000 IU/L are generally due to acute ischaemia, acute drug-induced liver injury (usually paracetamol) or acute viral hepatitis.

AIM: The aim of this study was to correlate the ALT activity > 1000 IU/L with aethiology and clinical outcome as a marker of severity of illness.

MATERIALS AND METHODS: This single-centre, retrospective, observational study was conducted in the Emergency laboratory of a tertiary referral university hospital over an 18 month period.

Using the Laboratoy Informatic System (LIS), all patients with ALT plasma concentrations > 1000 IU/L from January 2014 to June 2015 were selected. Clinical data for the final diagnosis and outcome were obtained from the medical electronic record.

ALT was measured by IFCC method in a Dimension Vista (Siemens HD).

RESULTS: During the 18-month study period a total of 207 patients with ALT levels > 1000 IU/L were included. Eleven patients were excluded because of their inconclusive diagnosis. The final study cohort consisted of 196 patients (100 males, 96 females), median age: 49 years (range 0-92).

Mean ALT activity was 2458±3152 IU/L in patients who died (n=74), and 2069±2342 IU/L in survivors (n=122) (p=0,001).

AETIOLOGY	N		%		%MORTALITY		MEAN ALT +/- 2DS
	<14 years	>14 years	<14 years	>14 years	<14 years	>14 years	
Ischaemic liver injury	5	41	11	27	60	66	2528 +/- 3330
Cholestasis	2	26	4	17	0	4	1586 +/- 1248
Hepatobiliar malignancy	5	14	11	9	0	43	1722+/- 1542
Cardiogenic Multiorgan failure	1	15	2	10	100	100	2112+/- 2828
Septic shock	1	14	2	9	0	71	2289+/- 2098
Drug induced	4	11	9	7	0	18	2777+/- 3142
Viral hepatitis	3	10	7	7	0	30	2282+/- 1896
Idiopathic	4	9	9	6	25	22	2150+/- 2022
Autoimmune hepatitis	6	3	13	2	0	0	2368+/- 2676
Other causes (osteosarcoma, biliary atresia, transplant,···)	14	8	31	5	10	8	1579+/- 1834

CONCLUSIONS: ALT activity in plasma above 1000 IU/L is not directly related to mortality in all aetiologies so it cannot be considered an independent factor for illness severity. When interpreted together with the patient diagnosis it may play a role as a prognostic factor of mortality, with the highest rates linked to cardiogenic shock, septic shock and ischaemic liver injury and the better prognosis in autoimmune hepatitis, cholestasis and hepatobiliar malignancy patients.

B-298

Evaluation of CKD-EPI equations using cystatin c in elderly population

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Background: the assessment of renal function with the 24 hours urine creatinine clearance (Cl-Crea) in elderly population leads to a wrong urine collection. Besides the muscle mass frequently decreased in these patients do not reflect the values of serum creatinine. Recently, cystatin C (cysC) has become an important marker of kidney damage. This peptide is synthesized in all cells regardless of body muscle mass. It is freely filtered at the glomerulus and it is completely catabolized at the renal tubules which allow its use as a marker for renal filtration. Equations were proposed for estimating Glomerular Filtration Rate (GFR) using both cysC or creatinine (Crea) and cysC. Aim: To evaluate the use of CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ equations in an elderly population. Methods: we studied 140 patients with a mean age 71±11. We measured creatinine (compensated Jaffe IFCC-traceable) in serum and 24 hours urine and cystatin C (Immunoturbidimetry) in serum. Cl-Crea was calculated and patients were divided by GFR <60, 60-89 and > 90 ml/min/1.73m^2. We applied in these ranges the CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ equations. Results: the Cl-Crea correlated with both equations (r=0.80 and r=0.85, respectively; p <0.001). Cl-Crea and formulas in the range below 60ml/min/1.73m^2 were not different. In the 60-89 range, the Cl-Crea had higher values than CKD-EPI^{cysC} (77±9 vs 64±15; p=0.003), whereas Cl-Crea had no differences with CKD-EPI$^{cysC/crea}$ (p=0.17). Above 90 ml/min/1.73m^2, Cl-Crea had higher values respect to CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ (112±15 *vs* 71±17 and 112±15 *vs* 87±19, respectively; p<0.0001). Conclusion: both equations CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ have a good correlation with Cl-Crea considered as the standard method. It would be advisable and acceptable to use the CKD-EPI$^{cysC/crea}$ in elderly population up to the range of 90 ml/min/1.73m^2 corresponding to the clinical criteria that GFR below 90ml/min/1.73m2, is associated with impaired renal function.

B-299

Performance Evaluation of an Albumin BCP Assay on the Atellica CH Analyzer*

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Introduction: Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys. The Atellica™ CH Albumin BCP (Alb_P assay)* from Siemens Healthcare is intended for the quantitative measurement of albumin in human serum and plasma. In this assay, serum or plasma albumin quantitatively binds to bromocresol purple (BCP) dye to form an albumin/BCP complex that is measured as a bichromatic endpoint reaction at 596/694 nm. The objective of this study was to evaluate the performance of the Alb_P assay on the Atellica CH Analyzer.

Methods: Assay linearity was evaluated according to Clinical and Laboratory Standards Institute (CLSI), protocol EP06-A. Limit of quantitation (LoQ) was evaluated according to CLSI protocol EP17-A2. Precision was evaluated according to CLSI protocol EP05-A3. Three levels of commercial control and a human serum pool were tested with albumin concentrations ranging from 2.7 to 7.1 g/dL. Each sample was assayed two times per run, two runs per day, for at least 20 days. A method comparison study (n = 130 serum samples) was conducted between the Alb_P assay and the ALBP assay on the ADVIA® 1800 Clinical Chemistry System according to CLSI protocol EP09-A3.

Results: The Alb_P assay is linear from 0.5 to 8.0 g/dL (5–80 g/L). LoQ was determined to be 0.4 g/dL (4 g/L) based on 225 determinations, with an interassay precision of ≤10%. Repeatability ranged from 0.73 to 1.73% CV, with within-lab precision of 1.02 to 1.79% CV. The method comparison study yielded a regression equation of y = 0.99x - 0.01 g/dL, with r of 0.996, versus the ALBP assay on the ADVIA 1800 system. No significant interference (bias <10%) was observed from 600 mg/dL hemoglobin, 30 mg/dL conjugated bilirubin, 30 mg/dL unconjugated bilirubin, or 500 mg/dL lipemia (Intralipid).

Conclusion: The Albumin BCP assay for the Atellica CH Analyzer enables measurement of albumin in human serum with excellent precision and accuracy.

*Under development. Not available for sale.

B-300

Can supplementation of aspartate aminotransferase (AST) assays with pyridoxal-5'-phosphate (P5P) be useful in identifying macroenzyme aspartate aminotransferase (macroAST)?

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Background: Serum macroenzyme aspartate aminotransferase (macroAST) is a complex of AST and immunoglobulin that exhibits reduced clearance from blood due to its large size and results in increased serum AST activity. Confirmation of macroAST, which is benign, is critical to reduce unnecessary and invasive diagnostic procedures. Most methods used to identify macroAST involve precipitating or selectively removing large immunocomplexes from the serum sample. These methods require manual sample manipulation and the use of specialized reagents. Several case studies, including cases from our laboratory, have documented that macroAST samples show dramatic increases in AST activity upon assay supplementation with pyridoxal-5'-phosphate (P5P) suggesting this may be a unique characteristic of macroAST. **Objective:** To determine if the % increase in AST activity upon P5P supplementation can be used to identify macroAST in patient serum samples. **Methods:** Residual waste serum samples from physician-ordered macroAST or AST testing were used. AST activity was measured on a Roche Cobas® 6000 (Roche Diagnostics, Inc.) using both P5P-supplemented AST reagent (ASTLP, reference #:04467493190) and unsupplemented AST reagent (ASTL, reference #:20764949322). MacroAST was identified using a clinically validated polyethylene glycol-precipitation method developed at the Mayo Clinic (Rochester, MN). Three cohorts of samples were investigated: 11 positive macroAST samples, 20 samples from patients with isolated elevations of AST and negative for macroAST (AST >100 U/L and total bilirubin, alkaline phosphatase, and alanine aminotransferase within the reference interval), and 456 samples that had physician-ordered AST testing performed but no suspicion of macroAST. The % increase in AST activity upon P5P supplementation was calculated for each sample and the mean, median and distributions of the % increase in AST activity in each cohort were compared. Statistical significance was determined using the Student's t-test as well as the non-parametric wilcoxon test. Receiver operator characteristic (ROC) curve analysis was performed to determine the optimal % increase in AST activity cut-off for detecting macroAST. **Results:** The mean % increase in AST activity upon P5P supplementation in samples with confirmed macroAST was 223% (range 8-1590%; median 46%). In samples with isolated increases in AST but without macroAST, the mean % increase in AST with P5P supplementation was 29% (range 3-142%, median 24%). The cohort of samples with physician-ordered AST and no suspicion of macroAST had a mean increase in AST activity of 15% (range 0-125%, median 11%) upon P5P supplementation. The macroAST cohort showed a significantly larger % increase in AST activity upon P5P supplementation than the other two groups (p <0.0001). ROC curve analysis indicated the optimal diagnostic cut-off for identifying macroAST was 216% increase in AST activity which achieves a sensitivity of 27% and a specificity of 100%. **Conclusions:** In cases of confirmed macroAST, we have demonstrated that the AST activity associated with macroAST increases significantly upon P5P supplementation of reagent compared to non-macroenzyme AST. An increase in AST activity of ≥216% upon P5P supplementation is 100% specific for macroAST. However, given significant overlap across cohorts and poor sensitivity, it is unlikely this approach will have clinical utility as diagnostic test for macroAST.

B-301

Neoptrin and BACE1 as Novel Biomarkers in Alzheimer Disease

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Dementia is a disorder characterized by impairment of memory and at least one other cognitive domain (aphasia, apraxia, agnosia, executive function) .Dementia is one of the major causes of disability in later life. Alzheimer disease is a progressive neurodegenerative disorder of the brain that causes dementia, which is a gradual loss of memory, judgment, and ability to function, it is the most common dementia affecting elderly people .As our population continues to age, the increasing prevalence of Alzheimer disease will have an even greater impact. Neopterin is a catabolic product of guanosine triphosphate (GTP), a purine nucleotide; it is a sensitive marker for monitoring Th1-cell immune response. BACE1 (β-site APP Cleaving Enzyme 1) that initiates Aβ formation and increase is presentyears before AD symptoms arise, suggesting that Aβ42 is likely to initiate AD pathophysiology. It was addressed whether the BACE1 elevation observed in AD as an end product of advanced neurodegeneration and cell death or whether it is actively involved in disease progression. **Aim:** The aim of the present work was to study the use of serum neopterin concentration and Serum BACE 1 as new diagnostic markers in cases of Alzheimer's disease and correlate their concentration with the severity of Dementia of the Alzheimer type (DAT).**Patients**: One hundred subjects were enrolled in this study subclassified into fifty Alzheimer disease patients and fifty normal subjects. **Methods:** The concentration of neopterin was measured using DRG® Neopterin ELISA kit. BACE1 is measured derived from Immuno-Biological Laboratories Co., Ltd. **Results:** In the present study there was a significant positive correlation between Neopterin as well as BACE1 and Alzheimer disease, AUC was 0.926 with P<0.001for Neoptrin and 0.79 with P<0.01 for BACE1.The results suggest that Neopterin levels increase with increasing disease severity . Therefore, Neopterin levels may be a useful marker to follow progression of AD. However, itremains undetermined as to which is the initiating event, Aβ elevation and deposition or increased BACE activity, however As the rate-limiting enzyme in Aβ generation, BACE1, in principle, is an excellent therapeutic target for strategies to reduce the production of Aβ in AD.

Key words : AD , BACE1, Neoptrin, DAT(Dementia of Alzeimer type).

Wednesday, August 3, 2016

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

TDM/Toxicology/DAU

B-302

Development of Latex-Enhanced Immunoturbidimetric Assays for the Detection of Ethyl Glucuronide, the Synthetic Cannabinoids UR-144/XLR-11 and Their Metabolites in Urine Samples

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Background: Latex-enhanced immunoturbidimetric assays due to short reaction times and their application to automated analysers, are valuable analytical tools for the high-throughput screening of samples.

Ethyl glucuronide (EtG) is a metabolite of ethanol which has found application as a biomarker for the detection of recent alcohol consumption. The detection of alcohol consumption has a number of important applications, such as diagnosis and treatment of alcohol intoxication or poisoning, monitoring of individuals in professions with zero-tolerance policies with respect to alcohol, determination of legal impairment and forensic judgement.

UR-144 [(1-pentyl-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone] and its fluorinated version XLR-11 (5-fluoro UR-144) are the new generation of synthetic cannabinoids. Both compounds are potent and addictive and as they continue to be sold there is a need for screening tests in the detection process.

The aim of this study was to develop latex-enhanced immunoturbidimetric assays, applicable to a variety of automated clinical chemistry analysers, for the detection of EtG and the synthetic cannabinoids UR-144/XLR-11 and their metabolites in urine.

Methods: In these assays, the corresponding analytes are coated on the latex particles and bind to the specific antibody in the solution, which causes agglutination. When a sample containing the analyte is added the agglutination is inhibited to an extent proportional to the amount of the analyte in the sample. The change in turbidity caused by agglutination is measured as a change in absorbance, which is inversely proportional to the concentration of the analyte in the sample.

The assays are qualitative [cut-off: 500 ng/mL (EtG assay) and 10 ng/mL (UR-144/XLR-11 assay)] and are applicable to a variety of analysers. The results reported were obtained with an RX daytona analyser.

Results: EtG assay: the assay was standardised to EtG, methyl ethyl glucuronide was also detected with 61.5% cross-reactivity. The limit of detection(LOD) in urine (n=20) was 213 ng/mL. The intra-assay precision (n=20) was<3.5% for 3 different concentration levels of EtG (375 ng/mL, 500 ng/mL and 625 ng/mL). Recovery was assessed with a set of authentic human urine samples (n=32) and 94% were correctly reported as negative and positive with respect to values determined by LC-MS.

UR-144/XLR-11 assay: the assay was standardised to the metabolite UR-144 N-pentanoic acid, UR-144 presented a cross-reactivity(%) value of 25. Other metabolites and synthetic cannabinoids were also detected [cross-reactivity(%) ranging from 28 [UR-144 N-(5-chloropentyl) analog] to 94 (A-796260). XLR-11 metabolites were also detected [cross-reactivity(%) ranging from 33 [XLR11 N-(4-pentenyl)analog] to 49 [XLR11 N-(3-fluoropentyl) isomer]. The LOD in urine (n=20) was 2.8 ng/mL. The intra-assay precision (n=20), was<6.5% for 3 different concentration levels of UR144 N-pentanoic acid (5 ng/mL, 10 ng/mL, 15 ng/mL). The mean recovery for all the samples (n=20) at the three concentrations was between 98-105%. All replicates were correctly reported as negative and positive

Conclusion: The results indicate applicability of these latex-enhanced immunoturbidimetric assays to the detection of EtG and UR-144/XLR-11 and their metabolites in urine. The assays are applicable to different automated analysers using ready-to-use reagents, which ensures the reliability and accuracy of the measurements and facilitates the testing procedure.

B-304

The Effect of Sample Storage Conditions on Therapeutic Monitoring of Immunosuppressive Drugs

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Background: Conditions of storage and durations between sample collection and analysis may affect the stability of the samples and may lead to false results. We investigated the stability of immunosuppressive drugs cyclosporin, tacrolimus, sirolimus, and everolimus in samples and determined their best storage conditions.

Methods: Whole blood specimens in K_2EDTA tubes sent for drug monitoring (n=10 for each drug) were portioned into 13 aliquots. One sample was analyzed immediately for baseline value, three samples were stored at room temperature (RT), three samples at 2-8°C, and the rest at -20°C, all in dark. The stored samples were analyzed on 1st, 3rd and 7th days with LC/MS/MS (Shimadzu Co., Japan). The samples kept at -20°C were additionally analyzed on 1st, 3rd, and 6th months. The extracts of the samples analyzed for baseline value were kept at 2-8°C and re-analyzed on 1st, 3rd, 7th days.The analytical CV's (CVa) were determined for each drug with quality control materials (ClinChek, Recipe, Germany). The amount of change observed for the conditions tested were calculated as relative bias percentages (RBP) by the formula [(concentration of any time - concentration of baseline)/concentration of baseline] x 100. The medians of RBP for each drug which were less than the CVa were considered stable and not affected by the tested condition (Table 1).

Analyte	CVa %	Storage Condition	Storage Times and RBP		
Cyclosporin	6.0		1st day	3rd day	7th day
		RT	6,7	20,9	7,8
		2-8 °C	3,3	10,8	6,6
		After extraction at 2-8 °C	-11,4	-6,2	-9,7
		-20 °C short term	-0,6	5,3	4,8
			1st month	3rd month	6th month
		-20 °C long term	-1,9	-18,9	-24,9
Tacrolimus	10.5		1st day	3rd day	7th day
		RT	14,5	-2,7	-3,1
		2-8 °C	15,5	-5,4	-2,9
		After extraction at 2-8 °C	-4,3	-5,9	-17,6
		-20 °C short term	6,8	-8,9	-5,2
			1st month	3rd month	6th month
		-20 °C long term	-0,2	-8,7	-8,9
Sirolimus	7.4		1st day	3rd day	7th day
		RT	1,4	1,6	7,7
		2-8 °C	16,6	2,0	22,7
		After extraction at 2-8 °C	-7,5	4,9	2,5
		-20 °C short term	-10,5	3,4	19,9
			1st month	3rd month	6th month
		-20 °C long term	11,1	-8,4	-22,2
Everolimus	8.6	RT	7,5	5,3	-0,4
		2-8 °C	2,0	8,3	10,1
		After extraction at 2-8 °C	23,6	21,7	17,6
		-20 °C short term	-2,6	-5,4	1,2
			1st month	3rd month	6th month
		-20 °C long term	1,8	-8,1	-17,3

Results: Best storage conditions determined are given below.

Cyclosporin: 2-8°C for 1 day or -20°C for 1 month

Tacrolimus: After extraction at 2-8°C for 3 days or -20°C up to 6 months

Sirolimus: RT for 3 days

Everolimus: RT for 7 days, 2-8°C for 3 days or -20°C for 3 months

Conclusion: A single common storing condition couldn't be determined for the samples of these drugs. For patients who use more than one immunosuppresive drug, the drawn sample should be portioned and kept under the most appropirate condition to avoid changes caused by instability.

B-305

Evaluation of the ARK Diagnostics Levetiracetam Immunoassay on Beckman AU400 for Therapeutic Drug Monitoring

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Background: Therapeutic drug monitoring has been applied to the first generation antiepileptic drugs. The newer antiepileptic agents such as Levetiracetam are often well tolerated, have wider therapeutic ranges, and fewer serious adverse effects. Therapeutic drug monitoring of Levetiracetam is warranted in patients with clinical conditions such as renal failure that may alter the pharmacokinetic characteristics. **Objective:** Validate the Ark Diagnostics homogenous immunoassay for the quantitative determination of Levetiracetam levels in plasma, and to compare the immunoassay to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. **Method:** ARK Levetiracetam Assay is a homogeneous immunoassay based on competition between the unlabeled levetiracetam in the patient sample and levetiracetam labeled with the enzyme glucose-6- phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance, by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a Hitachi 917 chemistry analyzer and an LC-MS/MS method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, and comparison studies. **Results:** The assay showed excellent correlation to the Hitachi 917 chemistry analyzer (y = 0.994x - 0.33; r2=0.9967; N=40), and LC-MS/MS (y = 0.991x - 0.23; r2=0.9980; N=16), with an analytical measurement range of 2.0-100µg/dL. The analytical sensitivity was 2.0µg/dL. Both within-run (4.6%-4.6%) and between-run (2.6%-5.0%) imprecision were within acceptable limits, and the assay exhibited no carryover (<1%). **Conclusions:** The ARK Levetiracetam immunoassay is suitable for clinical use. This method was successfully used for therapeutic drug monitoring of the novel antiepileptic drug levetiracetam. The method provides rapid turn-around-time (2hours), compared to 2-3 days as a send out test. It is successfully used to optimize therapy for patients with altered renal function.

B-306

Performance evaluation of mycophenolic acid (MPA) on Siemens Dimension Xpand

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Background: Mycophenolic acid (MPA) is an immunosuppressant by inhibiting de novo purine synthesis in T- and B- lymphocytes. It is widely used alone or in combination with calcineurin inhibitors and corticosteroids to reduce acute rejection in transplantation. Although the relationship between MPA exposure and its toxicity is weak, its association with early postoperative efficacy, the great inter-patient pharmacokinetic variability and the influence of concomitant immunosuppressants necessitate the therapeutic drug monitoring (TDM) of MPA. The objective of this study was to assess the analytical performance of MPA on Dimension Xpand.

Methods: MPA was determined with a homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINIA). Basically, MPA in the sample competes with the MPA conjugated on the synthetic particles for monoclonal MPA specific antibodies, and the rate of aggregation of the antigen-antibody complex on the particles is measured using bichromatic turbidimetric analysis at 340nm and 700nm, which is inversely proportional to the MPA concentration in the sample. The evaluation was performed following CLSI guidelines. The performance was evaluated for precision, linearity, lower limit of detection, and accuracy. The within-run and between-run precisions were assessed by analyzing QC material at two levels of concentrations. The correlation between MPA on Dimension Xpand and liquid chromatography-tandem mass spectrometry (*LC/MS/MS*) was assessed.

Results: The within-run CVs for MPA were 3.2% and 3.3% at the levels of 2.1µg/mL and 13 µg/mL, respectively. The between-run CVs at low and high levels were 3.3% and 3.6%, respectively. The analytical measurement range was determined to be linear between 0.2 - 30.0 µg/mL. The lower limit of detection was 0.12 µg/mL using measurable value obtained from zero standard + 2SD (n=20). Comparison of MPA on Dimension Xpand with MPA on LC/MS/MS showed that the slope was 1.156 (95% CI = 1.080 to 1.232) with intercept of 0.28 and correlation coefficient r = 0.981 (Deming). The mean bias was 0.80.

Conclusion: Our data demonstrate that the MPA on Dimension Xpand analyzer has an excellent precision of performance with good linearity. There is a good correlation between serum MPA tested on Dimension Xpand and MPA tested on LC/MS/MS. Serum MPA concentrations can be precisely and accurately measured and monitored on Dimension Xpand in patients receiving MPA.

B-307

In vitro formation of ethyl glucuronide in meconium specimens

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Background: Among all known ethyl alcohol metabolites detectable in specimens of neonatal origin (fatty acid ethyl esters and ethyl sulfate, etc.), ethyl glucuronide (EtG) in meconium has been sought as the most promising biomarker of prenatal alcohol exposure. However, sources of ethanol alternative to maternal drinking behavior, such as ethanol-producing microorganisms, may be present in meconium and could potentially bias the testing results. The **objective** of our study was to investigate the possibility of *in vitro* EtG formation in authentic meconium following alcohol fortification.

Methods: The method for EtG quantitation in meconium consisted of sample homogenization, strong anion-exchange solid phase extraction, and liquid chromatography-tandem mass spectrometry using D_5-EtG as the internal standard. The limit of detection and the lower limit of quantification (LLOQ) were 6 and 12 ng/g, respectively. According to a calibration model established by a 30 ng/g single-point calibrator, the linearity range was 12-600 ng/g (y=1.028x-4.109, r^2=0.9800; coefficients of variation 11.2 - 13.4% at 24, 160, and 480 ng/g). Individual 0.1 g aliquots from each of the 20 authentic meconium specimens selected were directly subjected to EtG analysis. At the same time, separate 0.5 g aliquots from the same specimens each was thoroughly mixed with 50 microliters of anhydrous 200-proof ethanol, and then stored at room temperature. At 24- and 48-hours, 0.1 g aliquots of ethanol-fortified meconium samples were taken and analyzed for their EtG content.

Results: At time zero, all 20 meconium specimens showed EtG below the LLOQ, but after 24-hour ethanol exposure, 18 of them presented EtG concentrations significantly exceeding the recently recommended cutoff of 30 ng/g (Table).

Conclusion: The amounts and rates of EtG synthesized *in vitro* following the same level and time of ethanol exposure varied significantly between meconium specimens. Therefore, EtG content in meconium is not an ideal biomarker for maternal alcohol intake during pregnancy.

EtG formation in 10 representatives out of 20 meconium specimens following ethanol exposure

Sample number	EtG Concentration (ng/g)		
	0 hr	24 hr	48 hr
1	ND	12	14
2	ND	23	35
3	ND	107	226
4	ND	279	318
5	ND	369	802
6	ND	713	653
7	ND	829	2643
8	ND	867	959
9	ND	936	2413
10	ND	1150	2076

ND: Not detected (below limit of detection).

B-308

A Serum Fluoride Method by Ion Chromatography and an Assessment of Patient Population Results

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Background:

Exposures to fluoride can occur through its therapeutic and occupational uses. Fluoride is added to drinking water to prevent tooth decay. It can be administered orally to those children who do not have fluoride added to their water. Fluoride is also found in several adult dentifrices used for oral hygiene. Occupational exposures to hydrogen fluoride and its inorganic salts can occur in among other things the manufacturing of fluorocarbon chemicals, in stainless steel pickling, in aluminum production,

in petroleum alkylation, in uranium chemical production, and in the production of fluorinated chemicals including pharmaceuticals. Accidental or intentional ingestions, inhalation, or dermal contacts of fluorides can be life threatening following acute exposures. Chronic exposures to low level concentrations may lead to fluoride induced toxicities of the skeletal system. The determination of fluoride in serum can be a useful means of assessing exposures to fluoride. Accordingly, the objectives of this study are to present a method for measuring fluoride concentrations in serum by ion chromatography and to assess the population data of 395 patient samples from subsequent analyses.

Methods:

In the current method, fluoride concentrations are determined in serum by Ion Chromatography with suppressed conductivity detection. Patient samples were measured with a Thermo/Dionex ICS-5000 or Dionex DX-500 platform with a hydroxide mobile phase gradient and an AG-15/AS-15 guard/analytical column set. Samples were diluted 10x in deionized water and proteins were removed by ultrafiltration before injection onto the column.

Results:

A method of determining fluoride concentrations in serum by Ion Chromatography was validated in our laboratory. The low affinity for fluoride to ion exchange sites requires a high capacity column with optimized chemistry and a relatively low mobile phase strength. The AS-15 analytical column and AG-15 guard column were found to perform the best in separating fluoride from other early eluting anions such as acetate and lactate. Serum fluoride concentrations from 395 patient samples ranged from <0.05 to 2.7 mg/L with a median concentration of <0.05 mg/L. Of the 395 patient samples, 320 of them were less than the reporting limit value of 0.05 mg/L. The available demographic information revealed 187 males and 206 females were tested with the ages ranging from 1 to 96 years. Age-based values showed 43 patients from 1 - 19 years had serum fluoride concentrations ranging from <0.05 to 0.3 mg/L (median <0.05 mg/L); 286 patients from 20 - 65 years had a range of <0.05 to 2.7 mg/L (median <0.05 mg/L); and 59 patients >65 years had a range of <0.05 to 0.29 mg/L (median <0.05 mg/L).

Conclusions:

A method for analyzing elevated fluoride serum concentrations by Ion Chromatography is presented. Careful selection of the analytical column is key in separating fluoride from potentially interfering anions such as acetate and lactate. Although the majority of patient results reported here are below the reporting limit (0.05 mg/L), elevated levels as high as 2.7 mg/L were observed. Ion chromatography has been shown to be an effective analytical tool in determining serum fluoride levels which can be used to assess fluoride exposure.

B-309

Analysis of Everolimus Blood Levels Using a Unique Quantitative Microsphere System (QMS) on the Siemens Dimension EXL Instrument

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Background: Everolimus is a macrolide immunosuppressant belonging to the MTOR class of drugs (MTOR, mammalian target of rapamycin). Everolimus is used primarily for maintenance immunosuppressive therapy in renal transplantation to prevent acute rejection of renal allografts. As with many immunosuppressant therapies, everolimus is potentially toxic and has a very narrow therapeutic range making drug monitoring a requirement. Accordingly, we validated the Thermo Scientific QMS everolimus assay on our existing Siemens Dimension EXL chemistry analyzer. Methods: An everolimus comparison study was performed between our QMS/EXL method and a LC-MS/MS reference method using whole blood samples from twenty transplant patients. Also, a linearity range study of our QMS/EXL method was performed by analysis of six samples with known everolimus levels. Within and between run precision studies were based on replicate analysis of quality control materials. EP Evaluator (Data Innovations, Ft Myers, FL) software was used to determine the accuracy, linearity, precision, and range of our QMC/EXL everolimus assay. Results: A comparison regression study was performed between our QMS/EXL method and a LC-MS/MS reference method with everolimus blood levels ranging from 2.7 ng/ml to 13.4 ng/mL and yielded the following regression line: (n= 20, QMS/EXL = 1.04*LC-MS/MS - 0.687; r^2= 0.999). Precision data on quality control samples were as follows: Level 1, mean= 3.87 ng/mL,(95% CI,3.76 to 3.97), SD= +/- 0.225, CV= 5.8%; Level 2, mean=8.07 ng/ml (95% CI, 7.81 to 8.33) SD= +/-0.559, CV= 6.9%; and Level 3, mean= 15.3 ng/ml (95% CI, 15.0 to 15.6), SD= +/- 0.680, CV= 4.5%. Linearity samples covered the assay range of 0.238 to 20.1 ng/mL (n= 6) were assayed by our QMS/EXL method yielding the following results: regression line slope = 0.988, y-intercept = 0.110 and an observation error of +/- 0.13 ng/ml or 1.1%, well within limits of our allowable systemic error (SEa) benchmark of 1.23 ng/ml or 10.5%. A maximum deviation of 9.1 % was observed from the target of 100% recovery with six of six mean recoveries with all accurate within our allowable limit of +/- 10%. Conclusions: There is good to excellent correlation between our Siemens Dimension QMS/EXL everolimus method and the LC-MS/MS reference method. Other validation studies on our QMS/EXL method show excellent precision, linearity, accuracy, and a clinically acceptable reportable range. We conclude that our Dimensions QMS/DXL everlimus method is acceptable for use in clinical therapeutic drug monitoring on transplant patients.

B-310

Measurement of plasma Apixaban by LC-MS/MS

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Background: Direct oral anticoagulation agents (DOACs: apixaban, dabigatran, rivaroxaban) are of relatively new use, and are intended for use without need for routine concentration monitoring. However, there is arguably need for availability of measurement of DOACs for patients awaiting surgery, to ensure achievement of appropriately low levels after cessation of routine administration. For apixaban, this safety threshold is generally regarded as <30 ng/mL. In this context, we were asked specifically to make available an assay for plasma apixaban at our hospital. Subsequently, an LC-MS/MS assay was developed as a hybrid of methods from the literature (Noguez & Ritchie, 2016, PMID 26660170; Schmitz et al., 2014, PMID 25142183). Because numerous other hospitals are also likely to want to establish apixaban assays in the near future, we report our experience in design and validation studies for measurement of plasma apixaban by LC-MS/MS using the AB Sciex API 3200MD. **Methods**: Apixaban standards (0, 55, 118, 274, 615 ng/mL) were purchased from Hyphen Biomed; d4-rivaroxaban, used as the internal standard (IS), was purchased from Santa Cruz Biotechnology. Plasma samples were prepared as follows: 200 ul of MeOH containing IS was added to 100 uL of sample; after vortexing and centrifugation, 100 uL of supernatant was diluted with 100 uL of starting LC binary mobile phase mixture (50:50 A:B; A = H2O, 2 mmol/L ammonium acetate, 0.1% formic acid; B = MeOH, 2 mmol/L ammonium acetate, 0.1% formic acid). 20 uL of the sample preparation was injected for analysis by LC-MS/MS. LC (Shimadzu UFL Prominence) was performed using a Phenomenex Kinetix C8 column (100 A, 5 um, 50 × 4.6 mm) at 34C, with binary mobile phases having fixed flow rate of 0.4 mL/min. Mobile phase gradients were as follows (time (min), %B): 0, 50%; 1, 50%; 2.5, 98%; 4.5, 98%; 4.6, 50%; 5.0, 50%. MS/MS was run using positive electrospray ionization. Multiple reaction monitoring utilized transitions 461->77 for apixaban, and 440->145 for IS. Retention times were 3.3 min (IS) and 3.4 min (apixaban). Injection-to-injection time was 6.5 min. Quality control samples were prepared in pooled human plasma from apixaban purchased from Cayman Chemical. **Results**: The calibration curve was linear across all calibrators (up to 615 ng/mL; r >0.99). Interassay precisions for quality control samples were 8.4% (30 ng/mL) and 4.7% (200 ng/mL). Accuracy of calibration was verified by 1:1 correspondence between measured results and calculated spiked sample concentrations using apixaban obtained from an independent source (Cayman Chemical). LLOQ was 4 ng/mL. Recovery for spiked samples (comparison of measured results to calculated results) was 101±2.6%. No analytical interferences were observed across an array of therapeutic drugs, drugs of abuse, pooled plasma, and individual patient plasma samples. No ion suppression effects were observed. **Conclusions**: A plasma apixaban assay by LC-MS/MS was developed using the AB Sciex API 3200MD. Analytical performance characteristics of the assay were judged to be suitable for routine use in the laboratory for measurement and reporting of plasma apixaban.

B-311

Comparison of paired umbilical cord tissue and meconium samples for detection of *in utero* drug exposure

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Background & Objective

Prenatal exposure to pharmaceuticals, both illicit and prescribed, has many health consequences for neonates. One of the most severe is the development of neonatal abstinence syndrome, which can require weeks of treatment in a neonatal intensive care unit. Drug-exposed neonates are identified based on clinical presentation and history of maternal drug use, but diagnosis is often confirmed using toxicology testing on specimens from the neonate. Meconium has long been considered the gold

standard for neonatal drug testing, but practical considerations make it an unattractive specimen. Umbilical cord tissue has several advantages over meconium, and limited evidence suggests that it may be used as an alternative specimen. The goal of this study was to compare the detection of drugs in paired meconium and umbilical cord tissue samples collected from babies born at Vanderbilt University Medical Center between 10/1/13 and 4/8/15.

Methods

Patients whose providers ordered both umbilical cord and meconium toxicology testing and whose charts were available for review were included in this IRB-approved study. All toxicology testing was performed by a national reference laboratory using a combination of immunoassay and chromatography-mass spectrometry techniques. Drugs that were only available in either the cord or the meconium panel were excluded from the analysis. Toxicology results were gathered from the laboratory information system and clinical information was collected by chart review.

Results

Paired umbilical cord tissue and meconium results were available for 217 neonates. Of these, 77 pairs were concordant negative and 36 pairs were concordant positive. The remaining 104 pairs of samples had at least one discordant result. When metabolites and parent drugs were grouped together, 66 of these became concordant. For the remaining 38, the results from cord indicated a different drug exposure than the results from meconium. When considering drugs by class, the overall agreement between cord and meconium ranged from 75% to 100%. For the 6 individual drugs where adequate data was available, the concentration of drug measured in meconium did not correlate with qualitative detection in cord.

Conclusions

Accurate drug detection and interpretation of toxicology results is important for identification and treatment of drug-exposed neonates. This study demonstrates different sensitivities of drug detection in umbilical cord tissue and meconium, which indicates that these specimens are not completely interchangeable. These results can be used to help clinicians and laboratorians select the most appropriate test to confirm *in utero* drug exposure, and if testing is performed in multiple matrices, to interpret any discordant results.

B-312

Evaluation and Clinical Validation of a Handheld Digital Refractometer for Urine Specific Gravity Measurement

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Objectives: Manual refractometers are used by drug testing laboratories in urine specimen validity testing. They are also valuable in assessing hydration status in sports medicine and athletics. Few comprehensive performance evaluations are available demonstrating refractometer capability from a clinical laboratory perspective. The objective of this study was therefore to conduct a clinical validation of a handheld digital refractometer (Palm Abbe™ model PA202X; MISCO) used for human urine specific gravity (SG) testing.

Methods: Validation experiments followed Clinical and Laboratory Standards Institute® (CLSI) guidelines. Precision studies were conducted using BioRad quality control (QC) material over 20 days. Accuracy studies were conducted over 5 days using residual patient urine specimens spanning the analytical measurement range of four urine solutes: Cl^-, glucose (GLU), total protein (TP), and creatinine (Cr). Accuracy was assessed by comparison of Palm Abbe SG results to SG from a manual refractometer (URC-NE; Atago) and two methods of alternative evaluation: 1) urine osmolality (Advanced® Model 3250, Advanced Instruments), and 2) a "sum of solutes" score defined as the sum of urine Cl^-, Cr, GLU, K^+, Na^+, TP, and urea nitrogen measured for each specimen on a Roche cobas 8000 system (converted to mg/dL). Linearity was assessed using high patient pools diluted with either low patient pools (measuring range SG 1.0060 - 1.0418) or ddH_20 (measuring range SG 1.0001 - 1.0418). A limit of quantitation (LOQ) study was conducted using diluted urine specimens. Carryover was evaluated using low and high concentrations of QC material and patient urine pools. Previously published reference intervals (RI) were validated using 20 random urine samples from healthy donors. Traditional interference testing was not applicable, however, as all dissolved solutes contribute to SG by definition. Modified "interference" studies evaluating the extent of SG changes (with increasing concentration of NaCl, GLU, and bovine albumin) were conducted. Data was analyzed using EP Evaluator (Data Innovations), StatisPro (CLSI & Analyze-it), and SigmaPlot (Systat).

Results: Significant carryover was not observed (% differences ≤ -0.02%). A wash step was still included in our procedures as good laboratory practice. Low imprecision (average 0.0% CVs) was demonstrated using low and high QC material. Accuracy studies showed good correlation to manual refractometry (Deming regression, $y=1.018x-0.019$, $r^2=0.92$; overall bias, -0.1%). Regression after repeat testing of two outliers resulted in improved linear fit (Deming regression, $y=0.983x+0.016$, $r^2=0.99$; overall bias, -0.1%). Outliers were attributed to inadequate specimen mixing. Linear correlation was demonstrated between SG, osmolality, and sum of solutes. Linearity of Palm Abbe performance was verified with observed error of ≤0.1%. The LOQ study demonstrated acceptable performance at the SG level tested (1.0005). This level was selected as it is lower than our expected target for the low end of the clinically reportable range (1.0010). A random urine RI of 1.0020 to 1.0300 was verified. Increases in SG were observed with increasing concentrations of NaCl, GLU, and albumin ($r^2=0.99$, each).

Conclusions: The Palm Abbe digital refractometer was a fast, simple, and accurate way to measure urine SG. Our extensive CLSI-based validation studies demonstrated acceptable clinical performance of this handheld instrument.

B-313

Validation of a LC/MS-MS Method for Pain Management Confirmatory Drug Testing of 55 Drugs and Metabolites

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Background: Urine drug testing is routinely performed in pain management and other healthcare settings to ensure patient compliance and detect problems such as illicit or non-prescribed drug use, diversion, misuse, and abuse. We developed a LC-MS/MS assay for an academic hospital laboratory in consultation with pain management physicians to simultaneously detect/quantitate 55 drugs and metabolites including tapentadol, buprenorphine, carisoprodol, zolpidem and the following groups: opiates, synthetic opioids, benzodiazepines, amphetamines, barbiturates, anticonvulsants, sedatives, and illicit drugs. **Methods:** Fifty-five compound standards and fifty-two internal standards were monitored using scheduled multiple reaction monitoring on an SCIEX API 5500 mass spectrometer with electrospray ionization in a positive/negative ion switching mode with two ion transitions per analyte for identification and quantitation. Reversed-phase HPLC separation was performed using a biphenyl column (100x2.1 mm, 2.7 µm) with a binary mobile phase (A: 0.1% formic Acid in water; B: 0.1% formic acid in acetonitrile) by gradient (5-100% B) with a 0.6-0.8 mL/min flow rate. Seven calibrators were prepared in drug free urine between 1-5000 ng/mL (exact range varied by compound). Samples were prepared by diluting 100 µL patient urine or calibrator with 60 µL of 1:3 internal standard:β-glucuronidase solution, incubating for 30 minutes at 55°C, centrifuging, and diluting the supernatant 1:10 with 90:10:0.1 of water:acetonitrile:formic acid (*v/v*) in a fresh vial before injection. **Results:** The method validation test did not show any significant sample carry-over, matrix effects, or sample interferences. The compounds at the lowest cut-off had inter-day precision ranges between 4.3-18.5% with 49 of 55 compounds being ≤10%. All but two compounds had an intra-day precision ≤10% at their respective low cut-off concentrations (CV range=2.3-17.3%). The lowest cut-off for each compound was less than or equal to the cut-off used by the send-out reference lab, which utilized a reflex testing algorithm to screen urine samples by immunoassay and confirm positive screens by GC-MS or LC-MS/MS methods. In a qualitative method comparison of 71 patient samples comprising the 33 compounds tested by the reference lab, our LC-MS/MS method was 96.1% (199/207) in agreement with the positive reference lab results; the 8 negative compounds included 2 illicit, 1 benzodiazepine, 5 opiates. We detected and quantified 29 additional positive results (14 were above the screening cut-off) that were not reported by the reference lab. One hundred additional positive results among the 22 compounds not tested by the reference lab were detected by our LC-MS/MS method. When 20 CAP toxicology survey samples were tested, our LC-MS/MS method detected 100% of the drugs reported and the correlation between concentrations by Deming regression was acceptable ($R^2=0.9966$; $y=1.014x-0.0591$). **Conclusions:** Our lab developed a robust, single injection LC-MS/MS method capable of detecting and quantifying 55 drugs and metabolites in urine with minimal sample preparation, acceptable precision, and overall agreement to the results of the reference lab currently used by our hospital as well as to CAP toxicology survey samples. Ongoing method validations using alternative sample matrices are being pursued to monitor compliance using oral fluid and clinical efficacy using serum.

B-314

Quantitation of 32 Drugs in Serum by LC-MSMS

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Background: Blood drug testing is an effective tool for compliance monitoring, dose correlation and clinical tolerance. The aim of this study was the development of a fast and cost-efficient LC-MSMS method for simultaneously detecting and quantifying thirty-two drugs in serum or plasma. The panel includes opiates, amphetamines, cocaine, benzodiazepines, cannabinoids, analgesics, methadone and barbiturates. Deuterated analogs were used as internal standards.

Method: Fifty microliters of patient serum/plasma was placed into a polypropylene tube. Fifty µL of a 100 µg/mL internal standard mixture, containing deuterated standards in methanol, was added followed by 200 µL of acetonitrile. The solution was vortex mixed for 15 s and centrifuged at 13,000 rpm for 5 min at room temperature. The supernatant was transferred to a disposable glass tube and evaporated to dryness at 37°C for about 20 min using a gentle stream of air. The extracted sample was reconstituted with 200 µL of mobile phase A, vortex mixed, transferred to an autosampler vial, and injected onto a RESTEK Ultra bi-phenyl analytical column (5 µm, 50 x 2.1 mm) maintained at 40°C. Mobile phase A was 0.1% formic acid in water (1:1, v/v). Mobile phase B was 0.1% formic acid in acetonitrile (1:1, v/v). The acquisition method utilized 10 µL injection volume, 0.6 mL/min flow rate, and a gradient program of 98% A, increased to 95% B over 5.5 min, held for 0.8 min, decreased to 2% B over 0.2 min, and re-equilibrated at 2% B for 1.5 min. Run time was 8 min (injection to injection). The HPLC system consisted of Shimadzu pumps and autosampler. MSMS was performed on a Sciex API 4500 triple quadruple mass spectrometer with an electrospray source monitored in positive and negative ion modes.

Results: Specificity was assessed by retention times and unique quantifier/qualifier transition peak area ratios. Total imprecision (40 runs, two concentration levels in duplicate per run) averaged 11% CV with low-level drugs, and 9% CV with medium-level drugs. LOD (s:n ratio of 3:1) ranged from 0.1 to 11.0 ng/mL, and LOQ (s:n ratio of 10:1) ranged from 0.3 to 40.0 ng/mL. Linearities ranged from 0.5 to 2000 ng/mL Extraction efficiencies for native and deuterated analytes were greater than 87%. Matrix effects of native analytes were similar to corresponding deuterated analogs and did not affect quantitation. No carryover, endogenous or exogenous interferences were observed, with analyte stability at room temperature for 24 h. Qualitative correlation between our procedure and a commercial LC-MSMS method showed 100% agreement at cutoffs. Quantitative correlations showed less than 25% differences.

Conclusion: We present the development and validation of a LC-MSMS procedure for the quantitative determination of 32 drugs/metabolites in serum or plasma at therapeutic levels employing a small amount of a single sample, deuterated internal standards, and a single extraction - without derivatization, additional chromatographic resolution, or preliminary immunoassay screening. This novel method is suitable for routine clinical use.

B-315

Determination of Benzodiazepines Clonazepam, Clobazam and N-desmethylclobazam in serum by liquid chromatography electrospray ionization tandem mass spectrometry for therapeutic drug monitoring.

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Benzodiazepines are among the most frequently prescribed drugs worldwide and it therapeutic monitoring is very important to optimize the individual dosage of each medicine. The Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) is a reference technique for therapeutic drug monitoring TDM. Therefore, a LC-MS/MS method using a simple liquid-liquid extraction was developed and validated for a therapeutic monitoring of clonazepam, clobazam and clobazam metabolite (N-desmethylclobazam) in serum. For this, 200 µL of serum samples were spiked with internal standard temazepam and extracted with ethyl acetate. Detection was performed using a Waters Alliance-Quattro Micro tandem mass spectrometer triple quadrupole operate in a positive mode. Chromatographic separation was obtained on a Symmetry C18 column with an isocratic mobile phase containing methanol, water, acetonitrile and formic acid at a flow rate of 400 µL.min^{1}. The method had a chromatographic running time of approximately 6.0 min. The limit of detection (LOD) was 1.0 ng.mL^{-1} for clonazepam, 4.0 ng.mL^{-1} for clobazam and 35.0 ng.mL^{-1} for N-desmethylclobazam. The linear analytical range of the method was between 10.0 and 160.0 ng.mL^{-1} for clonazepam, 25.0 and 525.0 ng.mL^{-1} for clobazam and 100.0 and 5000.0 ng.mL^{-1} for N-desmethylclobazam. The precision studies were less than 10% for all analytes. The analytes were determinate with satisfactory sensitivity, accuracy, repeatability and linearity. In conclusion, the LC-MS/MS method has been applied successfully for the quantitative analysis and therapeutic monitoring of these benzodiazepines.

B-316

Determination of benzoylecgonine in urine using a Solid Phase Extraction and Gas Chromatography Mass Spectrometry for occupational evaluation.

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The Cocaine is one of the most stimulant drugs. In recent years, abuse of cocaine has become one of the serious social problems. Benzoylecgonine is the main biotransformation product of cocaine in urine and is one of the biological markers of cocaine exposure. In this work, we development a GC-MS method for determination of benzoylecgonine in human urine using a Strata-X solid phase extraction cartridge followed by BSTFA derivatization. Chromatographic separation was performed on an HP-5MS column (30m x 0.25 mm x 0.25 µm) and helium flux of 2,0 mL/min .The column temperature was held at 160 °C for 2 minutes, it was increased to 300 °C at 20 °C/min and maintained at 300°C for 1 minute. The injection was done in splitless mode and 0.75 min with the injector at 260 °C. Analysis was performed on a GC-MS PerkinElmer Clarus SQ 8T, EI mode, operated at 230 °C. The data is collected by selective ion monitoring mode of the ions of *m/z* 240 (quantifier) and two ions qualifiers for benzoylecgonine and *m/z* 243 (quantifier) and two ions qualifiers for benzoylecgonine-d3. The limit of detection was 15.0 ng/mL and the method was linear between 25.0 and 500.0 ng/mL. The medium range of recovery obtained was between 93.8 and 107.4%. The intra-day was less than 2.6% and inter-day precision was less than 4.1%. In conclusion, the GC-MS method has been developed and validated successful with a good precision and recovery and has been applied for occupational evaluation of the cocaine exposure.

B-317

Tobramycin Immunoassay Discrepancies - Comparison to Tandem Mass Spectrometry

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Objective: Tobramycin, an aminoglycoside, is primarily used to treat infections caused by aerobic gram-negative bacteria. Various tobramycin immunoassay methods exist on many clinical analyzers. A previous investigation of immunoassays used in Alberta revealed discrepancies in the results. This study aimed to investigate the accuracy of these assays compared to a tandem mass spectrometry (LC/MS-MS) assay.

Methodology: Fresh drug-free plasma and serum pools were prepared from two volunteers. Four pools of each sample matrix were spiked with tobramycin to obtain concentrations ranging from 1.0 to 10.0 mg/L. Aliquots (0.5 mL) of drug-free and spiked pools were prepared and distributed to four Alberta Health Services laboratories and to a reference laboratory for LC/MS-MS analysis. Samples were shipped refrigerated and analysed within 48 hours. Tobramycin analyses were performed as follows: Laboratory A – Beckman Coulter DxC 800 analyzer (Brea, CA, USA) using a particle-enhanced turbidimetric inhibition immunoassay (PETINIA); Laboratories B and C – Siemens Dimension Vista analyzers (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) with a PETINIA assay; Laboratory D – Roche Cobas c501 analyzer (Hitachi High-Technologies Corporation, Tokyo, JP) using a homogenous enzyme immunoassay (HEIA); Laboratory E – Thermo TSQ Quantum Ultra (ThermoFisher, Mississauga, ON, CA) with a LC/MS-MS method.

Results: Beckman Coulter DxC 800 results ranged from 3.7% to 15.1% and 15.6% to 31.8% higher than LC/MS-MS in serum and plasma, respectively. Biases in serum and plasma on the Siemens Vista assay were from -10.0% to 22.2% and -4.5% to

-12.0% respectively (Laboratory B) and -10.0% to -22.2% and -8.0% to 13.6%, respectively (Laboratory C), compared to the LC/MS-MS assay. The Roche Cobas c501 assay gave results from -17.0% to 20.0% and -8.0% to 22.2% compared to LC/MS-MS in serum and plasma, respectively. For the Beckman PETINIA assay, 25% of the spiked samples (2/8) gave results >20% higher than LC/MS-MS. For the Siemens PETINIA and Roche HEIA assays, one spiked sample gave results >20% lower and >20% higher than LC/MS-MS, respectively. For the 5.0 and 10.0 mg/L spiked samples (serum or plasma), clinically discrepant results were realized when comparing the Beckman PETINIA assay to both the Siemens PETINIA and ROCHE HEIA methods. Specifically, if these samples were peak/post-dose collections with a target range of 5.0 – 10.0 mg/L, the 5.0 mg/L spiked serum sample gave a concentration within target by the Beckman assay (6.1 mg/L) and below target by the Siemens and Roche assays (4.4 mg/L); the 10.0 mg/L spiked sample gave a concentration exceeding target by the Beckman assay (11.0 mg/L), while the Siemens and Roche assays gave concentrations within target of 8.8 and 9.5 mg/L, respectively.

Conclusions: Although most immunoassay results were within ±20% of LC/MS-MS values, some results were clinically discrepant. This, in turn, could lead a physician or pharmacist to take different clinical courses of action in regards to dosing. Diagnostic companies have an obligation to clinical laboratorians, physicians, pharmacists and above all, patients, to improve the accuracy of their tobramycin immunoassays through standardization with a non-immunogenic reference method like LC/MS-MS.

B-318

False Positive Carbamazepine Results by Gas-Chromatography Mass Spectrometry (GC-MS) and by VITROS 5600 Carbamazepine Assay Following a Massive Oxcarbazepine Ingestion

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Background: Carbamazepine (CARB) and Oxcarbazepine (OCBZ) are used as monotherapy and adjunctive therapy for the treatment of partial and generalized seizures. Both drugs are metabolized to their active metabolites. CARB is metabolized to 10,11-epoxide CARB, and OCBZ is metabolized to 10-hydroxy-10,11-dihydrocarbamazepine (DHC). For therapeutic drug monitoring of CARB, both parent drug and metabolites are measured as both compounds accumulate to significant concentrations. Because conversion of OCBZ is very rapid and the parent drug is present in very low concentrations, only DHC is measured for therapeutic drug monitoring. Reference ranges for CARB and DHC are 4-12 and 15-35 µg/mL respectively. A 23 month old patient with a seizure disorder presented to our emergency department after a suspected unintentional ingestion of his seizure medication. He had significant central nervous system depression and seizure-like movements. At the time of presentation, it was not clear if the patient was on Tegretol (CARB) or Trileptal (OCBZ) or if the patient had ingested another drug. Broad spectrum urine drug screening by gas-chromatography mass spectrometry (GC-MS) and CARB by immunoassay were ordered.

Methods: Broad spectrum drug screening was performed by an alkaline liquid-liquid extraction followed by GC/MS analysis. CARB assay was performed using Ortho Diagnostics VITROS 5600 Integrated System. HPLC linked to a UV detector was used for the assay of CARB, OCBZ and their metabolites.

Results: Urine drug screening by GC-MS showed the presence of CARB and OCBZ. CARB concentration measured by immunoassay was 7.9 µg/mL. Since CARB was reported on both GC-MS drug screening and VITROS 5600 immunoassay, it was initially thought that the patient ingested both drugs. Further investigation suggested that the patient ingested a large amount of OCBZ (estimated 4.5 grams) and had no access to CARB. This raised the possibility of a false positive carbamazepine by both GC-MS and immunoassay. To establish the absence or presence of CARB in the sample, a HPLC assay that detects CARB, OCBZ and their metabolites was performed. The HPLC assay did not show the presence of CARB or its metabolite. However, OCBZ and DHC were detected at concentrations of 20.4 and 49.3 µg/mL respectively. To further investigate the interference of OCBZ and/or DHC, plasma samples were prepared with different concentrations of OCBZ and DHC (12.5, 25, 50, 100 and 200 µg/mL), and analyzed by VITROS 5600 CARB assay. CARB values for the samples spiked with OCBZ were <3, 4.3, 8.9, 16.3 and >20 µg/mL respectively. CARB values for all the samples spiked with DHC were <3 µg/mL.

Conclusion: The false positive CARB result by GC-MS was likely due to thermal conversion of DHC to CARB in the injection port. OCBZ interferes with VITROS 5600 CARB assay. The finding is particularly important in overdose situations when OCBZ concentrations can be high.

B-319

A new high sensitive and specific assay for the determination of acetaminophen on clinical chemistry analyzers

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OBJECTIVE

Acetaminophen (also known as Paracetamol or APAP) is the most frequently used analgetic drug to treat mild or moderate pain caused e.g. by headache, muscle aches or toothaches. It also is frequently used to treat colds and fevers. In contrast APAP toxicity is the foremost cause of acute liver failure in the Western world and accounts for most drug overdoses. Due to its hepatotoxicity it is, by far, the most common cause of acute liver failure in both the United States and the United Kingdom. Currently available assays for the determination of an acetaminophen overdose in clinical chemistry laboratories do show clear problems with icteric or hemolyzed samples. Current tests also do interfere with the APAP antidote (N-Acetylcysteine, NAC).

METHODOLOGY

Here we present a new ready-to-use, two component reagent to measure acetaminophen on clinical chemistry analyzers (CCA). The new reagent is based on a colorimetric test, coupling the APAP specific enzymatic reaction to a new colorimetric chemistry principle. The new reagent shows an excellent performance using two point end kinetics and a low sample volume of only 4 µL. It is also not affected by so far known interferences like NAC, hemolyzed or icteric samples.

RESULTS

The new test shows a wide linear range from 3 to 600 mg/L APAP and allows for the robust determination of acetaminophen values in serum or plasma samples on routine CCA without prior dilution. Using a Hitachi 917 analyzer system, the test demonstrated an extraordinary precision of < 1% within run at a cut off concentration of 5 mg/L analyte. No significant interferences, at an acetaminophen concentration of 5 mg/L, with bilirubin (conjugated 30 mg/dL and unconjugated 12 mg/dL), NAC 1200 mg/L, hemolyzed samples 800 mg/dL, glucose 1000 mg/dL and intralipid 1000 mg/dL are given for the assay.

CONCLUSIONS

The new acetaminophen test is a very sensitive and highly specific method, which clearly demonstrates diagnostic utility for monitoring regular acetaminophen treatment. It also is very suitable for the diagnosis of patients with an APAP overdose or patients undergoing NAC antidote treatment. For this reasons the new chemistry test of acetaminophen offers a simple, robust and very reliable possibility to diagnose acetaminophen in the clinical laboratory routine.

B-320

New Emit II Plus Buprenorphine Assay with 5 ng/mL Cutoff

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Background: Buprenorphine is a semisynthetic opioid analgesic compound with a chemical structure similar to morphine. It is used as a substitution drug for treatment of opioid addiction but has a high potential for abuse and addiction. A new assay for measurement of buprenorphine has been developed by Siemens Healthcare on the Viva-E® Drug Testing System. The Emit® II Plus Buprenorphine assay has a cutoff of 5 ng/mL. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results.

Methods: Precision was evaluated at the cutoff and multiple levels according to CLSI EP5-A2. Analytical recovery was studied by spiking buprenorphine into human urine at levels that span the assay range (0-25 ng/mL). On-instrument stability was assessed by testing the assay controls over time. Specimens (127) were analyzed and the results compared to those of LC-MS/MS. Cross-reactivity with structurally related drugs was assessed at a concentration of 100,000 ng/mL. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of buprenorphine at the control levels of 3 and 7 ng/mL.

Results: Qualitative repeatability CVs (rate) for all levels ranged from 0.38 to 0.58%, and within-lab CVs ranged from 0.77 to 0.94%. Semiquantitative repeatability CVs (ng/mL) ranged from 1.68 to 4.38%, and within-lab CVs ranged from 2.23 to 6.92%. The assay's limit of detection was found to be 0.7 ng/mL. Semiquantitatively, the assay quantified buprenorphine-spiked samples between 2 and 25 ng/mL within ±10% of nominal values. The assay's measuring interval was between 0.7 and 25 ng/mL. At the 5 ng/mL cutoff, the percent agreement of specimens between the Viva-E analyzer and LC-MS/MS was 94%. Discordant samples were within ±25%

of the cutoff by both methods. The assay reagents demonstrated similar detection of buprenorphine and norbuprenorphine in urine with minimal cross-reactivity (<0.01%) to the structurally related opioids. Potentially interfering substances gave acceptable results relative to the 5 ng/mL cutoff. The reagents were stable onboard the Viva-E analyzer for a minimum of 4 weeks.

Conclusion: The Emit II Plus Buprenorphine assay on the Viva-E analyzer is a suitable screening method for urine specimens at the cutoff level of 5 ng/mL for both qualitative and semiquantitative analysis of buprenorphine.

B-322

Metabolic Patterns of Drugs and Drug Metabolites Observed in Urine, Serum or Plasma

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Background: Urine and serum specimen types are commonly used to assess patient adherence for prescribed drugs for pain management or for drug abstinence programs. Random specimen collections are typically used to determine whether patients are positive for the prescribed drug(s) and negative for the drug(s) not prescribed. Drug testing is performed by qualitative or quantitative analysis; however, hydration status and urine output volume can impact the drug concentration in various matrices. Quantitative analysis of parent and metabolites are routinely performed to assess adherence over time. However, there is minimal information to characterize the positivity rate of parent drug and metabolite(s) for tramadol, tapentadol, meperidine, fentanyl and methylphenidate, in urine and serum/plasma, to assess patient adherence.

Methods: Deuterated internal standards were added into an aliquot of the specimen. Drugs and metabolites were recovered from the urine, serum or plasma by solid phase extraction. The elution solution was evaporated and reconstituted in water. The solution was then injected into an ultra-high pressure liquid chromatograph coupled to tandem mass spectrometer (Waters Acquity UPLC TQD, [UPLC-MS/MS]). The instrument was operated in an ESI interface using multiple reaction monitoring (MRM) mode. There were four mass spectrometry methods that were validated for clinical use. Cutoff concentrations used to distinguish between positive and not detected varied between drugs. The following concentrations were used as cutoffs: 50 ng/mL (tramadol and tapentadol), 100 ng/mL (tramadol and tapentadol metabolites), 2 ng/mL (meperidine), 5 ng/mL (normeperidine), 10 ng/mL (methylphenidate) and 100 ng/mL (ritalinic acid) in urine and serum/plasma, respectively. For fentanyl and norfentanyl, 1.0 ng/ml was used as a cutoff for urine specimens, and 0.1 ng/ml was for serum or plasma specimens. Retrospective data were evaluated from the laboratory information system at ARUP laboratories (Salt Lake City, UT), a national clinical reference laboratory. The results were evaluated for positivity rates of the drugs and patterns of parent drug and drug metabolite(s).

Results: For urine specimens, the positivity rate was the highest for methylphenidate (61.8%, n=3948), followed by tramadol (43.8%, n=7972), fentanyl (40.9%, n=11136), tapentadol (35.7%, n=1948), and meperidine (9.5%, n=1678). Among positive samples, both parent drug and metabolite(s) was detectable in 95.2% of meperidine samples, 94.4% of tramadol samples, 93.6% of fentanyl samples and 86.6% of tapentadol samples. For serum or plasma specimens, the positivity rate was the highest for methylphenidate (74.2%, n=775), followed by fentanyl (57.3%, n=211), meperidine (55.7%, n=61), tapentadol (50.4%, n=129), and tramadol (30.1%, n=752). Similar metabolic patterns were found in serum or plasma. Of positive results, both parent drug and metabolite(s) were found in 98.3% of fentanyl samples, 79.8% of methylphenidate samples, 79.4% of meperidine samples, 70.8% of tapentadol samples, and 43.4% of tramadol samples.

Conclusion: Our data demonstrates the metabolic patterns of 5 drugs from a random urine or serum/plasma collection in patients. The data presented can be used to guide clinicians to determine drug adherence by assessing the positivity rates of the parent drug and corresponding metabolites. Of note, the lack of metabolites in serum is not indicative of noncompliance.

B-323

Red Blood Cell Methotrexate Polyglutamates are Stable for Over a Decade When Stored at Sub-Zero Temperatures (-80 Degrees C)

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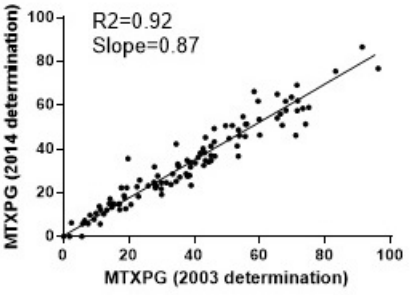
Objective: The establishment of long-term frozen stability of analytes is an important aspect of quantitative bioanalysis in therapeutic drug monitoring (TDM). In the present study we sought to establish the long-term stability of red blood cells (RBC) long-chain methotrexate polyglutamates (MTXPG3) from a group of 108 rheumatoid arthritis subjects previously enrolled to establish the performance characteristics of this TDM test (Arthritis Rheum. 2004 50:2766-74).

Methods: RBC MTXPG3 concentrations were originally measured in 2003 using a validated HPLC assay coupled with a post column photo-oxidation technique and fluorimetric detection (Clin Chem. 2003 49:1632-41). All patients enrolled in this study (from December 2002 to April 2003) consented for the long-term storage of their specimen. A total of 104 specimen were stored at -80˚C in our biorepository and available for re-analysis in February 2014 using the same analytical method. Results were expressed as nmol/L packed RBCs. Analysis consisted of linear regression with slope.

Results: As presented in the Figure there was a good concordance between RBC MTXPG levels determined in 2003 and those determined in 2014. RBC MTXPG levels determined in 2003 were 38±2 nmol/L (average±SEM) compared to 33±2 nmol/L 11 years later. Regression slope was 0.87 thereby indicating an acceptable bias of 13%. Regression coefficient was 0.923.

Conclusion: This is the first study establishing the long-term stability of RBC MTXPGs over a decade of storage at subzero temperature.



B-324

Prevalence of Gabapentin Abuse Among Clinical Patients

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Background:

Prescription medication abuse has drastically increased in the US over the last three decades. While toxicological analysis of clinical specimens is common practice among physicians, expanded testing to identify inconsistencies associated with non-prescribed drugs is less common.

During routine urine toxicology testing it was noted that a significant amount of the patient population was testing positive for Gabapentin when not prescribed by the physician. At the request of a single physician, a clinical investigation was conducted to assess the rate of misuse among his patients to determine the potential dangers associated with non-reported Gabapentin use.

Methods:

The purpose of this study was to determine the prevalence of Gabapentin misuse among a subset of clinical patients. LC/MS/MS testing was conducted on urine specimens when requested by the treating physician. The range of testing includes opiate/opioids, drugs of abuse, Gabapentin, and Pregablin, in addition to other substances. The method used for testing underwent a complete validation study where accuracy, precision, linearity (AMR), interference, carryover, ion ratio, retention time, LLOQ, matrix effect, suppression and specificity were assessed.

Using data mining, the laboratory identified all of those patients whose urine drug analysis indicated illicit Gabapentin use.

Results:

A total of 323 patients were tested over a five month period. Of these patients, 240 (74.30%) were compliant with their prescription drug regimen: positive for prescribed substances, negative for non-prescribed substances. In addition, 13 patients (4.03%) were non-compliant, testing negative for prescribed Gabapentin. A total of 70 patients (21.67%) were positive for non-prescribed Gabapentin.

Of those patients who tested positive for non-prescribed Gabapentin, 66 patients were on prescription drug regimens that included other substances: Opiate/Opioid (55.7%), Opiate/Opioid and Cyclobenzaprine (8.57%), Opiate/Opioid and Benzodiazepines (18.57%), other substances (8.57%). Four patients were not prescribed medications but tested positive for other substances in addition to Gabapentin.

Discussion/ Conclusion:

Gabapentin (Neurontin) is a known analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and is used for its anticonvulsant properties. While most widely used as an antiepileptic, in recent years it has also been used to treat neuropathic pain. Little is understood regarding the mechanism of action, however it is thought to have some effect on voltage activated calcium channels. Side effects include dizziness, ataxia, somnolence, nervousness and fatigue.

Little information exists regarding the significance of Gabapentin abuse among clinical patients. Until recently, it was considered to have little potential for abuse however this review has shown that a significant amount of patients are taking Gabapentin without physician consent. This could be due to the fact that recent studies have revealed that Gabapentin may potentiate the "high" obtained from other central nervous system acting drugs.

While Gabapentin is relatively safe and has a low potential for serious adverse effects, even in large doses, negative effects may occur when concomitant use with other CNS depressants occurs. Our study demonstrated the importance of performing full confirmatory testing in order to effectively identify at-risk patients.

B-325

Automating the Initial Chromatogram Review of a Liquid Chromatography Tandem Mass Spectrometry-based Broad Spectrum Urine Toxicology Screening Procedure with Peak Integration Software

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Background: Our regional reference laboratory performs qualitative broad spectrum urine toxicology screening via a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) procedure. This protocol uses defined positive/negative cut-off concentrations to screen urine for the presence of 63 different licit and illicit drugs. Volume for this test has grown ~30% since it was introduced in September 2013. This growth combined with our current process of requiring a medical laboratory technologist (MLT) to manually review the quality of all chromatographic results prior to release has impacted the tests turn-around-time. The commercially available ASCENT® software from Indigo bioAutomation is an algorithm-based peak picking and chromatographic data review system. This software deploys an exponentially modified Gaussian equation best fit model for standardized peak integration and offers the potential to automate the initial chromatogram review. **Objective:** Validate the ASCENT® software and its user-defined quality assurance peak integration rules by evaluating the reporting accuracy of the automated procedure relative to the current manual process. **Methods:** The validity of the ASCENT®-based protocol was assessed through parallel batch specimen processing and a comparison of the software-reviewed and MLT-reviewed data. Accuracy of the automated process was assessed by evaluating: (a) the relative agreement of all positive/negative results for all tested analytes within *N* >1500 patient specimens; and (b) the correctness of external proficiency testing challenge results. Although this test is reported qualitatively, the relative agreement of the derived analyte concentrations for all positive results was also compared. Within the tested patient specimens, the positivity rates for all analytes (*N*=63) included within the screen respectively ranged from 0 to 75.3%. The software's ability to accurately integrate low prevalence analytes was verified via its review of standard, quality control (QC) and spiked patient material with known analyte concentrations spanning their respective cut-offs and linear measuring ranges. **Results:** The derived concentrations of the calibration and QC standards in all tested batches were within our defined quality acceptance criteria when reviewed by either the MLT or software. A 99.9% (97009/97146) overall agreement between the software-reviewed and MLT-reviewed positive/negative patient results was observed. 82.5% (113/137) of the discordant results had analyte concentrations within ± 20 ng/mL and/or 20% of their respective positive/negative cut-off concentrations. Classifications of the remaining 17.5% (24/137) of discordant results were attributed to: (a) gross analyte concentration differences between the MLT-reviewed result and software-reviewed result (*N*=6); (b) MLT-review reporting error (*N*=3); and (c) software-review reporting error due to deficiencies in the implemented quality assurance peak integration rules (*N*=15), which were corrected prior to implementation. The software-reviewed results of all external proficiency testing challenges (*N*=5) were 100% accurate. The median percent concentration difference of all positive screening results (*N*=5868 from *N*=53 analytes) within the tested patient specimens was 4.1% (range: 0.01 to 36.2%). **Conclusion:** The automated ASCENT® software-review of our LC-MS/MS-based broad spectrum urine toxicology screening chromatograms had the equivalent accuracy and quality of our initial MLT-review process. Laboratories seeking opportunities to automate LC-MS/MS-based testing may consider using the outlined validation procedure to evaluate the ASCENT® software as a potential process enhancement.

B-326

Validation of an LC-MSMS method for nicotine reveals that the tobacco alkaloid anabasine is of limited clinical utility in differentiating active smokers from patients taking nicotine replacement therapy

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Intro: The need to provide accurate and quantifiable data in the detection of patient adherence to smoking cessation programs necessitates the utilization of high-resolution instrumentation. Reliance on immunoassay-based approaches can lack the sensitivity and specificity to provide a complete clinical picture in the context of various nicotine replacements and smoke cessation therapies. Surprisingly, during our validation of a new LC-MS/MS assay, we determined that this utilization resulted in a very high false negative rate when compared to patients' self-reported smoking status. Our results therefore call into question the clinical utility of anabasine as a biomarker of active tobacco use.

Methods: Patient urine samples were obtained from our orthopedics and transplant centers. Samples analyzed by automation were purchased from UTAK. Interfering matrix components were removed using supported liquid extraction (SLE) columns either by vacuum manifold or on the Extrahera (Biotage®) automation station. The extracted analytes (nicotine, cotinine, trans-3'-hydroxycotinine and anabasine) were chromatographically resolved over 7 minutes on a Waters 50 x 2.1, 1.7 µm BEH-C_{18} column prior to injection and analysis by tandem mass spectrometry (Waters Corp.). Linear responses were evaluated across the typical range reported for routine tobacco users. Patients smoking status was self reported to Emory clinicians and obtained by analyses of the electronic medical record. Patients were categorized as either active smokers (nicotine > 30 ng/mL; cotinine > 50 ng/mL; trans-3'-hydroxycotinine > 120 ng/mL; anabasine > 3 ng/mL) or non-smokers if they did not fulfill the above criteria. [3].

Results: The quantification limits of the method were 1-5 ng/mL and the limits of detection ranged from 0.6-5 ng/mL. The linear range for all analytes was confirmed over 5-5000 ng/mL. Interday and intraday precisions for all analytes had a C.V. of <15% at the low end of the quantification limit. Method comparison studies were performed with either GC-MS (within institution method) or by LC-MS/MS (ARUP) correlation coefficients were determined to be between (0.95-0.99). Under the auspice of automation, recoveries were determined to be 107, 95, 53, and 107 percent for nicotine, anabasine, trans-3'-hydroxycotinine, and cotinine, respectively. Moreover, sample processing times (completed in batches of 24) decreased by 50% using the Extrahera™ automation station when compared to manual extractions. Most surprisingly we determined that 64% of our patients that self declared as active smokers and tested positive for nicotine had anabasine levels below <3 ng/ml.

Conclusions: Nicotine, cotinine, trans-3'-hydroxycotinine and anabasine can be simultaneously accurately quantified in human urine by LC-MS/MS to distinguish between smokers and non-smokers. Moreover, workflow efficacy improved significantly when automation was integrated into the pipeline. Interestingly the clinical utility of anabasine as a means of detecting active tobacco use is highly questionable, as up to 64% of admitted smokers had no detectable levels of the alkaloid.

B-327

Validation of Analytical method for quantification trans-trans muconic acid (t,t-MA) urinary as a biomarker exposure to benzene

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Background: The primary purpose of biological monitoring is to protect the health of employee, preventing them from excessive exposure to chemical products. The t,t-MA is a benzene biotransformation product and has been recommended as exposure biomarker adopted by the Brazilian legislation for the monitoring of occupational exposure to solvent. This study was conducted in order to validate the urinary t,t-MA detection method, for their use in biomonitoring of employees exposed to the product.

Methods: We used high performance liquid chromatograph Hewlett-Packard® HP 1100 brand model consisting of autosampler, quaternary pump and UV detector with variable wavelength. Detection was accomplished at 227 nm. For the chromatographic separation employed Hypersil GOLD aQ® analytical column (125 x 4 mm, 5µm) and maintained at 30 °C during the analysis. The mobile phase was water:methanol (94:6, v/v) with a flow rate of 1.0 ml/min. The linearity was observed in the expected concentration range. Urine was used as a biological matrix for the study and t,t-MA standard.

Results: The linearity was studied using enriched samples of t,t-MA from 0.1 to 3.00 mg/L and the urine samples were evaluated by six times, each. The limit of detection (L.D.) and the limit of quantification (L.Q.) were defined as the lowest concentration, whose the coefficient of variation did not exceed 20% and 15%, respectively. The intra-assay precision was demonstrated by average coefficient of variation (CV%) for urine sample analyzed from seven different concentration and by six times, each one, in the same moment of the study. To determine inter-assay CV% mean analyzed three different concentrations for three days. The accuracy of the method was verified by analyzing samples of known concentration and expressed in percentage. The retention time (RT) of 3.3 min was obtained and the total analysis time was seven min. The use of solvents such as ether extraction and acetonitrile in the mobile phase composition, ensure simplicity and low cost in the exam. Linearity was studied in the concentration range 0.1 to 3.0 mg/L with a coefficient of determination (R2) of 0.99374. The CV% average intra-assay accuracy and precision obtained for the range of concentration of 0.2 to 3.0 mg/L was 2.86%. For the concentration range between 0.5 and 1.5 mg/L mean inter-assay CV% was 5.10%. The method's accuracy is between 87% and 109%. The samples used in determining these parameters were prepared from urine obtained through donation and previously submitted to the proposed method, proving the absence of the substance.

Conclusion: The method was quick and efficient in determining urinary t,t-MA. The efficiency and selectivity combined with the technical robustness can be employed to t,t-MA dosage in the control of occupational exposure to benzene.

B-328

Validation of a Broad Spectrum Drug Screening Method Using High Resolution LC-TOF

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Introduction: Urine drug screening is among the most widely used procedures in clinical toxicology laboratories. Immunoassay is the primary method used for drug screening in many laboratories followed by confirmation through tandem mass spectrometry (LC-MS/MS). LC-MS/MS isolates the protonated analyte [$(M+H)^+$] of interest followed by fragmentation in the collision cell and monitoring of one or more product ions. However, one of the major limitations of LC-MS/MS is that it difficult to perform non-targeted screening. Recently, high resolution mass spectrometry (HRMS) such as time of flight-mass spectrometry (TOF-MS) has been proposed as an alternative for non-targeted drug screening. We validated a UPLC-TOF HRMS method for drug screening by assessing reproducibility (within run and between run precision), patient comparison studies and carry over effects. **Methods:** Sixty one drugs were divided into six groups at three different concentrations (100 ng/mL, 1000 ng/mL and 5000 ng/mL) and within run (five injections), between run (twenty injections over 20 days) and patient comparison studies were performed. Identification criteria for a positive result involved accurate mass (within 5 ppm), one fragment (within 10 ppm) and retention time match (within 0.2 min). Compound separation was achieved using Waters and detected through TOF-MS (Xevo G2 TOF; Waters Corp). A MRM-based LC-MS/MS (UPLC-Xevo TQ-S) was used to confirm positive results and served as the reference method. **Results:** Within run studies revealed that out of 5 injections, norpropoxyphene, EDDP and tramadol were not identified at a low concentration of 100 ng/mL; however, detection greatly improved at the higher concentrations of 1000 ng/mL and 5000 ng/mL. The remaining drugs were all identified 5/5 times at all three concentrations. Between run precision studies revealed that, as in the case of within run precision, norpropoxyphene and tramadol were not identified at 100 ng/mL. In addition, other drugs such as morphine, norbuprenorphine, propoxyphene, EDDP and MDA were identified 17/20, 14/20, 17/20, 6/20 and 18/20 times respectively at a concentration of 100 ng/mL. Upon increasing the concentration of these drugs to 1000 ng/mL and 5000 ng/mL the detection rate improved significantly. Initial patient comparison studies for 23/61 drugs in the panel were performed. The LC-MS/MS method identified a total of 244 compounds while the TOF-HRMS method identified 190 and 188 compounds respectively when samples were run in duplicate. The drugs with the highest false positive rate of identification were oxycodone, EDDP, hydrocodone, lorazepam and methamphetamine. Drugs with the highest false negative rate identification were: fentanyl, morphine, norfentanyl, hydroxyalprazolam, amphetamine, norfentanyl, methamphetamine and nordiazepam. TOF-HRMS was able to identify a number of drugs that were not part of the normal confirmation panel through LC-MS/MS. **Conclusion:** Our UPLC-TOF HRMS method for drug screening offers the advantage of performing non-targeted drug screening (compared to MRM based LC-MS/MS) and has the potential for higher sensitivity and specificity than conventional immunoassays. Current validation studies show that at low drug concentrations, some of the drugs such as norpropoxyphene and tramadol were missed, mainly because due to the lack of fragment identification. However, upon increasing the drug concentrations the identification status improved significantly.

B-330

Non-FDA-Approved Benzodiazepine Analogs: Clonazolam and Etizolam. Reactivity with the ThermoFisher Immunoassay, Elimination Half-Life, and Incidence in Benzodiazepine Positive Urine Samples.

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Background: Benzodiazepines are widely used for treatment of anxiety and insomnia, however, this class of drugs is also commonly abused. Many different benzodiazepines and analogs have been produced that are not FDA-approved. We recently encountered two of these, clonazolam and etizolam, in clinical toxicology cases in the San Francisco Bay Area. The objective of this study was to determine the reactivity of these two benzodiazepine analogs with the immunoassay that we use in the clinical lab and determine the incidence in urine samples that were analyzed for drugs-of-abuse. We also determined the elimination half-life in a case of dual ingestion of clonazolam and etizolam.

Methods: For immunoassay cross-reactivity studies, 0-800 ng/mL of clonazolam or etizolam was prepared in drug-free urine. Samples were analyzed in triplicate using the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. For incidence studies, we collected 211 urine samples that were previously determined positive on the ThermoFisher Benzodiazepine Assay at the Zuckerberg San Francisco General. Samples were analyzed using a previously validated liquid chromatography high resolution mass spectrometry (LC-HRMS) method. Also, serum samples from a case of dual ingestion of clonazolam and etizolam were analyzed using this LC-HMRS method. Preparation of serum samples consisted of protein precipitation by acetonitrile, drying of the sample, and reconstitution in 2% mobile phase B. Urine samples were diluted 1:9 using 2% B. HRMS data was acquired with an ABSciex TripleTOF®5600 system in positive ion mode, collecting full scan data with IDA triggered acquisition of product ion spectra. Chromatographic separations were performed on a Phenomenex Kinetex C18 column (50 x 3.00 mm, 2.6 µm). Mobile phase A was 0.05% formic acid in 5 mM ammonium formate. Mobile phase B was 0.05% formic acid in 50% methanol 50% acetonitrile. The elution gradient was ramped linearly from 2% to 100% B over 10 minutes. Data analysis was done using PeakView® and MasterView® software (version 2.0, AB Sciex).

Results: Clonazolam and etizolam both showed significant cross-reactivity with the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. When using our current cut-off of 200 ng/mL, a concentration greater than 113 (95% CI: 107-120) ng/mL clonazolam, or 195 (187-204) ng/mL etizolam, was calculated to lead to a positive test result. However, none of the 211 analyzed urine samples, which were previously determined positive on the ThermoFisher Benzodiazepine Assay, were found to contain clonazolam, etizolam, or their metabolites. In a clinical toxicology case of dual ingestion of clonazolam and etizolam, the highest concentration found in serum was 10.2 and 281 ng/mL, respectively. The elimination half-life was 216 (95% CI: 136-521) and 285 (227-386) minutes, respectively.

Conclusion: Clonazolam and etizolam significantly cross-react with the ThermoFisher CEDIA® High Sensitivity Benzodiazepine Assay. Although recently encountered in clinical toxicology cases, clonazolam and etizolam were not detected in 211 benzodiazepine positive urine samples. The elimination half-life for etizolam was similar to previously reported values. An elimination half-life for clonazolam was not reported earlier.

B-331

Up in Smoke: Uncovering a Lack of Evidence for Proton Pump Inhibitors as a Source of THC Immunoassay False Positives

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Background: False positives in immunoassays can result from cross-reactivity of antibodies. This becomes an issue in interpretation of drug screens, and it is recommended that false positive screens be followed up with more specific confirmatory testing. Since 2005 the drug package insert for pantoprazole has included a statement of reports of false positive urine screening tests for tetrahydrocannabinol (THC) in patients receiving proton pump inhibitors (PPIs). However, no data is given nor a specific screen mentioned. A search of literature and the internet did not result in concrete data. There is an adverse event report of a positive THC meconium screen from an infant whose mother was taking pantoprazole, but the urine screen was negative. Recently, a case report was published claiming a false positive THC from a young patient who presented with cyclic vomiting syndrome and was given ondansetron, pantoprazole, and diazepam. The urine drug screen obtained 2.5 h after administration of pantoprazole was positive, but confirmatory testing via gas chromatography-mass spectrometry was negative. However, testing in the absence of pantoprazole was not done and there was no mention of the assay or cutoff used. A higher number of false positive THC urine screens in infants compared to adults has been attributed to metabolite differences. **Methods and Results:** Although the number of THC positive screens in our lab that do not confirm is low, we wanted to investigate the possibility of PPIs contributing to these. A THC-negative urine sample was spiked with pantoprazole (10,000 ng/mL) and analyzed using the EMIT II Plus Cannabinoid assay. This detects the 11-nor-Δ9-THC-9-COOH metabolite (THC-COOH) with a 20 ng/mL cutoff. A negative result was obtained. Since pantoprazole is metabolized in vivo and almost 80% of an oral dose is excreted as metabolites, we also investigated urine samples from 32 patients currently taking a PPI (dexlansoprazole, n=4; exomeprazole, n=3; lansoprazole, n=2; omeprazole, n=17; pantoprazole, n=6). These samples were obtained under IRB approval from volunteers or from samples previously submitted for drug testing to our lab for which current drug history was also available. These also resulted in no positives at the 20 ng/mL cutoff. Investigation of raw data identified one sample close to the cutoff value; it confirmed as positive for THC by a liquid chromatography-tandem mass spectrometry method with a 5 ng/mL cutoff. Since the number of samples available was low, we used computational techniques to determine whether a pantoprazole metabolite shares any structural or electrostatic similarities with THC-COOH. Pantoprazole sulfate, the major metabolite, and THC-COOH were built in Maestro and tautomeric states determined using Epik in water at a pH of 7 (±2). A 25K step mixed torsional/low mode conformational search was performed and electrostatic potential energy was calculated in an OPLS3 forcefield. Comparison of structures shows very little overall similarity in shape or electrostatics between the two molecules. **Conclusion:** We find no supporting evidence of pantoprazole or its metabolites as the cause of false positives in the EMIT II Plus Cannabinoid assay and caution the use of PPIs as a possible explanation for THC immunoassay false positives.

B-332

Trends in Heroin Related Deaths and 6-MAM Concentrations from the Hennepin County Medical Examiner's Office Between 2004 and 2015

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Background: Over the past decade, prescription opiate use and widespread abuse have become major concerns driving changes to opiate prescribing practices and prescription monitoring programs. These changes have accompanied increases in both the use of heroin and in heroin related deaths. This study evaluated the trends in heroin related deaths and associated concentrations of the heroin metabolite 6-monoacetyl morphine (6-MAM) in urine, blood, and vitreous humor. **Methods**: We retrospectively reviewed the cause of death (COD) for cases from the Hennepin County Medical Examiner's Office between 2004 and 2015 where heroin was suspected as a potential contributor to COD either based on investigative information or as identified by blood or urine drug screen. **Results**: We identified 287 heroin related deaths. From 2004 to 2006 there were an average of 12 heroin related deaths per year. This significantly increased (p=0.009) to a mean of 57 heroin related deaths per year from 2012 to 2014. 79% of the decedents were male and the mean (SD) decedent age was 36 (12) years. Mean (SD) 6-MAM concentrations were significantly different between sample types: urine 0.540 (0.439) mg/L; blood (various sources) 0.025 (0.027) mg/L; vitreous humor 0.064 (0.063) mg/L. Mixed drug toxicity was the COD in 46% (n=130) of cases. Ethanol was also present in 104 (36.2%) cases, with a mean (SD) concentration of 0.113 (0.124) g/dL. Mean (SD) blood concentrations of morphine were: total 0.899 (1.08) mg/L and free 0.233 (0.278) mg/L. In addition to opiates, cocaine (n=81) and benzodiazepines (n=61) were the most frequently identified drug classes on screening. Accident was the manner of death (MOD) in 94% (n=265) of the identified cases, with the remaining cases' MOD evenly split between natural, homicide, and suicide. **Conclusion**: We found the number of heroin related deaths increased nearly 5-fold between 2004 and 2015. Postmortem urine, blood, and vitreous humor specimens are all useful in identifying 6-MAM to confirm heroin related deaths.

B-333

Evaluation of a New ELISA Based Test for the Determination of Pregabalin in Biological Samples

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Background: Pregabalin is a gamma-aminobutyric acid analog used for the treatment of neuropathic pain and partial seizures, as well as generalised anxiety disorder. It is also used to treat fibromyalgia in the USA and is currently listed as one of the top 30 prescribed medications. Pregabalin is eliminated from the systemic circulation primarily by renal excretion as unchanged drug with a mean elimination half-life of 6.3 hours in subjects with normal renal function. In recent years, pregabalin abuse has increased, especially among heroin addicts and prison inmates.

As the demand for a method suitable for high-volume screening of pregabalin is increasing, this study reports a new Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of pregabalin in urine and whole blood. This is relevant for compliance monitoring of pain patients and for clinical toxicology applications. **Methods:** Pregabalin was derivatised and conjugated to immunogenic carrier material (Keyhole Limpet Haemocyanin). The resulting immunogen was administered, via intramuscular injection, to adult sheep, to intiate polyclonal antiserum production. The antiserum generated was employed in the development of a competitive colorimetric immunoassay. Ig fraction derived from the antiserum was immobilized and stabilized on a 96-well micro-titre plate. The analyte, if present in the sample, competed with horseradish peroxidase labelled pregablin conjugate for antibody binding sites on the microtitre plate. Absorbances were read at 450nm.

Results: Specificity was determined by calculating percentage cross-reactivity with the major metabolite N-methylpregabalin and a range of therapeutic compounds. The assay exhibited specificity for pregabalin and < 1% cross-reactivity with the other compounds tested. The analytical range of the assay was 0 - 50 µg/mL pregabalin with Limits of Detection (LOD) of 0.05 µg/mL for urine and 0.159 µg/mL for whole blood. Recovery and inter-assay precision were assessed using spiked negative urine and whole blood at the cut-off and +/- 50% cut-off (1, 2 and 3 µg/mL). Three replicates of each level were assessed over 5 separate runs. For each level the recovery was within the target range of 100 +/- 20%, while inter-assay precision expressed as CV(%) was ≤ 10. Additionally, 20 authentic urine samples, 10 positive for pregabalin and 10 for gabapentin, were assessed and correlated with the concentration obtained via liquid chromatography mass spectrometry (LC-MS). Specificity for pregabalin was confirmed and the assay exhibited 100% agreement with the LC-MS method.

Conclusion: This evaluation indicates applicability of this new ELISA to the detection of pregabalin in blood and urine. The LODs for both matrices were below the cut-off concentration of 2µg/mL. The assessment of samples showed optimal concordance whilst maintaining specificity to pregabalin with minimal detection of gabapentin. Thus, the ELISA represents a highly specific and useful analytical tool, which could be applied within a clinical setting to compliance monitoring of pain patients and toxicology applications.

B-335

Long-term Storage Stability of Opioid Drugs in Urine, Dependence on Sample pH

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Background: Urine analysis of opioids is employed for a variety of purposes, including pain management, employment eligibility, and diagnosis of drug intoxication. There is often a need for long-term storage of urine samples prior to analysis. We observed that most opioids were stable in frozen (-20 C) urine, with two notable exceptions: buprenorphine and 6-monoacetylmorphine (6MAM) fell below acceptable limits prior to 30 days of storage. The recovery of other analytes suggested this was an analyte-specific phenomenon. We hypothesized that the pH of the sample pool was outside of the normal range of 6-8, leading to premature degradation of sensitive analytes.

Methodology: Discarded urine samples containing buprenorphine-glucuronide or 6MAM were pooled and the pH was verified between 6 and 8. Samples were stored frozen in preservative free plastic cups. Buprenorphine-glucuronide samples were prepared by enzymatic hydrolysis and liquid-liquid extraction, then quantitated by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a method that performs within a 9.8% CV for between run replicates. 6MAM samples were prepared by solid phase extraction, then quantitated by LC-MS/MS using a method that performs within a 6.0% CV for between run replicates.

Results: Concentrations (ng/mL ± standard deviation) of sensitive analytes in routine and pH controlled stability experiments. *Indicates result below acceptable limit (80% of Day 0 mean). Data from the lower of two target concentrations is shown.

Day	Spiked Buprenorphine	Patient Buprenorphine, pH 6 to 8	Day	Spiked 6MAM	Patient 6MAM, pH 6 to 8
0 (n=5)	14.8 ± 0.6	15.8 ± 0.7	0 (n=5)	10.5 ± 0.1	20.6 ± 0.7
1 (n=3)	10.0 ± 0.3*	13.4 ± 0.4	1 (n=3)	10.4 ± 0.2	
2 (n=3)	13.7 ± 1.1	12.8 ± 0.1	7 (n=3)	9.4 ± 0.1	
7 (n=3)	13.1 ± 0.5	13.0 ± 0.2	14 (n=3)	7.2 ± 0.2*	18.5 ± 0.3
14 (n=3)	8.5 ± 0.7*	13.0 ± 0.3	30 (n=3)	7.6 ± 0.4*	18.3 ± 0.4
30 (n=3)	10.4 ± 1.2*	16.5 ± 0.4	60 (n=3)		19.8 ± 0.4

Conclusions: Free buprenorphine exhibits variable recovery when spiked into urine. By utilizing positive patient samples and maintaining pH between 6 to 8, the standard deviation of replicates was reduced, giving a more accurate determination of stability. Selecting samples with normal pH was also beneficial for determining the stability of 6MAM in urine. Clients are now advised that published stability data is applicable to urine samples with pH of 6 to 8 for tests that include sensitive analytes.

B-336

Evaluation of the ARK Diagnostics Lamotrigine Immunoassay on Beckman AU 400 for Therapeutic Drug Monitoring

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Background: Lamotrigine is an anti-epileptic drug used as an adjunct therapy for partial seizures, and has since gained indications as monotherapy for partial seizures and also as treatment for bipolar disorder. Lamotrigine is commonly used in pregnant women with epilepsy due to its solid safety record in pregnancy. Objective: Validate the Ark Diagnostics homogenous immunoassay for the quantitative determination of Lamotrigine levels in plasma, and to compare the immunoassay to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Method: ARK Lamotrigine Assay is a homogeneous immunoassay based on competition between the unlabeled lamotrigine in the patient sample and lamotrigine labeled with the enzyme glucose-6- phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance, by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a Hitachi 917 chemistry analyzer and an LC-MS/MS method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, and comparison studies. Results: The assay showed excellent correlation to the Hitachi 917 chemistry analyzer (y = 0.989x - 0.32; r2=0.9956; N=20, with an analytical measurement range of 1.0-40µg/dL. The analytical sensitivity was 2.0µg/dL. Both within-run (3.5%-3.9%) and between-run (4.8%-5.6%) imprecision were within acceptable limits, and the assay exhibited no carryover (<1%). Conclusions: The ARK Lamotrigine immunoassay is suitable for therapeutic drug monitoring of the novel antiepileptic drug lamotrigine. The method provides rapid turn-around-time (2hours), compared to 2-3 days as a send out test.

B-337

Analytical evaluation of cyclosporine and tacrolimus on the Roche automated electrochemiluminescence immunoassay platform

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Objectives: Cyclosporine (CSA) and tacrolimus (TAC) are immunosuppressant medications that require careful therapeutic drug monitoring following organ transplantation. This study evaluated the analytical performance of the semi-automated Roche Elecsys immunoassays for CSA and TAC on the Roche e411 platform. **Design and Methods:** Precision was evaluated using manufacturer and BioRad Whole Blood Immunosuppressant controls. Linearity across the measuring range was assessed using CAP Linearity Survey material. Method comparison studies comparing Roche e411 with Abbott Architect and LC-MS/MS were performed using patient samples spanning the analytical measuring range. Analytical sensitivity and lot-to-lot assessment were also assessed. **Results:** Precision ranged from 3.4 to 8.0% for CSA and 4.1 to 9.9% for TAC. Linearity was verified from 48.0 µg/L to 960.9 µg/L for CSA and from 1.4 µg/L to 27.1 µg/L for TAC. The functional sensitivity met the manufacture's claim and was determined to be 44 µg/L for CSA and 0.7 µg/L for TAC (CV ≤ 20%). Deming regression analysis of the Abbott Architect method comparison (n = 102) yielded slopes of 0.92 (95%CI: 0.89-0.95) for CSA and 0.92 (95%CI: 0.88-0.97) for TAC. Deming regression analysis of the LC-MS/MS method comparison (n = 20) yielded slopes of 1.33 (95%CI: 1.17-1.50) for CSA and 0.92 (95%CI: 0.84-1.01) for TAC. Lot-to-Lot comparison (n = 20) yielded slopes of 0.998 (95%CI: 0.97-1.03) for CSA and 0.97 (95%CI: 0.94-1.01) for TAC. **Conclusions:** The Roche Elecsys CSA and TAC assays have acceptable precision, linearity, and functional sensitivity and are comparable to Abbott Architect and LC-MS/MS methods and is fit for purpose for the therapeutic drug monitoring of CSA and TAC.

B-339

An Investigation of Illicit Fentanyl Substances in a Clinical Setting

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Background

Acetyl-fentanyl is a powerful opioid analgesic that is an increasing concern in the treatment of patients in the pain management and addiction settings. More information is coming to light regarding the introduction of fentanyl and acetyl-fentanyl into adulterated pills and street drugs. The DEA has confirmed 52 acetyl-fentanyl associated deaths between 2013 and 2015. Heroin related overdose and death has reached epidemic proportions in the United States. Mainstream media frequently associates this increase in overdoses with the possibility of fentanyl and/or acetyl-fentanyl laced heroin but little scientific research has been published investigating these theories. Acetyl-fentanyl laced cannabis is also a concern with the changing legalities surrounding recreational and medicinal cannabis, the suspicion of laced cannabis may be falsely decreased. Illicit sources of the drug will not have the stringent testing requirements that most surely will blossom in the changing cannabis landscape. LC-MS/MS confirmation must be performed to identify acetyl-fentanyl in urine as immunoassays cannot differentiate it from fentanyl.

Objectives

To determine the prevalence of acetyl-fentanyl, fentanyl, and norfentanyl in urine of patients confirmed positive for 6-acetylmorphine and THC.

Methods

A total of 365 urine specimens were analyzed. The sample group was comprised of all 6-acetylmorphine confirmed specimens (positive cut off 5.0 ng/mL) from a 90 day period and all THC samples confirmed > 500 ng/mL over a 30 day period. All samples were tested by LC-MS/MS (Agilent, Santa Clara CA) for the presence of free and conjugated forms of acetyl-fentanyl, fentanyl, and norfentanyl, employing IMCSzyme recombinant beta-glucuronidase pretreatment, with a reporting concentration limit of 2.5 ng/mL for all compounds.

Results

Of the 156 6-acetylmorphine urine specimens tested, 3 (1.9%) confirmed positive for acetyl-fentanyl, 19 (12.2%) confirmed positive for fentanyl, and 20 (12.8%) confirmed positive for norfentanyl. Fentanyl prescriptions were not disclosed in any of the 6-acetylmorphine cases. None of the 209 THC urine specimens tested confirmed

positive for acetyl-fentanyl however, 2 (1.0%) confirmed positive for fentanyl, and 3 (1.4%) confirmed positive for norfentanyl. Fentanyl prescriptions had been disclosed in 3 (1.4%) of the THC positive cases.

Conclusions

Due to the lack of disclosure of illicit substance use the source of acetyl-fentanyl in our study cannot be determined. In all positive cases acetyl-fentanyl was present in addition to fentanyl and/or norfentanyl. Adulterated or mislabeled illicit pills are an increasing presence in the street drug market. Our experiment filtered samples for positive heroin use in an attempt to investigate low level norfentanyl positive, negative fentanyl results seen in tandem with 6-acetylmorphine in our patient population. The results obtained however cannot rule out the additional use of illicit fentanyl pills. Although acetyl-fentanyl was identified in 6-acetylmorphine samples, the lack of acetyl-fentanyl in THC positive samples indicates the acetyl-fentanyl lacing of cannabis may not be as high of a concern as with heroin. While acetyl-fentanyl is present in specimens our lab received, the low prevalence does not warrant the addition of this assay to our current testing panel at this time.

B-340

Evaluation of a point of care test for urine fentanyl screning

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Introduction: Fentanyl is a synthetic opioid that is estimated to be 100X more potent than morphine and 40X more potent than heroin. Deaths due to fentanyl have dramatically increased in Canada in the past 2 years, especially in Alberta where in 2015, over 250 deaths were associated with fentanyl. The fentanyl crisis has impressed the need for a screening method. There are few fentanyl immunoassay kits available that can be used to screen for the drug. Mass spectrometry will be the choice for confirmation, if needed. Here we present the evaluation of a point of care fentanyl strip for use in urine drug screening.

Methods: The point of care strip (Innovacon) is a qualitative lateral flow immunoassay calibrated against norfentanyl, a major metabolite of fentanyl. The analysis time is 15 minutes after specimen application. The cutoff is 20 ng/mL. Results are read manually with the appearance of two lines indicating a negative and one line indicating a positive. The strip performance was compared to our quantitative opioid confirmation assay which consists of dilute and shoot followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis that detects fentanyl and norfentanyl with a lower limit of quantitation of 10 ng/mL for each.

Results: 50 samples (2 drug free urine blanks, 4 QC, 44 patients) were compared using the fentanyl strip and LC-MS/MS. The fentanyl and norfentanyl concentrations in the patient specimens ranged from <10 to 4584 ng/mL and <10 to 11197ng/mL, respectively, as determined by LC-MS/MS. Overall, results generated by the point of care (POC) kit agreed well with those generated by LC-MS/MS analysis (both positive and negative), with agreement for 45/50 samples (90%). Four of the remaining 5 specimens with fentanyl or norfentanyl concentrations of 12 ng/mL or lower (by LC-MS/MS) were also positive by POC kit; however, the 5th specimen with norfentanyl concentration of 25 ng/mL and fentanyl concentration <10 ng/mL by LC-MS/MS was a false negative by the POC kit.

Conclusion: The point of care fentanyl strip compares well with our LC-MS/MS opioid confirmation assay and may be used for qualitative analysis of fentanyl in the lab.

B-341

Comparison of cobalt and chromium results from two Canadian laboratories

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Introduction: Metal on metal (MoM) prosthetics for hip replacement have proved popular as they exhibit less wear than their metal on polyethylene counterparts. Following MoM joint replacement, it is common for the levels of prosthetic metals (cobalt and chromium) to become elevated in the blood and are used by the surgeon as indicators of wear and tear and/or to assess for possible toxicity. At present, there is little consensus on the use of blood cobalt and chromium measurements, especially with respect to the specimen of choice (whole blood vs serum). Our lab recently switched referral labs for trace metals testing from Lab A, who performed cobalt and chromium in whole blood, to Lab B, who measures cobalt in whole blood and chromium in serum. Here we present the results of a study comparing the cobalt (whole blood vs. whole blood) and chromium (serum vs whole blood) results from these two labs.

Materials & Methods: Both Lab A and B use inductively coupled plasma-mass spectrometry (ICP-MS) for trace element analysis.

Patients with existing MoM hip replacement being monitored by orthopedics were included in the study. Following their regular blood draw for whole blood cobalt and serum chromium which was sent to Lab B as per normal laboratory protocol. An aliquot of the whole blood tube was taken and sent to Lab A for whole blood chromium and cobalt analysis. Results were collated and analyzed using our in house patient comparison spreadsheet. So far, results have been obtained from 68 patients with a MoM joint replacement .

Results: Preliminary results showed that comparison between Lab A and B had an R2 value of 0.9969 (y=0.79x + 2.163) and displayed a bias towards Lab A. For whole blood cobalt concentrations below 100 mmol/L, the percent bias ranged from +10% to -30%. Above 100 ng/mL, the percent bias was consistent at -20%. This was of interest as we expected better agreement between the two labs when using the same specimen type and technology.

The comparison of whole blood chromium (Lab A) to serum chromium (Lab B) showed a clear positive bias towards Lab B (R2 = 0.9944; y=1.41x-5.05). The majority of chromium values obtained were below 100 ng/mL and exhibited a % bias ranging from -30% to +60%. This is consistent with previous studies which have shown poor correlation between serum and whole blood chromium.

Conclusion: Cobalt and chromium measurements can differ substantially between labs, even when using the same specimen type and analytical method.

B-342

Optimization and validation of qualitative testing for prescribed medications in urine using liquid chromatography-mass spectrometry

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Background: Urine qualitative testing for prescribed medications by liquid chromatography-mass spectrometry (LC-MS) provides scientific data that a clinician can use to assess patient medication management. In this study, we statistically evaluated the performance accuracy of qualitative testing methods by LC-MS. By optimizing the cutoff values and instrument data processing, we further lowered cutoffs, enhanced test sensitivity, and reduced the false negative rate.

Methodology: Patient data, along with LC-MS results and instrument integration threshold parameters, were collected and statistically evaluated with Receiver Operating Characteristics (ROC) analysis. The data from 50 to 80 patients with positive and negative results for each drug were collected. The documented information on patient prescribed medications, combined with higher resolution and sensitivity high performance liquid chromatography-tandem mass spectrometry, were used as gold standards to confirm result accuracy. R statistical programming was used to construct ROC curves and curves of accuracy across the range of possible cutoffs for multiple drugs. Area under the curve (AUC) for each ROC, sensitivity and specificity for each possible cutoffs, positive and negative predictive values (PPV and NPV), and accuracy were calculated by running the written R scripts.

Results: The AUCs of ROC curves for most of drugs in the test panel ranged from 0.96-0.98. At optimal cutoff, the sensitivity and specificity were 97-98% and 94-95%, respectively.

Conclusion: The setup for optimal cutoffs and instrument parameters for qualitative LC-MS drug testing significantly enhances the test accuracy and sensitivity, and reduced the false negative rate.

B-343

LC-MS/MS quantification of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy- Δ9-tetrahydrocannabinol-glucuronide in urine

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Introduction: Cannabis is the most commonly used illegal substance worldwide, with 7.5% of American's older than 12 years of age and 10.2% of Canadians older than 15 years of age projected to have used cannabis in the past year. Such high prevalence of usage and its association with the initiation of illicit drug use has required the detection of cannabis and its metabolites by clinical toxicology laboratories engaged with addiction screening and drug treatment programs. The quantification of the

main psychoactive cannabinoid, Δ9-tetrahydrocannabinol (THC), as well as its metabolites, 11-hydroxy-THC (THCCOH) and 11-nor-9-carboxy-THC (THCCOOH) predominately rely upon either GC-MS or LC-MS/MS analysis, with these methods requiring hydrolysis of 11-Nor-Δ9-THC-9-carboxylic acid glucuronide (THCCOOH-glucuronide) to measure total THC.

Objective: Development of a LC-MS/MS quantitative method for the quantitative analysis of THCCOOH and THCCOOH-glucuronide in patient urine, including the investigation of the source of THCCOOH and THCCOOH-glucuronide interferences.

Material and Methods: A low volume and sensitive LC-MS/MS method has been developed for the quantification of THCCOOH and THCCOOH-glucuronide in 200 µL of urine. Quantitative analysis for both THCCOOH and THCCOOH-glucuronide analytes is simultaneously achieved on an Agilent 1290/6460 LC-MS/MS without the need for hydrolysis. Chromatographic separation utilized an acetonitrile/water gradient on a Restek Ultra Biphenyl II (2.1 x 100 mm, 5 µm) column, with a total run time of 4.5 minutes. Detection of each analyte and isotopically labelled internal standard relied upon multiple reaction monitoring (MRM); THCCOOH (343.2 ◊ 299.1/245.1), THCCOOH-D3 (346.2 ◊ 302.1, 248.1) THCCOOH-glucuronide (519.2 ◊ 343.2/299.2) THCCOOH-glucuronide-D3 (522.2 ◊ 346.2/302.1). Quantification relied upon the ratio between the cps intensity of the MRM quantifier transitions of the analyte and internal standard, while analyte confirmation relied upon the ratio between the MRM quantifier and qualifier transitions.

Results: Preliminary results show that both THCCOOH and THCCOOH-glucuronide analyte were chromatographically separated and displayed excellent peak resolution. The lower limit of quantification (LLOQ) was assessed to be 10 ng/mL for THCCOOH using both calibrators prepared in blank urine and patient samples analyzed using GC-MS, while the LLOQ of THCCOOH-glucuronide was assessed as 10 ng/mL using calibrators prepared in blank urine. Linearity for both analytes was from 10 to 2000 ng/mL. During analysis of patient samples an unidentified compound was found to interfere with the ratio of the MRM quantifier and qualifier for THCCOOH-glucuronide; we are investigating this finding.

Conclusion: We developed an accurate and cost effective LC-MS/MS method to quantify both THCCOOH and THCCOOH-glucuronide in patient urine with a linearity of 10-2000 ng/mL; work is in progress to fully evaluate this assay and will be presented at the meeting.

B-344

Comparison of a Test Strip Versus an Automated Assay for Urine Specimen Validity Testing

A. Ridgway, D. R. Bunch, S. Wang. *Cleveland Clinic, Cleveland, OH*

BACKGROUND: Ensuring urine specimen validity is essential to proper urine toxicology testing. Products and information are available online to help a person subvert a urine drug testing. To counteract these products and techniques, laboratories utilize testing to confirm the absence or presence of common adulterants such as bleach and vinegar, as well as techniques such as excessive hydration. This study compares Intect® 7, a urine adulteration test strip to a Roche SVT assays for adulteration testing on Cobas 311. **METHOD:** The Intect 7 method is a colormetric test strip which allows for subjective judgment while the SVT assays give quantitative values. Individual patient urines (n=30) without preservative were collected and assayed on the C311 for specific gravity (SVTSG) and pH (SVTpH). A second group of patient urine (n=24) was used for the quantitative creatinine assay. A pool of patient urine was spiked with 1:10 v/v bleach:urine then serially diluted to 1:20, 1:40, 1:80, and 1:160. The resulting samples were assayed by the SVTOx assay which detects bleach and other oxidants. Meanwhile, all samples were tested using the Intect 7 strips. **RESULT**: The specific gravity ranged from 0.999 to 1.023 on the C311 and from 1.000 to >1.020 by the Intect 7method. The reference interval for urine specific gravity is 1.005-1.030. The average difference between the two assays was -0.0003 without the >1.020 values and 0.0003 if the >1.020 were set at 1.020. For samples <1.005 (low abnormals) by Intect 7, 5 out of 10 agreed. Both assays determined the pH of the urine samples to be between 5-10. The creatinine values ranged from 10.89 to 346.47 mg/dL by C311 with 19 of 24 samples matching the Intect 7 ranges. Of the 5 discrepant samples, four were higher on the C311. Intect 7 creatinine classification ranges were <10, 10-20, 20-50, and >50 mg/dL. Intect 7 was only able to detect bleach to the 1:80 dilution while the C311 detected bleach at all dilution levels. While the comparisons were acceptable, there is an element of subjectivity and color discrimination that may make the cut-offs softer than a calibrated quantitative assay. **CONCLUSION**: The C311 had an accepatable comparison to Intect 7 for the detection of specific gravity, pH, and creatinine, and had higher sensitivity for bleach than the Intect 7 assay.

B-345

A Case of Mitragynine Adverse Effect

L. Liu, S. Giannoutsos, J. A. Rymer, R. Venkataramanan, K. Tamama. *University of Pittsburgh Medical Center, Pittsburgh, PA*

Background: Mitragynine is the primary psychoactive alkaloid of the plant kratom indigenous to South East Asia. Being illegal in some countries, mitragynine has started to emerge in the U.S. as a legal psychoactive product on numerous websites. Pharmacologic studies have shown that mitragynine produces stimulant effects at low dose, but sedative narcotic effects at high dose by acting as a selective and full agonist of the µ-subtype opioid receptor. Animal studies and user experiences indicate an addictive potential with cognitive impairments, which suggest its classification as a harmful drug. Although restricted in some states in the U.S., mitragynine is not scheduled under the Controlled Substances Act at the federal level. As of now, there is no immunoassay available for mitragynine detection, and our clinical experience and knowledge of mitragynine abuse in U.S. are very limited.

Case Report: A 26-year-old male with a prior history of polysubstance abuse presented to the emergency department with severe constipation and urine retention. His symptom started approximately 3 weeks before when he had difficulty in having bowel movements. Over-the-counter laxative was not helpful. One week later, he started to have difficulty in urinating and subsequently developed abdominal and lower back pain. At the time of presentation, he can only dribble urine. He denied saddle anesthesia, fever, or vomiting. His abdomen was moderately distended. CT scan of abdomen showed severe constipation with fecal impaction. Foley catheter placement returned two liter of urine. Aggressive bowel regimen including enema was employed. A comprehensive urinary untargeted drug screening by gas chromatography-mass spectrometry (GC-MS) revealed the presence of mitragynine in urine.

Conclusion: This is the first detection of mitragynine by GC-MS based untargeted drug screening at our institution. The severe constipation and urine retention in this individual is likely caused by mitragynine-induced activation of opioid receptors in the gastrointestinal and urinary systems. As mitragynine gains popularity in the U.S. in the recent years, it has caused more serious clinical manifestations, including psychosis. The mass spectrometry-based mitragynine assay plays a key role in the diagnosis and detection of mitragynine abuse. Its usage monitoring by national drug abuse surveys might also be warranted.

B-346

Fully automated analysis platform for the routine determination of immunosuppressant drug compounds in whole blood

H. Shibata. *Shimadzu, Columbia, MD*

Background: LC-MS/MS shows higher sensitivity and superior specificity compared to immunoassay-based approaches for the analysis of Immunosupressant medications. Nevertheless, LC-MS/MS approaches lack in standardization and the necessary throughput for the application in routine analysis. We report a fully automated, high-throughput platform for the quantitation of four major immunosuppressant (cyclosporine A, tacrolimus, sirolimus and everolimus) in whole blood sample. Method: The analysis of immunosuppressant was performed using a fully automated LCMS preparation Unit (CLAM-2000, Shimadzu) online with HPLC-LCMS (NexeraX2-LCMS8050, Shimadzu) starting from Standard Blood Collection Tubes using "MassTox®" kit (Chromsystems). Samples (EDTA whole blood), calibrators, Extraction Buffer, Precipitation Reagent, and Internal Standards (Chromsystems) were loaded into the CLAM-2000. The fully automated preparation/analysis procedure consisted of: I) 25 µl of sample was dispensed in filtration-collection vial; II) 50 µl of Extraction buffer was added (liquid-liquid extraction); III) 12.5 µl of internal standards (IS) were added; IV) stirring (10 sec) and incubation (2 min); V) 125 µl of precipitation reagent were added; VI) stirring (1 min) and Filtration (deproteinization); VII) injection by HPLC autosampler (5 ul) and quantitation following MRM for drugs and IS. Preliminary results: Usually LC-MS/MS analysis of whole blood samples require some manual preparation steps for extraction and protein precipitation before the injection. With the aim to reduce the operator involvement, to increase the throughput and the data quality, we completely eliminated the manual sample preparation procedure by the use of a novel automatic preparation unit (CLAM-2000, Shimadzu). First, we tested if the LOQ and linearity of our method were compatible with suggested therapeutic intervals for immunosuppressant (100-350 ug/L Cyclospirin A, 3-8 µg/L everolimus, 3-20 µg/L sirolimus, tacrolimus 4-20 µg/L). The LOQ for all the compounds resulted <0.5 µg/l (Cyclosporin A 0.45 µg/l, tacrolimus 0.15 µg/l, everolimus 0.5 µg/l and sirolimus 0.25 µg/l, with S/N>10). We

used a wide range of quantification (6 levels) for all the compounds (up to 930 µg/l for Cyclosporin A, and 45 µg/l for everolimus, sirolimus, tacrolimus). Also, we obtained good linearity over the entire range (r^2>0,997). Since the difference in concentration between the highest calibration level and the LOQ was remarkable we tested the carry-over effect, and it was negligible. We tested the intra-day precision (repeatability) of the method, by analyzing 4 reference samples (spanning from low concentration to high concentration levels). Interestingly, the automated sample preparation resulted in providing low CV% values for the compounds (<2.5% Cyclosporin, <6% sirolimus, <8% Everolimus, <3.5% tacrolimus). Also the inter-day precision (reproducibility) was in accordance with CLSI guidelines , with CV% <12% over all 4 levels of concentration. The method showed good correlation and accuracy to the reference material for all compounds (Bias% <5% for everolimus, tacrolimus, and sirolimus, <10% for cyclosporine A). Finally, results on whole blood samples showed a strong agreement with immunoassay results.

Conclusion: The completely automated quantification method for Immunosuppressant drug compounds allows routine analysis with high data quality/precision, reduced time, increased throughput and safety.

B-347

Quantitation of Urine Fentanyl and Norfentanyl by UPLC-MS/MS

B. Wright, D. Giacherio, H. Ketha. *University of Michigan, Ann Arbor, MI*

Background: Fentanyl, a powerful synthetic opiate analgesic more potent than morphine has a high abuse potential on its own or in combination with other prescription and street drugs. The abuse of fentanyl at the present time is high in the state of Michigan and has risen in recent years. Available immunoassays show poor cross reactivity towards fentanyl leading to false negative results.

Relevance: Measuring urine concentrations of fentanyl and its metabolite norfentanyl is of clinical value, for diagnosing acute overdoses, monitoring compliance and forensic investigations.

Objective: The first objective was to develop and validate a liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assay for measuring urine fentanyl and norfentanyl concentrations. The second objective was to monitor urine concentrations of patients taking fentanyl.

Method and Validation: An UPLC-MS/MS assay for measuring urine fentanyl and norfentanyl was developed. Briefly, 200 µl of urine was added to 100 µl of methanol containing Fentanyl-D_5 (100 ng/ml) and Norfentanyl-D_5 (1,000 ng /ml), plus 750 µl ammonium acetate (3M, pH 5.5). The solution was passed through a conditioned cation exchange SPE column (Supelco Inc, PA, USA), washed and eluted with 5% ammonium hydroxide in methanol. The eluate was neutralized with 0.4M Acetic Acid, and 10 ul injected onto a Acquity UPLC BEH C-18 50mm x 2.1 mm column (Waters), and separated using an acetonitrile/0.1% formic acid and water/0.1% formic acid gradient over 3.0 minutes at 50 °C and analyzed on a mass spectrometer (Xevo TQ-S, Waters Corp., Milford, MA) using multiple reaction monitoring mode (MRM). Fentanyl transition used were (m/z 337.45>188.3) and norfentanyl transition used were (m/z 233.35>84.2). Fentanyl and Norfentanyl standards (10, 25, 100, and 1000 ng/ml for each) and quality control samples were prepared in fentanyl free urine. 50 samples from the Mayo Clinic were also analyzed.

Results: The UPLC-MS/MS assay was linear across an analytical measurement range of 5-1000 ng/ml with (r^2= 0.999). The intra assay imprecision for fentanyl was 2.0 % at 12 ng/ml, 2.0% at 30 ng/ml, 1.7% at 50 ng/ml (n=5) and 1.6% at 750 ng/ml. The intra assay imprecision for norfentanyl was 3.1% at 12 ng/ml, 2.0% at 30 ng/ml, 1.9% at 50 ng/ml, and 0.5% at 750 ng/ml (N=5 replicates each). The inter assay imprecision for fentanyl ranged from 2.7-2.8%. The inter assay imprecision for norfentanyl ranged from 5.0-7.1%. The limit of quantitation was set at 10 ng/ml for both fentanyl and norfentanyl. Our method compared well with the Mayo Clinic LC-MS/MS assay Fentanyl: $UM_{UPLC/MS} = 1.11*MAYO_{LC-MS/MS} + 1.67$ (r^2=0.97); Norfentanyl: $UM_{UPLC/MS} = 0.83*MAYO_{LC-MS/MS} + 7.62$ (r^2=0.99).

Conclusions: We have developed an UPLC-MS/MS assay for measuring Fentanyl and Norfentanyl concentrations in urine. In our initial cross sectional study, fentanyl and norfentanyl could be reliably quantitated in samples from patients taking fentanyl. In the future, studying the fentanyl/norfentanyl ratio in urine of patients is an attractive option in for compliance monitoring.

Wednesday, August 3, 2016

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

Technology/Design Development

B-348

A Novel Chemistry for Analyte Quantification by Immunoassay: A Case Study Using Spatial Proximity Analyte Capture Luminescence

M. J. Cameron, K. C. Pauly, D. P. Astry, W. F. Xie. *Beckman Coulter, Southfield, MI*

Background: SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence) is a chemistry that allows for the quantification of biomarkers from biological fluids in a homogeneous immunoassay format. The core of the novel chemistry is the interaction of horse radish peroxidase (HRP) with hydrogen peroxide and the release of free radicals that breaks chemical bonds within an acridan molecule, emitting light. The amount of light generated is proportional to the amount of analyte bound in a specific immune complex. The chemistry is proximity based and an anti-oxidant is used to control the distance of free radical travel, limiting background. The chemistry is highly flexible and configurable noting that acridan and HRP must be in the assay design, allowing for the possibility of multiple immunoassay formats. As proof of concept, an immunoassay was developed and validated for human interleukin 8 (IL-8) for use with human plasma samples.

Methods: Anti-human IL-8 antibodies were commercially sourced. Each antibody candidate was labeled with acridan and HRP. All antibody pair candidates were screened against recombinant human IL-8 for best pair determination. Assay development included the determination of dynamic range, minimum required dilution, duration of assay run time and optimization of key reagents (acridan conjugated anti-human IL-8, HRP conjugated anti-human IL-8 and the anti-oxidant). Individual lots of human plasma were screened for endogenous IL-8 levels. Plasma lots with non-detectible levels of endogenous IL-8 were used to make quality control samples (QC's). Validation included the accuracy and precision runs with six QC samples (200, 100, 50, 25, 12.5 and 6.25 pg/mL of recombinant IL-8 in human plasma).

Results: The IL-8 SPARCL immunoassay had a dynamic range of 3.125 - 4000 pg/mL (3 logs) and required a single incubation step of 30 minutes. Individual points in the standard curve, run in triplicate had CV's ranging from 1.9 to 14.0 %. Quality control samples were included and concentration values were interpolated off the standard curve after a dilution of 1:10. Individual concentration values showed a mean bias (accuracy) of 0.75, 3.5, 0.8, 3.6, 4.8 and 2.4% respectively for the six QC samples. Precision of the six QC samples was 6.1, 3.4, 1.9, 11.1, 4.9 and 7.7 percent respectively. The total error (mean bias plus percent CV) was 6.9, 6.9, 2.7, 14.7, 9.7 and 10.0 respectively. **Conclusion:** The novel chemistry is simple, allowing for rapid development of a biomarker assay from human plasma. The proof of concept IL-8 assay is accurate and precise. As the signal generated is luminescent, no specialized equipment is needed beyond a luminometer. The SPARCL chemistry may be applied to a broad range of biomarkers and immunoassay types (pharmacokinetics, detection of anti-biologic drug antibodies and therapeutic antibody development) with the advantages of improved workflow, lower workload and quicker turnaround time. The SPARCL chemistry may be amenable to point of care testing due to rapid test results, small sample volume requirements and no washing format.

B-349

Development of a software (SGP) for validation/verification registry and research management in a clinical laboratory

G. C. Lopes[1], N. L. Dias[2], E. Mateo[2], J. G. Assumpção[2]. *[1]Hermes Pardini Institute (Molecular Genetics Sector), Vespasiano, Brazil, [2]Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil*

Background: The development and/or implementation of a new test in a clinical laboratory involves several steps such as literature review, reagents and equipment acquisition, definition of suitable controls and sample size, performance measurements, comparison of results with those obtained with other methods, etc. Every stage needs to be carefully documented and assed at any time. Aims: The aim of this study was to develop a software that allows documentation of validation and verification processes, compilation of the results into a final report, and management of ongoing projects observing the requirements of regulatory agencies such as ANVISA (National Health Surveillance Agency), PALC (Brazilian Society of Clinical Pathology and Laboratory Medicine) and ISO (International Organization for Standardization). Methods: The software was designed for Web access on the Microsoft ASP.NET platform using C # programming language, and uses Microsoft SQL Server database 2012. All tools were developed upon demand to meet the registration requirements considering the needs of different productive areas of the laboratory; they were developed considering the normative ISO 9001-2008, Control item documents 4.2.3. Results: The software SGP (*Sistema de Gestão de Projetos*) allows project registration, including the features: 1) Project status management; 2) Financial viability and commercial analysis; 3) Daily registration of the test protocols; 4) Automatic assembly of partial and final report, 5) Technical advice tool; 6) Process full traceability.The daily protocol registration tool allows the user to edit text, including tables, graphs and images using editing tools in HTML page. This documentation can be generating at PDF format. Final reports generated in the validation process are automatically compiled in a default format, where the user can choose the daily protocol to be included in the technical memorial. Targeting information security, access to each project is only allowed to a project-designated manager, it has privileges to edit, finish and archive the data. All relevant changes are recorded in an access log and printed documents are controlled to be audited.The system allows the production of graphical reports that include productivity data, non-compliance of deadlines, access and documents printedlogs, and project status. This tool includes a feature that informs the manager all the relevant events in each project using automatic e-mail. Conclusion: The SGP software was a low cost solution when compared to commercial modular softwares available in the market. Due to its customized development, it fits best the laboratory's needs,improving our capability of recording and analyzing scientific data. It also decreased time consumed in elaboration of reports, created a follow-up tool for research management and avoided paper-based records that are expensive, difficult to keep and access.

B-350

HaploCYP: a software for *CYP2D6* genotyping and phenotyping

M. A. Pereira[1], R. G. C. C. Cardenas[2], E. Mateo[1], A. C. S. Ferreira[1], M. C. M. Freire[1]. *[1]Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil, [2]Torchmed - Software Development, Belo Horizonte-MG, Brazil*

Background: The Cytochrome P450 2D6 (*CYP2D6*) gene (MIM #124030), located on chromosome 22q13.1, is one of the most polymorphic pharmacogenes with more than 109 allelic variants reported up to date by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2d6.htm). The polymorphisms include single-nucleotide polymorphisms (SNPs), insertion/deletions (indels), copy number variations (deletions, duplications or multiplications), conversions and gene rearrangements. Due to this genetic polymorphism, *CYP2D6* exhibit notable inter-individual variability in enzyme activity and individuals can be divided into four phenotypic groups: poor (PM), intermediate (IM), extensive (EM) and ultrarapid (UM) metabolizers. **Objective:** Since *CYP2D6* genotype assignment and phenotype prediction are complex and of utmost importance into clinical practice, this work aimed to develop and validate a user-friendly software for *CYP2D6* genotyping and phenotyping using Sanger sequencing and copy number variation (CVN) data. **Methods:** HaploCYP combines a set of python modules, BLAST tooland a MySQL database system in a web interface running on an Apache web server. The workflow consists of BLAST alignment, polymorphism detections, genotype annotation and phenotype prediction. CNVinformation and fasta files from Sanger sequencing of *CYP2D6* gene are given as input.Variants are detected through BLASTn alignment with the*CYP2D6**1 reference sequence (Accession Number: AY545216.1). Mutation nomenclature and haplotypes are defined according to The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee, PharmGKB and LOVD databases. The haplotypes that best represents the set of polymorphisms and CNV information are defined and reported following the star-allele nomenclature system.In some cases, more than two haplotypes can be reported. At this point, the user needs to review the haplotypes proposed and choose the correct genotype. Then, the phenotype is predicted as PM, IM, EM or UM, based on *CYP2D6* diplotypes and the activity score system recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy. The software was validated with sequences from NCBI and ten real data with known *CYP2D6* genotypes. **Results:** HaploCYP was able to genotype correctly all the simulation datasets and nine real data. For one sample, the program was not capable to genotype,since two genotypes were equally likely. However, the expertise of the user was enough to solve this genotyping problem. A larger dataset will be used to test accuracy, specificity and sensibility. **Conclusions:** HaploCYP simplify and facilitate the genotyping and phenotyping process of *CYP2D6* into clinical diagnosis, where speed and precision

is of high importance. In addition, HaploCYP can be used for others *CYP* locus and any pharmacogenes that it is necessary to identify pharmacogenomics variants to individualize drug prescription and analyze drug efficacy and safety.

B-351

Human Factors Data and Methods for Designing Diagnostic Devices

I. J. Elson. *Abbott Laboratories, Irving, TX*

Background

Most new products start out as concepts and then prototypes that are revised several times as they pass through stages of product development that is defined by the manufacturer and regulatory agencies in healthcare. During this product development program over 573 customers or potential customers from many countries around the world accepted an opportunity to use, evaluate or discuss a concept or prototype developed for our next generation diagnostic devices over a period of several years. There were over 25 scientific studies or events organized to collect user input and over 12 formal reports written to manage or document the product design for the program or for compliance with national or international healthcare governing bodies. Lastly, many hardware and software developer questions were fielded along the way to shape the prototypes to meet user capabilities, limitations and preferences. All of this work was done by following a user-centered strategy by applying human factor methods and knowledge about people. The objective of this poster presentation is to educate manufacturers and users of these medical devices about the importance of human sciences to achieve a more usable and safer product, globally.

Methods

Human interactions with in vitro diagnostic devices are designed and evaluated using data from various knowledge domains such as anthropometry, biomechanics, visual science and design, auditory science, cognitive and learning sciences, information design and work physiology. The Human Factors methods used over several years of development included: ethnography, experience mapping, conjoint analysis, competitive benchmarking, heuristic and expert design evaluations, exploratory iterative studies of concepts and prototypes, design comparison assessments, use error failure mode and effects analyses (FMEA), formative usability assessments, cognitive walk-throughs, task/workflow analysis, field studies and final design validation assessments.

Results

Three designs are highlighted in this poster. (1) The field of anthropometry established the human dimensions required to satisfy the 5th percentile Japanese female measurement to the 95th percentile United States male measurement. This specification was used to provide an adjustable display monitor hung on an articulating arm which presented a comfortable viewing height for this broader range of users. This design was validated using formative usability assessments with short and tall users. (2) The field of biomechanics provided guidance for the sample and reagent tray design. The user can pick up the tray using a safer grip that keeps the wrists in a neutral posture to minimize cumulative trauma from repetitive motions. This design was validated using expert design evaluations. (3) The field of visual science provided research data that specified a comfortable blink rate for the LED indicator lights on the Reagent and Sample Manager device platform to avoid presenting a hazard to sufferers of photosensitive epilepsy by triggering a seizure. Blink rates were validated with the intended users with iterative prototype testing.

Conclusions

User populations around the world require and expect greater consideration of human factors/ergonomics in the design. This poster illustrates how human factors data was obtained and validated during design and development to guide the next generation of diagnostic equipment.

B-352

Next Generation Single Molecule Counting Scanning Reader in the Sgx Clarity™ Instrument has Equivalent Sensitivity and Analytical Performance to the Erenna™ System

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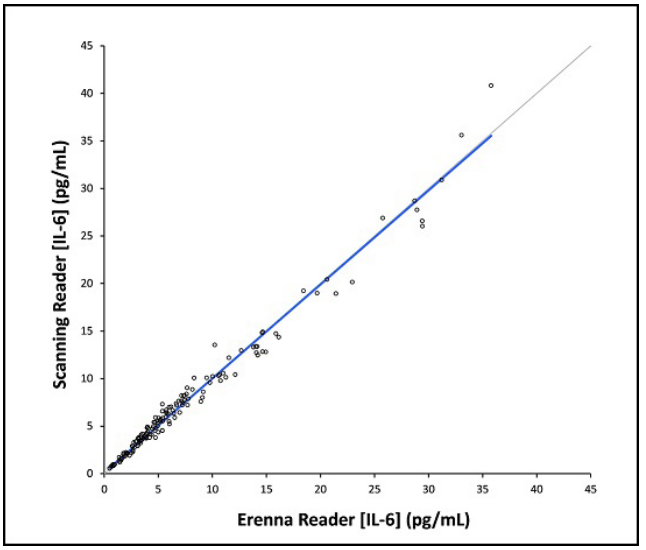
Background: Single Molecule Counting technology was introduced in 2007 with the capillary flow-based Research Use Only (RUO) Erenna® Immunoassay system. Since that introduction, immunoassays using the Erenna have allowed for the detection of low abundance biomarkers at levels that were previously undetectable. Several improvements on the usability of the Erenna have resulted in the development of an SMC™ scanning reader which forms the basis of the Sgx Clarity™ System, designed to be the most sensitive, fully-automated, next generation immunoassay platform available.

Objectives: To demonstrate the equivalence, in terms of analytical performance, between the Sgx Clarity System (in development) and the Erenna Immunoassay system.

Methods: Immunoassays for two different biomarkers, Interleukin-6 (IL-6) and cardiac troponin I (cTnI), were developed and run on both systems. 150 samples spanning the individual assay ranges were run over multiple days to assess the correlation between the two systems. In a further sub-analysis of the same data, low-end precision was demonstrated and functional sensitivity calculated.

Results: Excellent correlation existed between the matched sample results from the two systems. For IL-6, the Passing-Bablok regression slope was 0.99 and the Pearson correlation coefficient, R, was 0.990. For cTnI, the regression slope was 1.08 and the Pearson R was 0.998, thus demonstrating equivalent results between the two readers. The precision on duplicate sample readings was very similar between the two reading methods. Four replicates of each sample (two replicates from each system) were plotted to create a precision profile versus cTnI concentration to determine the 20% functional sensitivity, which was 0.16 pg/mL. This value compares very well with the determination of 0.13 pg/mL we previously reported for the Sgx Clarity cTnI assay (Shephard et al., 2015).

Conclusion: Immunoassays run on the new Sgx Clarity System have the same sensitivity and precision as when run on the Erenna Immunoassay system.



B-353

Clinical Evaluation of HER2 Molecular Diagnostic Assay with Internal Quality Controls in Breast Cancer

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Background: Determination of HER2 status before taking therapeutic regimens plays an essential role in breast cancer. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are currently working assays for HER2 testing. With the advantages of cost-effectiveness and high accuracy for IHC and FISH respectively, these two assays have been recommended as the routinely-used diagnostic tool. However, several studies have been reported that false signals and lab-to-lab variations are major unsolved issues for HER2 testing. Moreover, both IHC and FISH assays do not have the internal quality control mechanism, which might be the root-causes of the issues that we're facing.

Methods: Here, we developed and evaluated a novel HER2 testing assay with built-in internal quality control mechanisms by the principle of multiplex real-time PCR. Fifty FFPE samples were collected and analyzed for the evaluation.

Results: The result showed that the agreement between the developed assay and FDA certified FISH assay is over 90%, suggesting the developed Real-Time PCR

is accurate and reliable for HER2 testing. Furthermore, the quality of FFPE samples can be distinguished simultaneously, which can prevent false signals or lab-to-lab variations that resulted from poor qualified samples.

Conclusion: To sum up, the clinical efficacy of the developed HER2 molecular diagnostic assay shows great quality control ability and high analytical consistency with current assays for HER2 testing.

B-354

PMA-based High Resolution Melting to Rapidly Differentiate between Live and Dead Bacteria

L. Chou, L. Lee. *National Chiayi University, Chiayi, Taiwan*

Background: Due to the increase in foodborne outbreaks, foodborne pathogenic bacteria have emerged as a major public health problem and it highlights the need for a rapid and reliable methods for monitoring pathogenic bacteria. To detect and identify these bacteria, in addition to traditional cultural methods and sequencing (the gold standard), another alternative is the 16s based quantitative PCR (qPCR). Although 16s qPCR is rapid, accurate with high sensitivity, however, a practical issue often been questioned for qPCR is the positive results (signals) are from targeted live bacteria or from potentially existed background DNA. To eliminate potential false-positive results, recently developed viability PCR (v-PCR) that uses DNA-binding dyes such as propidium monoazide (PMA) to treat samples or PCR master mix, makes differentiation between live and dead bacteria cells possible. However, to our knowledge, the amount of dye used and assay detection dynamic range have not been thoroughly studied for the v-PCR. Therefore, to improve v-PCR, in this study we have designed an unique PMA based broad-range HRM assay, evaluated the amount of dye used, and investigated the detection dynamic range, to reliably differentiate PCR signals between live and dead bacteria (background DNA).

Methods: *Escherichia coli* (*E. coli*) K12 MG1655 was purchased from Bioresource Collection and Research Center (BCRC), Taiwan. For the live bacteria group, culture was grown under its respective growth conditions; for the dead (background) group, culture was lysed at 95°C for 5 min and checked by plate counting. All DNA extractions were done using QIAamp DNA mini kit. NanoDrop 2000 was used to check quality and quantity of isolated DNA. Broad-range HRM primers were design based on the variable region 6 (V6) of the 16s rDNA sequences and acquired from Integrated DNA Technologies. PMA concentration was evaluated at levels between 0.5 to 25 *u*M. All PCR reactions were carried out in LS32 high resolution melting instrument using FailSafe PCR master mix. PCR cycles were tested at 45, 55, 60, and 70 for determination of the lowest detection range. HRM analysis was performed at the temperature range between 65°C to 95°C with a 0.1°C/s ramp rate. All experiments were done in duplicate for three times.

Results: To PCR master mix, we demonstrated here that the maximum PMA concentration in a PCR reactions was 2 *u*M, without interfering the polymerase activity. Amplification from no template control was observed when PMA concentration was at 1.5 *u*M. For sample treatment, our data demonstrated PMA concentration of 50 *u*M was sufficient to remove more than 99.9998% background DNA. After sample treatment and DNA extraction, we verified the final PMA concentration in a PCR reaction was less than 2 *u*M, by comparing delta ct values. According to serial dilutions, the lowest detection limit for live bacteria of our PMA-HRM assay was 1 *pg* (200 genome copies).

Conclusion: To summarize, we presented a rapid differentiation of PCR signals between targeted live bacteria cells and background DNA (dead cells), with an unique combination of DNA-binding molecule, 16s broad-range primers, and DNA high resolution melting analysis.

B-355

Automated Integrated NGS and qPCR Workflow for *In Vitro* Diagnostics

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Background: Traditional dideoxynucleoside chain termination (Sanger sequencing) and PCR methods have been the standard molecular methods in clinical diagnostics for decades. Next Generation Sequencing (NGS) technology revolutionized the field of genomics, transcriptomics and metagenomics and is now swiftly becoming a routine method in different areas of clinical diagnostics such virology, oncology, non-invasive prenatal tests (NIPT), microbiology, precision medicine, etc.

Results: Vela Diagnostics developed an integrated automated multi-purpose *Sentosa* workflow, which consists of: 1) a robotic liquid handling system for nucleic acid extraction, PCR set-up and/or NGS library preparation (*Sentosa* SX101); 2) instruments for real-time PCR or template preparation and deep sequencing; 3) kits for nucleic acid extraction, target specific real-time PCR-based tests, NGS library preparation assays and reagents for deep sequencing; 4) assay specific applications, and 5) data analysis and reporting software. Different diagnostic applications employ the same robotic platform for qPCR set-up and preparation of NGS libraries. In less than 5 years Vela Diagnostics developed 7 NGS-based viral and oncology assays and more than 20 qPCR-based viral, microbial and oncology CE-IVD tests, which can be run on the same system. In addition, several extraction kits were developed to isolate nucleic acids from various types of clinical samples, including FFPE, whole blood, plasma/serum, swabs, sputum, stool and urine.

Conclusion: Combined automated qPCR and NGS Sentosa workflow appears as a reliable and efficient *in vitro* diagnostics (IVD) tool for the detection and/or quantitation of a wide range of bacterial and viral pathogens as well as gene mutations. These unique abilities of the Sentosa workflow provide complete and relevant information to aid clinical decision-making and patient management.

B-356

An Evaluation of the Analytical Performance of the New Beckman Coulter DxC 700 AU Clinical Chemistry System

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Background: The Beckman Coulter DxC 700 AU clinical chemistry analyzer* is the latest system from Beckman Coulter. It is a fully automated, random access analyzer, designed for medium to high throughput laboratories, with a throughput of 1200 tests/hour including ion selective electrodes. The purpose of this study was to evaluate the analytical performance of the new DxC 700 AU and to compare the performance against the current AU680 and AU5800 analyzers.

Methods: To assess the performance of the DxC 700 AU, Beckman Coulter assays were selected for evaluation that covered a range of sample types and assay methodologies. Precision was assessed using pooled human samples over 20 days following CLSI guideline EP05-A3. The linear range was assessed following CLSI EP06-A. The DxC 700 AU was compared to the AU680 and AU5800 analyzers using a minimum of 100 serum samples spanning the dynamic range based on CLSI guideline

EP09-A3.

Results: Estimates of repeatability and within laboratory precision were assessed at multiple critical analyte concentrations. Data from a selection of assays are summarized in the table below. All assays were shown to be linear over the respective assay's analytical range. All DxC 700 AU assays showed excellent correlation with the current AU680 and AU5800 analyzers with all slopes between 0.95 to 1.05.

Conclusion: The results of the study demonstrated excellent analytical performance of the new Beckman Coulter DxC 700 AU analyzer and confirms comparable performance to the AU680 and AU5800 analyzers.

Application	Mean	Within Laboratory %CV	Mean	Repeatability %CV	Within Laboratory %CV	Within Laboratory %CV
Glucose Serum	25.9 mg/dL	2.4	280.6 mg/dL	0.9	0.9	0.9
IgG Serum	486 mg/dL	1.4	1831 mg/dL	1.5	1.6	1.6
CRP Serum	0.49 mg/L	3.5	50.1 mg/L	0.6	0.8	0.8
Amylase Urine	42.2 U/L	1.8	1315.2 U/L	0.8	1.0	1.0
IgG CSF	4.4 mg/dL	4.2	34.8 mg/dL	0.9	1.8	1.8

*Product In development. Pending clearance by the United States Food and Drug Administration and achievement of CE compliance. Not currently available for *in vitro* diagnostic use.

B-357

HLA Genotyping and Pharmacogenomics with Whole Exome Sequencing

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Background: Pharmacogenomics (PGx), the study of genetic variation in drug response phenotypes; and whole exome sequencing, evaluation of the coding regions for germline variants associated with a genetic disorder, are the perfect pairing. For diagnostic odyssey, standard WES has a 20-45% detection rate for variants responsible for the underlying disorder. With nearly all individuals having a variant in a pharmacogene which may impact their care, PGx may be able to help with a patient's treatment, even if WES is unable to identify the genetic cause of the patient's phenotypic presentation.

Methods: Whole exome sequencing is performed on genomic DNA. The exome is captured utilizing a custom reagent developed by the Mayo Clinic and Agilent Technologies. Sequencing was performed on an Illumina HiSeq 2500 Next Generation sequencing instrument. Paired-end 101 base-pair reads were aligned to GRCh37/hg19 reference assembly from 1000 Genomes, using Novoalign. All germline variants were jointly called through GATK Haplotype Caller and Genotype GVCF, with each variant annotated using the BioR Toolkit and HLA analysis using manual review and Omixon HLA Target. Although a trio is required for the testing process, only the proband is evaluated for pharmacogenomic interpretation. The pharmacogenomic testing determines the presence or absence of a select list of variants/alleles in *CYP1A2, CYP2C9, CYP2C19, CYP3A4, CYP3A5, HLA-A, HLA-B, SLCO1B1, UGT1A1, and VKORC1*. Additionally, due to limitations of the current whole exome capture reagent, ancillary testing is performed to analyze *CYP2D6*. *CYP2D6*-specific genotyping is performed by Luminex-based allele-specific primer extension/bead hybridization with fluorescence detection. In approximately 3-5% of cases, the sample is reflexed through Mayo's CYP2D6 cascade process (copy number variant analysis, and targeted sequencing of specific alleles/hybrids) to determine accurate genotype and phenotype. A pharmacogenomic report is resulted out, and additional educational material is provided. The education report utilizes OneOme's proprietary algorithm, which provides the physician with information relevant to the PGx phenotype of the patient.

Results: Five trios were assessed using the methodology described above. All results obtained from WES data were confirmed using alternative methods available in the clinical laboratory. HLA validation underwent a phased review for the NGS data; first exploring validity of targeting specific tagging variants for *HLA-A* *31:01, *HLA-B* *15:02, *57:01, and *58:01. Second, a manual review of regions for each of the given alleles. Lastly, validation using Omixon software to rule out the presence or absence of the specific HLA allele; HLA-A and B calls from Omixon were compared the results obtained from Mayo's High Resolution Class I Phenotyping assay. All genotype results were concordant between the methods and concordant across all replicates and runs of the assay.

Conclusion: We have clinically validated a subset of PGx variants across 11 genes for use with whole exome sequencing at Mayo Clinic. By providing pharmacogenomics results from whole exome sequencing, we hope to assist care providers with current and potentially prospective treatment optimization for the patient.

B-358

Performance of Magnesium, Albumin BCG and Urine/CSF Protein assays on Abbott's next-generation immunochemistry analyzer

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Objective/Background: To evaluate analytical performance utilizing photometric technologies for detection of analytes in human plasma/serum, urine and cerebral spinal fluid on the next-generation immunochemistry analyzer. The sample is dispensed into a cuvette followed by reagents. The contents are mixed and incubated allowing for the reaction to occur. If a second reagent is required, the second reagent is added to the cuvette; the contents are mixed again and incubated. Absorbance readings of the sample are taken at regular intervals throughout the process at a primary and if applicable, a secondary wavelength. Data reduction generates a calculated absorbance based on the reaction mode of the assay (rate or end point) and measures the calculated absorbance using a calibration curve to generate a result.

Methods: Key performance testing including precision, linearity, limit of quantitation (LoQ), and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range across which acceptable performance for bias, imprecision and linearity was met.

Results: Total imprecision, LoQ, and linearity results along with the defined measuring interval are shown for representative assays in the table below. Results versus the on-market comparator assay demonstrated a slope 0.99 - 1.01 and r = 1.00.

Assay	Total %CV	LoQ	Linearity	Measuring Interval
Magnesium Serum	≤ 2.1	0.17 mg/dL	0.51 -11.44 mg/dL	0.60 – 9.50 mg/dL
Magnesium Urine	≤ 5.5	1.68 mg/dL	1.62 – 28.67 mg/dL	1.8 – 26.4 mg/dL
Albumin BCG	≤ 0.9	0.3 g/dL	0.0 – 11.3 g/dL	0.4 – 10.5 g/dL
Urine CSF/Protein	≤ 4.3	6.1 mg/dL	6.8 – 190.1 mg/dL	6.8 – 200.0 mg/dL

Conclusion: Representative clinical chemistry assays utilizing photometric technologies tested on Abbott's next-generation immunochemistry analyzer demonstrated acceptable precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market comparator analyzer.

B-359

Evaluating Improvements in Predictive Instrument Monitoring and High Throughput Testing in an Integrated Immunochemistry Platform

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Background: Integrated analyzer technology has progressed to a point where accurate interpretation of samples is expected, and focus is turning to early detection and prevention of what will be errant results caused by instrument malfunction along with automation of systems by minimizing productivity lost to routine tasks. A number of technological advances in these areas have culminated in Abbott's next-generation integrated immunochemistry platform. These key technological advancements aim to provide clinicians with faster and more reliable laboratory results for rapid diagnosis and treatment for the patient.

Methods: Technological development focused on two critical areas: errant results prevention via enhanced microparticle wash monitoring methods and increasing throughput by maximizing the amount of time systems can operate autonomously.

Results: Errant Results Prevention: The washzone monitoring system shows repeatable detection of liquid presence in reaction vessels from 400uL to 1000uL in 50uL increments. This type of monitoring alerts the user to incorrect dispense/aspiration volumes and helps determine root causes including loss of vacuum, clogged aspirate probes, flooded wash areas or even restricted nozzles that mobilize the correct volume but at the incorrect velocity.

Increased Throughput Area Of Improvement	Previous Generation Systems	Next Generation Immunochemistry System Design Goals
Pretreatment Assays Per Hour	100	200
Reagent Inventory	65 CC, 25 IA	70 CC, 47 IA
Downtime spent replenishing supplies	Up to 57 minutes	None, can resupply while processing
Reduction of Footprint (tests per hour per square meter)	54	165

Conclusions: Performance evaluation of the technological developments has been ongoing in concert with verification of assay accuracy to ensure that creating a predictive system capable of continuous use has not hampered the core functionality of biomarker and disease detection. Accurate results combined with the numerous technological developments present in the next-generation system make this platform a competitive alternative to traditional integrated immunoassay and clinical chemistry platforms.

B-360

ICT (Integrated Chip Technology) Assay Performance on Abbott's next-generation immunochemistry analyzer

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Objective/Background: To evaluate the analytical performance of the ICT (Na, K, Cl) assays for measurement in human serum/plasma and urine. Abbott's next-generation immunochemistry analyzer uses Integrated Chip Technology (ICT), a

solid-state flow through Ion Selective Electrode (ISE) module containing Sodium, Potassium and Chloride electrodes integrated with a silver/silver chloride reference electrode and a potassium chloride liquid junction. A diluted sample is aspirated into the module and held while the potentiometric differences of Sodium, Potassium and Chloride electrodes and the Reference Electrode are measured. The results are available within 3 minutes after aspirating the sample.

Methods: Key performance testing including precision, linearity, and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range which acceptable performance for bias, imprecision and linearity was met. Impact of common interferences was assessed at low and high analyte concentrations. Reference interval verification was performed using healthy subjects.

Results: Total imprecision, linearity, and defined measuring intervals are shown for ICT assays in the table below. Results versus an on-market comparator assay demonstrated a slope 0.99 – 1.05 and r = 1.00. The assays were not affected by endogenous or exogenous interferences.

Assay	Total %CV	Linearity	Measuring Interval
Sodium Serum	≤ 1.5	27 – 259 mmol/L	100 – 200 mmol/L
Potassium Serum	≤ 2.7	0.5 – 14.3 mmol/L	1.0 – 10.0 mmol/L
Chloride Serum	≤ 2.0	22 – 172 mmol/L	50 – 150 mmol/L
Sodium Urine	≤ 3.0	1 – 474 mmol/L	20 – 400 mmol/L
Potassium Urine	≤ 3.0	0.1 – 366.0 mmol/L	1.0 – 300.0 mmol/L
Chloride Urine	≤ 1.8	5 – 346 mmol/L	20 – 300 mmol/L

Conclusion: The ICT assays tested on Abbott's next-generation immunochemistry analyzer demonstrated excellent precision, linearity, and correlation with an on-market comparator assay. The ICT assays also showed minimal interference from hemolysis, lipemia, bilirubin and urine interferences.

B-361

First Virtual Congress on Laboratory Medicine: Opportunities, Achievements and Difficulties

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Background: The aim of this poster is to demonstrate that new technologies offer a novel site to held congresses and a unique opportunity to reach more professionals in an effective and easy way. Argentina Biochemistry Foundation (FBA) is a non-profit organization committed to spread laboratory medicine knowledge in the scientific community to favor public health outcomes. Since 2004, FBA is working in the field of new technologies and education.

Methods: A group of subject matter experts was called by the scientific committee as spkeakers to produce 24 conferences that covered many topics of laboratory medicine. Conferences were recorded using an interactive video presentation format (video and slides) powered by Present.me™. Each conference was uploaded to the congress website in combination with an individual discussion forum, a self-assessment quiz and a compilation of additional materials to go deep in the topic. A virtual poster session was established. Posters were presented in PDF format and explained to visitors through a video presentation on YouTube™. The industry sector was also present in the event in an special session called VirtualExpo. They offered information about products in digital formats and were in touch with visitors over Internet. A survey was conducted to evaluate the congress satisfaction among the attendants. A digital certificate of participation with a validation code was issued in a digital format. The first virtual congress was fully held on Internet from November 16th to December 2nd, 2015 http://www.virtualab.org.ar/

Results: 689 professionals attended the congress (90% from Argentina and 10% from others countries, especially from LA). Each one of the 24 conferences was available by pairs every 48 hours. During this period the discussion forum of each conference was active. Speakers moderated the conversations and replied questions and comments submitted by attendants. In addition, a Twitter account and a Facebook page were setup to encourage communication over social networks between attendants and general population. 14 posters were submitted and one of them was awarded. The satisfaction survey was submitted by 203 attendants and the results were extremely positive. All the responders said that they will participate in the next virtual event. 192 digital certificates of participation were issued among the attendants.

Conclusion: A virtual congress fully conducted on Internet is a cost-effective approach to spread new knowledge over laboratory medicine community. The tools used are very affordable and do not require special knowledge or infrastructure. New technologies offer an excellent opportunity for professionals associations to create innovative ways of scientific communication. One of the mayor challenges was to convince speakers, professionals and expositors about the benefits of this novel model of congress. However, this VirtuaLAB was a proof-of-concept that a virtual congress is reliable and cost-effective for knowledge distribution especially for people who cannot attend on site congresses.

B-362

Rapid Immunoassay Development, Employing Biolayer Interferometry

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Background: Well characterised antibodies are a prerequisite for the development of accurate diagnostic immunoassays and biotherapeutics. To facilitate rapid immunoassay development, surface-based analytical techniques, such as surface plasmon resonance and biolayer interferometry, have been applied to the rapid characterisation of large numbers of antibodies and the identification of optimal candidates. Biolayer interferometry generates similar data to surface plasmon resonance, however it is less susceptible to complex matrices (serum or blood) and considerable bulk refractive index changes. The binding interaction is continuously monitored by measuring changes in depth of the protein layer on a replaceable, optical biosensor.

The aim of this study was to develop a rapid, cost-effective biolayer interferometry-based technique for the selection of high affinity sheep monoclonal antibody (mAb) pairs, employing protein G biosensors. Protein G biosensors provide an ideal platform for assay development, as they permit rapid and stable immobilisation of antibodies. Furthermore, they can be easily regenerated, which keeps consumable costs low. This is a new application of this technology, which is relevant for the rapid characterisation and selection of antibodies for use in development of immunoassays for clinical applications.

Methods: Purified sheep mAbs were captured on a protein G biosensor surface employing the Octet RED96 instrument (ForteBio, USA) and ranked (off-rate ranking) based on the percentage of complex remaining after dissociation in assay buffer. The clones that exhibited the slowest off-rates were again captured on the protein G biosensor surface and employed to identify competing antibodies (epitope binning). Matched antibody pairs were subsequently evaluated using sandwich Enzyme-Linked Immunosorbent Assays (ELISAs).

Results: Forty-five anti-prolactin mAbs were off-rate ranked, using five commercial antigen (recombinant and native) sources. The top eighteen mAbs, exhibiting the slowest off-rates and highest binding response levels, were used in a series of epitope binning experiments, resulting in the screening of 324 possible antibody pairs and identification of three unique epitope bins. Using a sandwich ELISA format, several of the matched antibody pairs were used to successfully generate calibration curves, with one pair obtaining a sensitivity value of 0.06 ng/mL. The complete screening procedure was completed in 72 hours.

Conclusion: The results of this study demonstrate that biolayer interferometry can be employed to rapidly rank antibodies with low off-rates and to identify suitable antibody pairs for immunoassay development, through the application of epitope binning techniques. The method described can also be used to screen hybridoma supernatants and is compatible with higher throughput systems, which would facilitate the analysis of large numbers of antibodies on a daily basis. Thus, this new application of the biolayer interferometry technique results in more rapid identification of antibody pairs and a more rapid immunoassay development system.

B-363

Method comparison with Delfia Xpress immunoanalyzer and Cobas 8000 analyzer for free BHCG and PAPP-A parameters.

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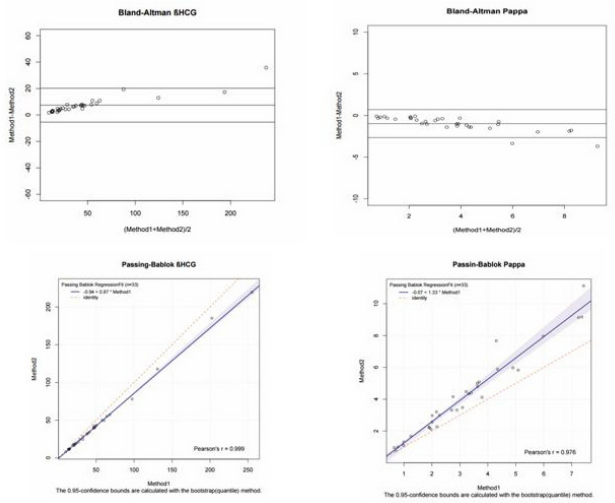
Background: Screening for the major fetal chromosomal anomalies is becoming increasingly widespread globally. Routine first-trimester screening is very useful due the superior detection rates and safer and less traumatic termination procedures available, if required. The aim of the study is to compare the results in Delfia Xpress Perkin Elmer® and Cobas 8000 Roche Elecsys® for pregnancy-associated plasma protein A (PAPP-A) and free beta human choriogonadotropin (free βhCG) parameters to determine the concordance of these results in both methods.**Methods:** The measurements were performed with 34 routine serum samples from pregnant at 11 to 13 weeks of gestation, which were processed in the Delfia Xpress and Cobas 8000.

The samples were processed in both methods in the same day. In all of them free βhCG and PAPP-A were determined. For the statistical analysis we used R statistic. We analyzed the concordance between the results in both methods, calculating the correlation by the Pearson´s coefficient and the concordance by Passing-Bablok regression and Bland-Altman plots. **Results:** We found strong to nearly perfect agreement in PAPP-A and free βhCG between both methods, r= 0.976 and r=0.999 respectively, using Pearson´s analysis. A mean bias of -0.98 was observed for PAPP-A and a mean bias of 7.41 was observed for free βhCG based on Bland-Altman plots. The Roche assay showed acceptable agreement with Perkin assay based on Passin-Bablok analysis for PAPP-A (intercept: -0.7 U/L, 95% CI: -0.51 to 0.11; slope: 1.33, 95% CI= 1.25 to 1.49) and for free βhCG (intercept -0.94, 95% CI: -1.77 to 0.17; slope: 0.87, 95% CI: 0.84 to 0.90). **Conclusion:** Both methods demonstrated acceptable performance, but the Roche Cobas assay demonstrated better performance than Delfia Xpress in the studied samples for PAPP-A and free βhCG.



B-364

Development and Validation of RP-HPLC Methods to Characterize Complex Reagent Mixtures used in Genotyping Analysis

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Background: Canon U.S. Life Sciences is developing genotyping assays that utilize PCR amplification of a target genomic sequence followed by high-resolution melting (HRM) analysis to distinguish clinically relevant mutations from wild-type sequences. Each of the assays requires both a reagent used for all assays and an individual, assay-specific reagent. The DNA dilution buffer (DDB), the common reagent, contains DNA polymerase, a fluorescent dye for HRM, and bovine serum albumin (BSA) in a buffered solution. Each primer mix (PM), the assay-specific reagent, contains dNTPs and oligonucleotide PCR primers as well as unlabeled oligonucleotide probes for some assays. Most previously reported quality control methods for PCR-based reagents are functional PCR assays; however, reversed-phase high-performance liquid chromatography (RP-HPLC) analytical methods provide quantitative information on the purity and identity of individual components that can supplement or even replace current quality control methods.

Methods: The analytical RP-HPLC methods for the DDB and PMs were developed using an Agilent 1260 Infinity Bio-Inert quaternary system with diode-array spectrophotometric detector. DDB testing utilizes a Phenomenex Aeris Widepore XB-C8 column (250 x 2.10 mm, 3.6 µm) and a 0.1% TFA/acetonitrile gradient to resolve the three critical components (DNA polymerase, fluorescent dye, and BSA). The dye absorbance is detected at 450 nm and the protein absorbance is detected at 280 nm. PM testing utilizes an Agilent PLRP-S column (2.1 x 50 mm, 3.6 µm) and a 0.1 M triethylammonium acetate/acetonitrile gradient to resolve up to four oligonucleotides and a dNTP mixture.

Validation Results: Linearity, range, accuracy, and limit of quantitation were evaluated for each method. The DDB method had linear ranges of 0.45-150 µg for BSA, 0.6-60 pmol for polymerase, and 0.9-300 pmol for dye with sensitivities of ≤0.6%, 2%, and ≤0.6%, respectively, of the recommended load. Repeatability and inter-assay precision with respect to area was ≤1% CV for BSA and dye and ≤5% for polymerase. The PM method had excellent selectivity, resolving oligonucleotides with the same number of base pairs, and demonstrated 98.8% area recovery in a spiking experiment with an oligonucleotide and its n-1 variant. The sensitivity was between ≤0.6-5% compared to the target load, depending on the oligonucleotide concentration. Repeatability and inter-assay precision, with respect to purity and area, were excellent for all oligonucleotides tested (CV ≤3%). The demonstrated linear range for oligonucleotides was 0.9-225 pmol, and the range for dNTPs was 1.4 - 225 nmol (sensitivity=0.6%).

Conclusion: We have developed and validated two sensitive and selective RP-HPLC methods that can resolve all critical components in the DDB and PM reagents. When used together, these methods can provide the quantitative data necessary to ensure a highly reproducible assay formulation and supplement functional testing for release of reagents required for genotyping analysis by PCR-based HRM.

B-365

Analytical Validation of a Clinical-grade Next-Generation Sequencing Assay for Assessing Allograft Injury in Solid Organ Transplant Recipients

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Background: Circulating cell-free DNA (cfDNA) is widely accepted as a biomarker in prenatal testing and is coming into clinical use within oncology. A by-product of cell apoptosis and necrosis, cfDNA can also be used to assess allograft injury, such as rejection, in solid organ transplants, where donor-derived cell-free DNA (dd-cfDNA) is measured against the background of recipient cfDNA. We developed a targeted next-generation sequencing (NGS) assay employing 266 SNPs to quantify dd-cfDNA in solid organ transplant recipients. A unique and practical aspect of the test is that prior knowledge of donor and recipient genotypes are not required to measure the proportion of cfDNA in the recipient's plasma that is released from the donated organ.

Objectives: Characterize and validate the analytical performance of a novel cfDNA-based NGS test for evaluating allograft health in solid organ transplant recipients and to validate the laboratory workflow from sample accessioning to result reporting.

Methods: Assay performance was characterized using both external reference materials and cfDNA extracted from plasma. Reference materials consisted of cell line genomic DNA fragmented by sonication to the approximate size of cfDNA. Three separate panels were created by mixing one "donor" cell line into a genetically different "recipient" cell line in proportions that mimic cfDNA present in the blood of organ recipients. Blood from healthy volunteers (HV) and transplant recipients was collected in Streck Cell-Free DNA BCT® blood collection tubes. Reference materials were used to determine the Linearity, Lower Limit of Detection (LLOD), and the Lower Limit of Quantification (LLOQ). Reproducibility was assessed within and across runs at 3 input amounts (3ng, 8ng, 60ng). Limit of Blank (LOB) was determined using the reference materials and cfDNA from HV. Mock transplant patient samples (constructed using HV samples by mixing either plasmas or cfDNAs from two individuals) were used to confirm linearity and assess the impact of common test interferents. Reproducibility from blood draw to final result was tested using paired specimens from transplant recipients. All testing was performed following approved study protocols based on CLSI guidelines. Process validation including accessioning, testing, cloud-based bioinformatic analysis and results reporting was performed using mock recipient samples.

Results: Analytical performance (reported as %dd-cfDNA) at 8ng: Linear range 0.2% to 16%; LOB 0.1%; LLOD 0.16% and LLOQ: 0.20%. Within-run standard deviation: 0.07% at 0.6% dd-cfDNA and 0.12% at 2% dd-cfDNA. Across-run CV: <20% for all samples tested. CV for independently processed sample replicates was similar to across-run CV demonstrating minimal variability from blood collection and cfDNA extraction. Amplification was not inhibited in samples containing common interferents. End-to-end process validation demonstrated accuracy and integrity of the test system.

Conclusions: We have developed a clinical-grade NGS-based test to measure the fraction of dd-cfDNA in transplant patients as an assessment of organ injury. Analytical validation demonstrated performance that met or exceeded specified requirements. End-to-end process validation confirmed specimens were accurately tracked throughout the entire test system and appropriately reported. Testing can be completed in <3 days providing the short turnaround time necessary for organ transplant surveillance.

B-366

A novel point-of-care immunoassay delivering clinical lab performance

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Background: Point-of-care testing (POCT) requirements (e.g., operation by untrained personnel, quick turnaround time, diverse sample type) can compromise analytical performance. As the clinical utility of biomarkers becomes established, there is increasing recognition of their value in the near-patient setting if stringent standards for sensitivity and precision can be met. We introduce the Pylon™ technology, which utilizes an antibody-coated probe that moves through reagent wells on a test strip and a novel signal amplification strategy to achieve central lab performance.

Objective: To assess analytical performance of Pylon immunoassays, focusing on biomarkers with demonstrated clinical need for POCT.

Methods: The small surface area of the probe tip offers the advantages of small sample volume (10-40 µL), low reagent consumption and, importantly, no interaction with red blood cells, enabling testing of whole blood. There are no reagent additions during the assay, simplifying instrumentation.

Pylon employs two signal reagents, a biotinylated secondary antibody followed by a Cy®5-streptavidin-polysaccharide conjugate. The high-molecular-weight polysaccharide (several million daltons) serves as an inert carrier of multiple Cy5-streptavidin molecules. Background signal is negligible since the polysaccharide has minimal interaction with the probe. Sensitivity is further boosted by cycling the probe between the biotinylated antibody and Cy5-streptavidin conjugate to form multiple layers. Cyclic amplification is reproducible, enabling high precision.

The versatility of the assay protocol is illustrated by a dual-sequence protocol to achieve a wide quantitation range without compromising sensitivity. The first sequence is optimized for high-concentration samples, while a second sequence is optimized for low concentrations.

Results: Results for PCT, BNP, CRP, D-dimer and β-hCG assays are summarized in the table.

Conclusions: Pylon meets clinical lab performance standards for sensitivity, precision and range. Small sample volumes and whole blood further facilitate POCT. Performance of the cTnI assay, summarized in a separate abstract, suggests potential for hs cTnI.

Pylon immunoassay performance data

	PCT	BNP	CRP	D-dimer	β-hCG
MDL	0.02 ng/mL	5 pg/mL	0.1 mg/mL	0.02 mg/L FEU	0.5 IU/L
LoD	0.035 ng/mL	10 pg/mL	0.2 mg/mL	0.05 mg/L FEU	0.8 IU/L
LoQ	0.1 ng/mL	30 pg/mL	0.3 mg/mL	0.2 mg/L FEU	2 IU/L
Measurable Range	0.02-400 ng/mL	5-10,000 pg/mL	0.1-300 mg/L	0.02-50 mg/L FEU	0.5-300,000 IU/L
Cutoffs	0.25 ng/mL	100 pg/mL	3 mg/mL	0.5 mg/L FEU	25 IU/L
CV% at Cutoff	<10%	<10%	<10%	<10%	<10%

B-368

Hematocrit independent collection of volume defined blood samples with a novel microfluidic DBS card for increased accuracy of quantitative DBS sample analysis

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Background: Dried Blood Spot (DBS) as a sampling method has gained large interest in recent years in several fields. However for precise quantification of analytes in DBS, unknown sample volume, varying hematocrit and uneven sample distribution can yield inaccurate results. **The objective of this study was** to demonstrate a new microfluidic DBS card to create fixed volume DBS spots in combination with whole spot analysis. **The study shows that the there is no hematocrit-induced bias** on the sampled volume and the analysis. **This enables accurate quantification for DBS analysis** while maintaining a simple sampling procedure which patient themselves may perform.

Method: Blood at four different hematocrit levels (26,37,47,60) was prepared and spiked at two QC levels. The volumetric sampling precision was determined for each sample by a gravimetric differential measurement. Microfluidic DBS card samples and pipetted reference samples were also analysed using a Thermo Scientific TSQ-MS with a DionexUltima 3000UHPLC. After drying, all DBS samples were extracted overnight in 200 µl methanol containing 1pg/µl internal standard. 150 µl extracts were transferred to lab tubes, evaporated to dryness and reconstituted with 100 µl mobile phase before injection.

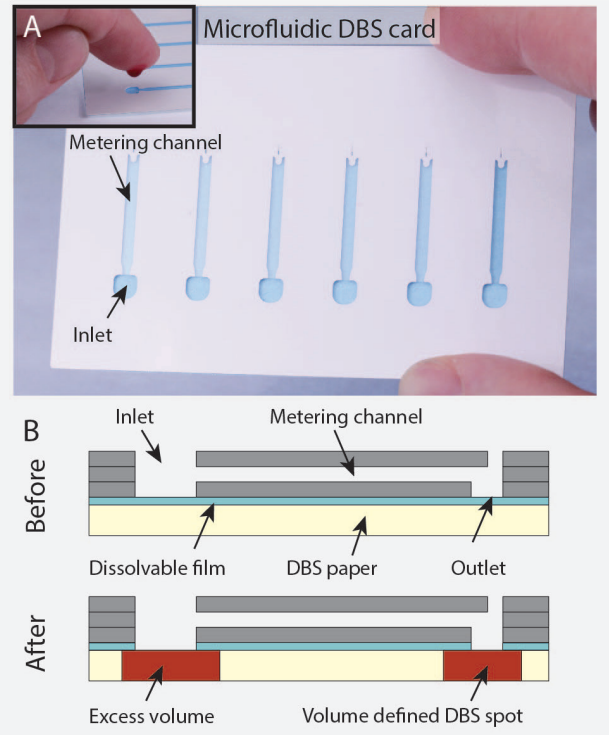

Figure 1: A) Microfluidic DBS card prototype with 6 metering channels. The insert shows the application of fingerprick blood to the inlet. B) Illustrated cross section through the DBS card along the channel before and after use. Applying a droplet of blood to the DBS card inlet initiates the microfluidic metering function, which is performed completely autonomous by capillary forces and results in the excess volume being absorbed at the inlet and the defined volume being absorbed at the outlet.

Results: The gravimetric measurements demonstrate good volume metering precision for the DBS card samples with hematocrit-specific CVs below 4% (n=9) while **the overall CV irrespective of hematocrit is 3% (n=51).** MS measurements demonstrate that there is **no bias caused by HCT** or the microfluidic DBS card (CV=0.5-7.2) and the performance is comparable to pipetting of DBS with a calibrated pipette (CV=0.3-3.6)

Conclusion: The demonstrated microfluidic DBS card can eliminate existing problems for the quantitative analysis of DBS by spotting a specific volume which is completely analysed. **The microfluidic DBS card offers an attractive solution for collection of fingerprick blood and hence enables patient self-sampling, home sampling and accurate analysis** without adding complexity for the patient or the analyst.

B-369

3D Cell Culture in Anchored Hydrogel Droplets for High Throughput Drug Screening

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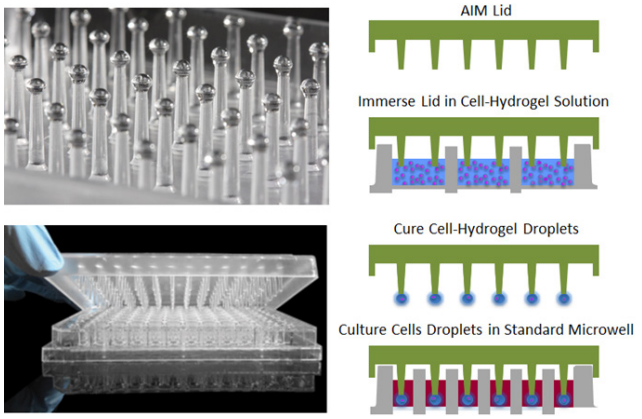
Background: Current drug-discovery approaches are highly inefficient, as <10% of new drugs entering the pipeline receive FDA approval. The tools used today may explain this low success rate as researchers typically study cells cultured in microplates; a non-natural 2D environment. Studying the response of cells cultured

in a more physiologically relevant 3D environment has recently shown promise for increasing the drug-discovery success rate. Similarly, isolated lymphocytes, cultured in 3D, are an important source of new biological drugs. Available tools for culturing cells in 3D are challenging to use and difficult to automate, especially for long-term culture studies. The lack of appropriate platforms for handling cells in 3D severely limits the development of 3D cell assays for new drug discovery. **Method:** ARLD has developed a new technology, composed of arrays of specifically designed surface structures, on which droplets of hydrogels containing cells can be anchored, as shown in Figure 1. These structures are built into ARLD's AIM lid which is compatible with standard 96/384 well microplates. Hydrogel droplets containing cells are dispensed on the AIM lid by immersing the surface features into a solution of hydrogel. After cure, the AIM lid is placed on a standard microplate, immersing the hydrogel droplets into the culture medium or any other solution contained in the microwells. The cells remain within the gel, but nutrients and gases can rapidly diffuse through the nano-porous gels. Medium changes, washing steps, staining and drug introduction are achieved by moving the lid to a new microplate containing fresh solution. **Results:** The long term culturing of cells (≥ 10 days) as well as the diffusion rate of peptides and proteins in the anchored 3D gel droplets have been studied using optical and high resolution (63x) florescence microscopy. The effect of hydrogel composition and cross-link density will also be discussed.



B-370

Evaluation of a *liquid* Lactic Acid Assay on the ARCHITECT *c*Systems Instrument

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OBJECTIVE: To convey the performance specifications as well as convenience of the *liquid* Lactic Acid assay on the ARCHITECT *c*Systems instrument.

RELEVANCE: A *liquid* Lactic Acid assay with improved precision and stability claims is a convenient alternative to the *lyophilized* ARCHITECT assay. The *liquid* assay eliminates end- user reconstitution of the *lyophilized* reagent and potential reagent handling error.

METHODOLOGY: The Lactic Acid assay utilizes lactate oxidase, to convert lactate in a fluoride/oxalate plasma sample to pyruvate and hydrogen peroxide. Next, peroxidase, in the presence of hydrogen peroxide produced in the first step, catalyzes the oxidation of a chromogen precursor to produce a colored dye. Production of this dye is proportional to the concentration of lactate in the sample and is monitored at 572 nm.

VALIDATION: The Lactic Acid assay displayed a LoQ of < 1.5 mg/dL. Deviations from linearity were ≤ ±0.9 mg/dL from 1.5 to < 9.0 mg/dL and ≤ ±10% within the range of ≥ 9.0 to 120.0 mg/dL of lactate. The 20-day imprecision evaluation utilizing 3 controls and 2 standards having lactate concentrations ranging from 4.1 - 119.4 mg/dL showed a total %CV of ≤ 4.0% for samples where the lactate concentration was ≥ 9.0 mg/dL (N = 60). Imprecision of samples having lactate concentrations < 9.0 mg/dL displayed a total SD of ≤ ±0.36 mg/dL (N = 60). These imprecision claims represent an improvement over the predicate *lyophilized* Lactic Acid

assay (total %CV ≤ 6.3%). Endogenous and therapeutic interferents were evaluated using the acceptance criteria of ≤ ±0.9 mg/dL (samples having [lactate] < 9.0 mg/dL) or ≤ ±10% (samples having [lactate] between 9.0 and 120 mg/dL) at two lactate concentrations (~7 and ~16 mg/dL). The following are the interferent concentrations where the indicated interferent was within the acceptable limits for each lactate concentration tested, respectively: 12.3 and 12.7 mg/dL unconjugated bilirubin, 3.5 and 3.4 mg/dL conjugated bilirubin, 525 and 525 mg/dL Intralipid, 424 and 424 mg/L N-acetylcysteine, 204 and 204 µg/mL acetaminophen, 1.5 and 1.5 mg/dL L- ascorbic acid, 105 and 105 mg/dL D-lactate, 102 and 102 mg/L phenobarbital, 691 and 691 mg/L salicylic acid, and 13.3 and 13.1 g/dL total protein. Evaluation of CAP and RCPA proficiency samples displayed a bias range of 5.0 - 15.7% and 3.8 - 14.4%, respectively, when comparing the results of the *liquid* and *lyophilized* Lactic Acid assays. Method comparison of the *liquid* and *lyophilized* Lactic Acid assays generated a Passing Bablok correlation of [*liquid*] = 1.08[*lyophilized*] - 0.2 (R^2 = 0.99) for samples ranging from 1.6 to 107.6 mg/dL (N = 126). Reagent on-board/calibration stability showed that the *liquid* Lactic Acid reagent could remain on the *c*Systems instrument for 30 days without recalibration, representing an improvement over the 7-day claim for the *lyophilized* assay. Furthermore, the new *liquid* assay has 12 months of shelf life stability.

CONCLUSIONS: The *liquid* Lactic Acid assay is a convenient, stable, sensitive, accurate, precise, and robust assay for the measurement of lactate in patient samples.

B-371

Molecular Identification of Phospholipids Implicated in Dementia

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Background

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. Recently, Mapstone *et al*. reported a panel of plasma phospholipids that predicted cognitively normal adults who later progressed to either mild cognitive impairment or dementia due to AD (Mapstone 2014). This study used a targeted metabolomics p180 kit (Biocrate, Life Science AG, Austria) that measures phospholipids by a triple Quadruple mass spectrometry. Phospholipids have many isomers comprised of different combinations of fatty acids. Because a triple Quadruple Mass Spectrometry (e.g., AB Sciex 6500) generally lacks the mass accuracy and resolution needed to easily distinguish the isomers, fatty acid molecular identify of the phospholipids remains unknown. Identification of the fatty acid molecular identity of the phospholipids implicated in AD is critical for two reasons: (a) one will not be able to determine the functions of these phospholipids and their contributions to pathophysiology of the disease; and (b) one need to determine the molecular identity before one can develop quantitative assays to measure the phospholipids in plasma.

Objective

The objective of this study is to determine fatty acid components of 7 phospholipids, phosphatidylcholine (PC) acyl-acyl (aa) C36:6, PC aa C38:0, PC aa C38:6, PC aa C40:1, PC aa C40:2, PC aa C40:6, and PC acyl-alkyl (ae) C40:6, in plasma using a high-resolution accurate mass liquid chromatography tandem mass spectrometry (LC/MS/MS) based approach.

Methods

A pool was made using plasma from 15 participants with normal cognition (n=5), mild cognitive impairment (n=5), and dementia (n=5). Twenty microliter of the pooled plasma was extracted using organic solvents of Chloroform, Methanol, and Water. Internal standards lysophosphatidylcholine 17:1 and PC 17:0/14:1 (Avanti Polar Lipids, Alabama) were added before performing the analysis by ultra high-pressure liquid chromatography (Dionex 3000-RSLC HPG) using a C30 prototype column (2.1 X 250 mm, 1.9 um) and Thermo Scientific Orbitrap Fusion Lumos mass spectrometry. MS and MS/MS data were obtained at 120,000 and 30,000 mass resolving power, respectively. The sample was run duplicate with positive and negative mode. The HPLC gradient was 60:40 ACN/water to 90:10 IPA/ACN (0.1% formic acid and 10mM ammonium formate) in 30 minutes. Thermo Scientific LipidSearch 4.1 SP1 software was used for lipid and fatty acid identification.

Results

Our method identified that plasma PC aa 36:6 contains fatty acids 14:0 and 22:6. PC aa 40:2 contains fatty acids 22:0 and 18:2; PC aa 40:6 contains fatty acids 18:0 and 22:6. Fatty acids of PC ae C40:6 could be either 18:0p ("p" stands for plasmalogen vinyl ether) and 22:5 or 20:1p and 20:4 ; fatty acids of PC aa C38:6 could be either 16:0 and 22:6, 18:1 and 20:5, or 18:2 and 20:4. However it did not confirm the fatty acid molecular identity for PC aa C38:0 or PC aa C40:1.

Conclusions

Identification of fatty acid components in these phospholipids will enable us to develop quantitative methods to measure them as prognostic biomarkers in AD.

References

Mapstone M, et al. Plasma phospholipids identify antecedent memory impairment in older adults. Nature Medicine. 2014;20(4):415-8.

B-372

The Benefits and Challenges of Utilizing Bulk Solvents in the Laboratory

T. V. Hartman, A. Guenther, A. Getchell, M. Hanley, D. Rollmann, P. Jannetto, L. Langman. *Mayo Clinic, Rochester, MN*

Objectives:

Due to the high volume of organic solvents used in the laboratory, a bulk-solvent delivery system was evaluated to determine if the system could reduce costs, eliminate waste, and improve work-flow and safety.

Background:

Solvents play a major role in sample preparation and analysis in the clinical laboratory. In order to perform testing, a reliable supply of solvent at the required grade/purity and suitable economic pricing are necessary. Safety and regulatory concerns must also be considered and addressed.

Design and Methods:

The bulk-solvent delivery system employs a fleet of re-usable 200L stainless steel containers which are returned to the vendor to be refilled after each use. The containers are sequestered for specific solvents and are not interchanged or filled with alternate solvents. Furthermore, the containers assigned to the Mayo Clinic fleet are used only in Mayo's system ensuring an uncontaminated and consistent supply.

The containers are connected to a high-purity nitrogen supply which applies positive pressure to dispense the solvents at approximately 4L per minute. Subsequently, the solvent supply has no exposure to the atmosphere prior to dispensing which allows the solvent to maintain its purity inside the containers.

Results:

The bulk-solvent delivery system was implemented for the 4 highest volume solvents: methanol, acetonitrile, hexane, and isopropyl alcohol. By purchasing these solvents in the bulk containers vs. 4L bottles, the total solvent cost per year was reduced by 13% or roughly $12k. This system also reduced the consumption of over 3900 glass bottles per year which no longer are being delivered, stocked, handled, and recycled on a daily basis. Additionally, the bulk-solvent delivery system reduces the risk of exposure to these solvents by reducing the events involving broken or mishandled bottles.

Many challenges arose with the implementation of the bulk -solvent system including: high initial implementation costs ($108k), flow-rate issues, hardware compatibility, hardware malfunctions, and container portability issues.

Conclusions

The implementation of the bulk-solvent delivery system has produced many benefits but has also presented significant challenges and issues. The initial costs of building and implementing the necessary infrastructure for the system was significant and the pay-back period will likely be several years calculated solely on the yearly cost-savings of the solvent, but other benefits were also weighed heavily in the decision to build and implement the system. The system is also providing significant work-flow, safety, and environmental benefits that will be realized for years to come.

B-373

Comparative Study of Three Chemistry Instrument for Testing the Level of BUN & CK in Finger Blood and Venous Blood from 40 Athletes

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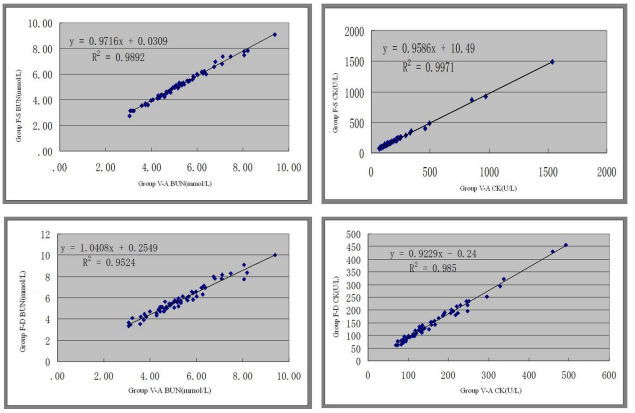
Background: To make a comparison and correlation between the level of blood urea nitrogen(BUN) & creatine kinase (CK) in finger blood monitored by semi-automatic chemistry analyzer (Group F-S) or dry chemistry analyzer (Group F-D) and those in venous blood monitored by automatic chemistry analyzer (Group V-A) from same samples. Methods: Finger blood and venous blood from 40 athletes were collected and tested for the level of BUN and CK. Finger blood was measured on MD-100 semi-automatic chemistry analyzer or reflotron.sprint system , but venous blood on 7020 automatic chemistry analyzer. Results: The result indicated that there was significant difference but significant correlation among three analytic methods. The linear regression equation is Y=0.9716X+0.0309 (p<0.001) and Y=0.9586X+10.49 (p>0.05) respectively for the level of BUN & CK between Group F-S and Group V-A, while the linear regression equation is Y=1.0408X+0.2549 (p<0.001) and Y=0.9229X-0.24 (p<0.001) respectively for the level of BUN & CK between Group F-D and Group V-A. Conclusion: The regression equations may be helpful for the monitoring of sport training in athletes.

The comparison on the level of BUN & CK in Group F-S and Group V-A

	Group F-S	Group V-A	*possibility*	regression equation
BUN（mmol/L）	5.10±1.29	5.22±1.31	＜0.001	Y=0.9716X+0.0309
CK（U/L）	212±231	214±222	＞0.05	Y=0.9586X+10.49

The comparison on the level of BUN & CK in Group F-D and Group V-A

	Group F-D	Group V-A	*possibility*	regression equation
BUN（mmol/L）	5.7±1.45	5.2±1.36	＜0.001	Y=1.0408X+0.2549
CK（U/L）	190±178	217±238	＜0.001	Y=0.9229X-0.24



AUTHOR INDEX TO ABSTRACTS OF SCIENTIFIC POSTERS, AACC MEETING, 2016

(Numbers refer to Poster Numbers; see pages S2 to S234)

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z